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RESEARCH

Assessment of the nutritional and effluent properties of potential fish-meal-free diets for rainbow trout (*Oncorhynchus mykiss* W.) in Iran

Hamed Salehi[1,2] · **Stefan Reiser**[1] · **Ulfert Focken**[1,2]

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Abstract
Research is needed on alternative ingredients for aquafeeds due to rising demand and limited fish meal availability. This study evaluated fish-meal–free diets for rainbow trout in two phases. The first phase focused on assessing the growth performance and nutrient digestibility of six cold-pelleted diets. Aside from the control diet, the test diets contained poultry protein concentrate, blood meal, low-ash poultry by-product meal, 20–40% canola meal, and feather meal (Goldmehl®). Juvenile fish, averaging 5.3 g, were fed the diets for 56 days in a random-block design with four replicates per feed. Feces were collected using the settling method to evaluate nutritional digestibility and phosphorus availability. The feed from the primary phase, which resulted in the least-cost feed conversion ratio, was extruded and fed to fingerlings (14.6 g) in the second phase. This trial added to the first run in measuring digestibility parameters over a 28-day period. In parallel, extruded feed was given to grow-out fish, averaging 172.5 g, for 55 days in triplicate completely randomized tanks to evaluate growth performance and nutrient effluents. Throughout all the experimental runs, a casein-based laboratory pelleted feed was used for the respective controls. Feeding rainbow trout with 35% canola meal in both pelleted and extruded forms showed comparable results ($P > 0.05$) to the control in terms of growth performance and apparent digestibility for lipid (> 91%), crude protein (> 87%), and organic matter (> 78%). The extruded feed in this study represents an eco-friendly option for the growing aquafeed sector.

Keywords Salmonids · Digestibility · Phosphorous availability · Sustainability · Eutrophication

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✉ Hamed Salehi
hamedsalehi1983@gmail.com

Stefan Reiser
stefan.reiser@thuenen.de

Ulfert Focken
focken_ulfert@yahoo.com

[1] Thünen Institute of Fisheries Ecology, Bremerhaven, Germany

[2] Present Address: University of Hohenheim, Institute of Agricultural Sciences in the Tropics, Stuttgart, Germany

Introduction

Iran has recently experienced a development in its aquaculture industry, and that sector remained unaffected by the COVID-19 pandemic. According to a decade-long statistical analysis, the culture of aquatic animals in Iran has risen significantly, from 296,514 tons in 2012 to 480,624 tons in 2022, representing a growth rate of over 60% during that period. Rainbow trout (*Oncorhynchus mykiss* W.) has been a primary focus of aquaculture in Iran (Fig. 1). Furthermore, this country has emerged as a global leader in rainbow trout production, with a total culture of 194,000 tons in 2022, accounting for roughly 19% of the world's reared rainbow trout (FAO 2024). This growing sector needs sustainable feed sources. According to Alltech (2024), around 300,000 tons of aquafeed were produced in 2023 in Iran. As a result, it has become essential to study commodities that are potentially available in local markets to ensure the fulfillment of the demands of the aquafeed industry.

Environmental studies revealed that the expansion of rainbow trout farming in Iran negatively affected aquatic ecosystems (Gholizadeh and Zibaei 2021; Tavakol et al. 2017; Soofiani et al. 2012). In the majority of aquaculture practices, feeds are recognized as sources of pollution. This is due to the fact that fish feces primarily consist of undigested portions of the feed, and any surplus nutrients absorbed by the fish are excreted through urine, gills, or feces. Therefore, the goal is to create environmentally friendly feeds that minimize nutrient discharge by enhancing the digestion, absorption, and retention of nutrients in cultured aquatic animals. This goal can be achieved by reducing dietary nutrient levels to the minimum required amounts and incorporating highly digestible feed components into the feed formulation process (Sugiura and Hardy 2000).

Fish meal is considered the prime source of protein for aquafeeds, but due to its limited availability from sustainable resources and the increasing gap between its supply and the demand for aquafeeds (Tacon and Metian 2015, 2008), alternative feed compounds are

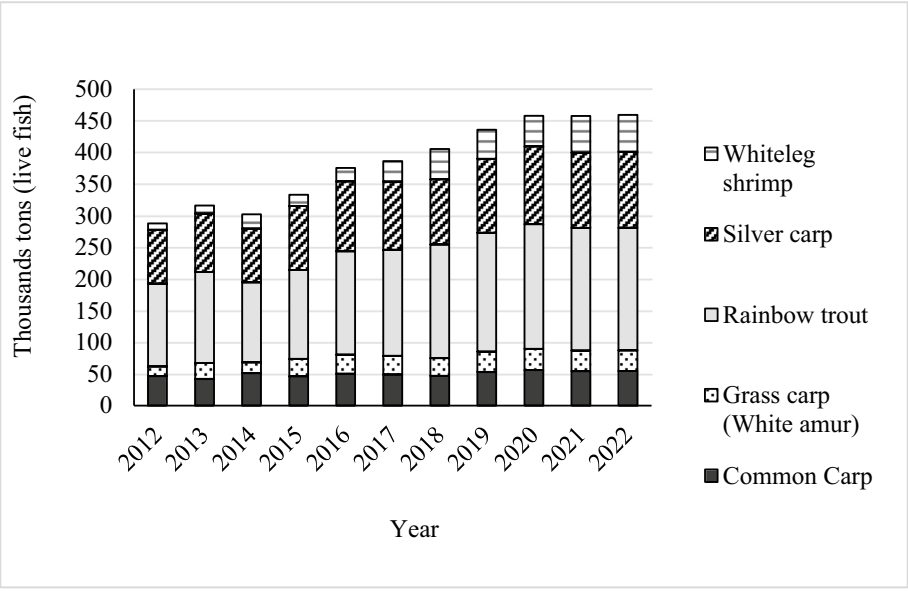

Fig. 1 Reared main aquatic species in Iran

needed. This is especially true for Iran, where in addition to the high price on the world market also other obstacles to the import of high-quality feed ingredients such as fish meal exist. The present investigation was designed to develop fish-meal–free diets using potential locally available feed ingredients in order to secure sustainable aquafeed for the growing aquaculture industry. To achieve this, the most digestible feed components from the recent publication of Salehi et al. (2023) were incorporated into five terrestrial protein-based diets with gradual levels of canola meal (CM) reaching up to 40% dry matter (DM) base. During the first run, the digestibility of experimental diets and growth performance parameters were assessed in juvenile rainbow trout. The purpose of the second run was to simulate practical conditions encountered in the rearing of market-size fish. This phase aimed to measure the reliability and applicability of the data obtained in the primary phase, specifically in relation to the aquafeed industry. Therefore, the experimental formulation that yielded the most cost-effective growth performance in the initial run was selected to undergo an extrusion process. In this phase, the in vivo digestibility of that feed was evaluated in fingerling rainbow trout in the aquaria setup. Meanwhile, a pelleted semi-synthetic control diet and extruded practical feed were fed to grow-out rainbow trout in fiberglass tanks to examine fish performance parameters, nutrient utilization productivity, and nutritional effluent. The findings of this study have the potential to inspire aquafeed manufacturers in Iran to develop low-emission environmentally friendly aquafeeds without fish meal.

Materials and methods

Experimental setups

This study was carried out in two different setups. In the first one, the experimental fish were placed in an aquarium system to monitor various parameters related to fish performance and feed digestibility. The second phase consisted of two parallel parts, which involved both aquaria and tanks. The aquarium setup was selected because fingerling fish were used for feed digestibility assessment and phosphorus availability, utilizing the settling method and gentle siphoning of feces to comply with animal welfare standards. In contrast, grow-out fish were stocked in tanks to allow for more space to grow, align with industrial production practices, and measure growth performance parameters as well as assess nutrient discharges for this size of fish.

Diet preparation

In order to assess growth parameters and feed digestibility in juvenile rainbow trout, highly digestible rendered poultry by-products and CM from the recently studied digestibility trial of Salehi et al. (2023) were used to formulate five fish-meal–free test diets with a graded level of 20 to 40% canola meal at the expense of animal digestible crude protein in the first run. All test diets maintained a consistent inclusion level of poultry protein concentrate (PPCon) (AquaTrac® sol SD) and low ash poultry by-product meal (PBM). However, the amount of blood meal (BM) and feather meal (FeM) varied across the test diets. The commercial poultry rendering manufacturers GePro Geflügel-Protein Vertriebsgesellschaft mbH & Co. KG and its poultry processing unit A&L Tierfrischmehl Produktions GmbH, located in Diepholz, Germany, supplied the animal-based ingredients used in the test diets of this study. These animal protein sources met the EU regulations outlined in (RE EU) No. 1069/2009 and 142/2011

Table 1 Ingredients of experimental diets[§] (g kg^{-1} dry matter)

Ingredients	Trial 1						Trial 2	
	Control	20%	25%	30%	35%	40%	Control	35%
Poultry protein concentrate[1]*		10.0	10.0	10.0	10.0	10.0		10.0
Blood meal[2]*		46.0	45.0	46.0	42.0	43.0		50.0
PBM_LA[3]*		110.0	110.0	110.0	110.0	110.0		110.0
Feather meal[4]*		204.0	190.0	175.0	164.0	148.0		160.0
Canola meal[5]		200.0	250.0	300.0	350.0	400.0		350.0
Wheat flour		182.9	150.2	117.1	84.4	52.1		147.8
Casein	400.0						384.3	
Gelatin	40.0						40.0	
Cellulose[6]	125.9						71.5	
Dextrin	95.0						100.0	
Pre-gelatinized corn starch[7]	65.0						122.0	
Fish oil	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Canola oil	154.0	144.9	145.0	144.8	144.8	144.5	180.0	84.0
DL-Methionine[8]**	23.7	14.7	14.7	14.6	14.4	14.4	6.5	7.2
L-Lysine[9]**		11.3	10.3	9.1	8.4	7.2		10.0
L-Arginine**	3.3						3.2	
L-Threonine[10]**	1.0	1.2	1.1	1.0	0.9	0.9		
Sodium carbonate	21.0						21.0	
Monocalcium phosphate	16.1	20.0	18.7	17.4	16.1	14.9	16.5	16.0
Phytase[11] (mg kg^{-1} feed as.is)		78.3	81.4	84.5	87.7	91.1		300.0
Choline chloride 98%					1.0			
Vitamin C 35%[12]					1.0			
Vitamin premix[13]					5.0			
Mineral premix[13]					3.0			
TiO2					5.0			

[§]Control: casein-based semi-synthetic laboratory standard diet; 20%, 25%, 30%, 35%, and 40% are canola meal inclusion rates in the designed fishmeal-free diets

[1]AquaTrac sol SD®

[2]Ultra-flash dried poultry blood meal

[3]Poultry by-product meal (low ash)

[4]Goldmehl®

[5]Provided by Teutoburger Ölmühle GmbH, Ibbenbüren, Germany

[6]Provided by Mikro-Technik GmbH & Co. KG, Bürgstadt am Main, Germany

[7]Provided by Kröner-Stärke GmbH, Ibbenbüren, Germany

[8]MetAMINO®

[9]Biolys®

[10]THREAMINO®

[11]Natuphos® E 5000 G. Provided by BASF SE, Ludwigshafen, Germany

[12]CUXAVIT CPHOSPHATE STAB, provided by Kaesler Nutrition GmbH, Cuxhaven, Germany

[13]Vitamin and mineral requirements of fish were fulfilled. Provided by Trouw Nutrition Deutschland GmbH, Burgheim, Germany

*Provided by GePro Geflügel-Protein Vertriebsgesellschaft mbH & Co. KG and A&L Tierfrischmehl Produktions GmbH, Diepholz, Germany

**Provided by Evonik Nutrition and Care GmbH, Hanau-Wolfgang, Germany

and were categorized as "Category 3" material. The CM was purchased from Teutoburger Ölmühle GmbH, Ibbenbüren, Germany. Table 1 illustrates the feed composition in the first and second runs of the study. In order to simulate practical conditions in rainbow trout culture, the test diet which resulted in the best price-efficient feed conversion ratio (FCR) during the initial run, which contained 35% CM, was selected to get extruded for feeding market-size fish in tanks during the second run. The feed formulations in the second phase were somewhat modified based on the changes in fish size, particularly for digestible amino acids (NRC 2011). The casein-based semi-purified control diets used in all phases of this study were developed based on the standard Guelph diet for rainbow trout. Test diets were also formulated based on the nutritional requirements for fish of varying sizes (Hardy and Barrows 2002; NRC 2011). With the exception of BM, PPCon, and PBM, all feed ingredients containing large particles were ground using a coffee grinder (KSW 3306, Clatronic International GmbH, Kempen, Germany). The macro feed ingredients were then sieved through a 1.0- to 2.0-mm screen, while the micro feed components through a screen smaller than 0.5 mm. For the assessment of digestibility of diets, titanium (IV) oxide (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was added as an indigestible marker to feeds. Exogenous phytase was added before pelleting and after extrusion with the amount of 1000 and 1500 FTU/kg of plant composition in the first and second runs, respectively. To produce semi-purified control diets in both runs as well as test diets in the initial run, the prepared micro feed ingredients were sequentially added to a kitchen blender (CNUM 80, Robert Bosch Hausgeräte GmbH, Germany) in increasing order of volume. Subsequently, the mixture was thoroughly combined with other macro feed components and oils, using a handheld concrete mixer to ensure precise mixing (DCD771 DEWALT, Czech Republic). The mixtures were pelletized using a cold pellet mill (Type 14–175, Amandus Kahl, Hamburg, Germany) with a 3.0-mm die to obtain the desired pellet size. In order to ensure the production of stable pellets, deionized water was sprayed onto the feed multiple times during the processing. Afterwards, the pellets were dried in an electronic oven at 30 °C for 24 h (T 12, Thermo Electron LED GmbH, Langenselbold, Germany). Prior to the extrusion of the test diet in the second run, all feed components except for oil and phytase were combined in a spiral mixer (Kronos 200 Pro, WP Kemper GmbH, Germany) in descending order of volume. The mixture was mixed for a duration of 6 min and subsequently extruded using a pilot-scale twin-screw extruder (EX-36, Bühler, Switzerland) across five barrels under a pressure of 18 bar and an average temperature of 112.5 °C for approximately 30 s. The pellets' temperature and diameter at the die were 110 °C and 3.5 mm, respectively. The final pellets were dried at 45 °C for 12 min with an industrial vibrator dryer until the dry matter content reached 95%. In order to ensure consistent distribution of the phytase enzyme on the extruded pellets, the phytase was dissolved in distilled water at a concentration of 1% of the feed and sprayed onto the pellets in a vacuum coater. Subsequently, the enzyme-treated pellets were coated with oil. The extrusion process was conducted at Technologie Transfer Zentrum in Bremerhaven, Germany. Both pelleted and extruded diets were stored in sealed plastic buckets at 4 °C until the feeding time of the fish.

Fish rearing

All trials were conducted within environmentally controlled indoor facilities at Thünen Institute of Fisheries Ecology in Bremerhaven, Germany. The experimental rainbow trout (*Oncorhynchus mykiss* W.) were obtained from the institute's own brood stock. For the aquaria investigations, 80 fish were randomly allocated to four 57-l aquaria for each diet. The aquaria were designed to settle feces, and they were part of a semi-recirculating

aquaculture system (RAS). The system was aerated by pressurized air from a compressor (LA-120A, Nitto Kohki Co., Ltd., Japan). Air was provided for every aquarium with a spherical-shaped airstone. To maintain water quality within the optimal limits for rainbow trout, the RAS was equipped with a pad filter, ultraviolet (UV) light, biofilter, and a water temperature controller (TECO®, Ravenna, Italy). The water inflow for the aquaria was adjusted to ensure an adequate supply of fresh water for the fish while also allowing for the collection of sufficient feces through the siphoning method. During the tank trial of the second run, the fish were allocated randomly to three 377-L fiberglass tanks for each diet. Every rearing tank was connected to a swirl separator, a water reservoir tank, and a biofilter tank. The inflow of preprocessed well water into the system was adjusted to ~ 15 L min^{-1}. A pump (DM-VARIO 10000, AQUA FORTE, Veghel, Netherlands) transferred water from the reservoir to the rearing tank. Apart from the swirl separator, the remaining tanks were aerated using an air compressor (VAU KDT 3.80/6–400, Becker, Wuppertal, Germany) through cylinder-shaped stone aerators. Water quality parameters such as oxygen and temperature were monitored using a probe (OxyGuard® International A/S, Farum, Denmark) 2 h after feeding in both the inflow and outflow basins of the aquaria system, as well as the rearing fiberglass tanks. Every 2 days, the water pH was measured with a probe (Type 7110, Xylem Analytics Germany GmbH, Weilheim, Germany) as well as NH_4^+, NO_2^-, and NO_3^- using a photometer (Spectroquant® test kit, Merck KGaA, Darmstadt, Germany) were analyzed in inflow basin water from the aquaria RAS and each fish rearing fiberglass tank. The aquaria and fish rearing tanks underwent cleaning every 2 weeks after recording the weight of the fish. However, in the second run, the out-flow collecting tanks required daily cleaning. For the aquarium trials, the fish for feeding trials and comparative studies were anesthetized with 2-phenoxyethanol (Merck KGaA, Darmstadt, Germany) and slaughtered by cutting the gill vessels. On the other hand, the fish from the fiberglass tanks in the second run were stunned by percussion and sacrificed by cutting the gill artery. An overview of all the experimental conditions is shown in Table 2. All experimental procedures were carried out in compliance with the European Directive 2010/63/EU, which governs the protection of animals used for scientific research purposes.

Feeding procedure

Commercial feed was used for rearing before stocking the fish in either experimental aquaria (Aller Futura EX GR 0.9–2.0 mm, Emsland Aller Aqua GmbH, Golßen, Germany) or tanks (Optiline F-3P 6.0 mm, Skretting/Trouw Nutrition Deutschland GmbH, Burgheim, Germany). The fish were individually weighed after 24 h of starvation at the beginning and end of each trial, as well as every 2 weeks during the trials. Weight determination every 2 weeks allowed us to adjust the amount of feed provided to experimental units. In the first run, after the fish were stocked into the aquaria, the daily feeding level was initially set at 0.5% of the biomass weight using a commercial feed (Aller Futura EX GR 0.9–2.0 mm, Emsland Aller Aqua GmbH, Golßen, Germany) during the acclimatization period. Subsequently, the commercial feed was replaced with experimental diets, and the feeding level was increased gradually up to a maximum of 2% of biomass once the diets were fully accepted by the fish. On the other hand, the fish from the second run, both fiberglass tanks and digestibility aquaria, were directly fed with experimental diets at a level of 1% of the biomass weight upon stocking in the experimental units and then was increased to reach 2% of the biomass weight. Once the minimum feeding level of 1% of the biomass weight

Table 2 Experimental setups of the two trials

	Trial 1	Trial 2	
	Aquaria	Aquaria	Tanks
Volume [L]			
Whole system	1500		850
Aquarium/tank**	57		377
Water inflow rate [L min^{-1}]			
Well water inflow into the system	6		15
Water flow to each aquarium/tank**	2		17
Water turnover rate [times/day]			
Whole system	5.8		25
Aquaria/tanks**	50.5		65
Water source	Preprocessed well water		
Number of aquaria/tanks** per diet	4		3
Type of aquaria/tanks**	Rectangular glass, tapered bottom		Cylinder-shape fiberglass
Photoperiod [light:dark]	12:12 h		
Type of light	LED tube		Fluorescent tube
Number of fish per aquarium/tank**	20	20	32
Number of reference fish	100	20	5
Room temperature [°C]	18	18	15
Average water temperature [°C]*			
Aquaria/tanks**	12.7 ± 0.06	13.8 ± 0.05	13.8 ± 0.07
System inflow	12.9 ± 0.31	13.9 ± 0.41	
System outflow	12.9 ± 0.31	13.9 ± 0.39	
Dissolved oxygen [mg L^{-1}]*			
Aquaria/tanks**	10.3 ± 0.04	10.0 ± 0.10	8.7 ± 0.24
System inflow	10.5 ± 0.23	10.1 ± 0.16	
System outflow	10.4 ± 0.23	10.0 ± 0.19	
pH*	8.2 ± 0.08	8.2 ± 0.06	8.0 ± 0.03

Table 2 (continued)

	Trial 1 Aquaria	Trial 2 Aquaria	Tanks
$NH4^+$ [mg L^{-1}]*	< 0.06 ± 0.02	0.11 ± 0.04	0.30 ± 0.05
$NO2^-$ [mg L^{-1}]*	0.13 ± 0.04	0.22 ± 0.10	0.20 ± 0.06
$NO3^-$ [mg L^{-1}]*	3.10 ± 1.37	7.46 ± 2.8	8.11 ± 0.32
PO4-P [mg L^{-1}]*			0.13 ± 0.02
Average stocking weight [g]*	5.3 ± 0.59	14.6 ± 1.04	172.5 ± 12.15
Initial average total length [cm]*			24.6 ± 1.57
Stocking density [kg m^{-3}]*	1.8 ± 0.02	4.9 ± 0.01	14.6 ± 0.03
Feeding level [%biomass/aquarium or tank**]	1–2		
Feeding time [hour]	8:00 and 13:00		9:00 and 14:00
Acclimation period [days]	2	0	0
Trial period [days]	56	28	55

*Reported mean values with standard deviation (± SD) **Experimental units

was achieved, the daily feed ration was divided into two equal installments and carefully hand-fed. The fish's feed intake (FI) was closely monitored, and feeding was stopped when the fish refused to consume any more feed. The remaining portion of the feed was then recorded. After conducting the experimental trials, the total consumed feed for each experimental unit was calculated. The aquaria trials were conducted in a randomized block design with four replicates ($n=4$). However, a completely randomized design was employed with three replicates for each diet in fiberglass tanks. Throughout the trials, any incidences of mortality were recorded daily, and the removed fish were stored in a freezer.

Sample collection, sample preparation, and chemical analyses

Before distributing fish in the experimental units and after 24 h of starvation, a number of fish were randomly selected as reference fish from the stock. These fish were then anesthetized by using 2-phenoxyethanol, weighed, and slaughtered by cutting the gill artery, and stored at − 21 °C for further comparative slaughter method analyses (Jobling 2001a). The same procedure was performed for the fish in the tank trial but after 48 h of starvation. Following the slaughter of market-size fish from the tanks, the liver of five fish within the range of modal value biomass weight was dissected, weighed, and frozen at − 21 °C for analysis of lipid content. The frozen fish samples were prepared for analysis by following a specific procedure. Firstly, the samples were defrosted in a fridge overnight at a temperature of 4 °C. Once defrosted, the fish samples were sliced and subsequently autoclaved (V-75, Systec GmbH, Linden, Germany) at 121 °C and 210.2 kPa for either 5 or 8 min, depending on fish size. The autoclaved fish samples were later ground and homogenized with a lab grinder (GRINDMIX, GM 200, Retsch GmbH, Haan, Germany). The preparation of frozen liver samples followed a similar procedure to the fish samples, with the exception of the autoclaving step. Both the homogenized fish bodies and liver samples were frozen at − 21 °C for at least 48 h. This freezing process ensured that the samples could be properly freeze-dried (GT 2, SRK-Systemtechnik GmbH, Riedstadt, Germany). After a 7-day feeding period with experimental diets, the collection of feces was performed using the passive settling method for the purpose of digestibility assessments. The feces were obtained by gently siphoning them from each aquarium before the morning feeding and approximately 2 h after feeding. This was carried out three times daily to minimize the potential leaching of soluble nutrients from the feces. Within the digestibility assessment period, the feces from every aquarium were collected daily, pooled, and frozen at − 21 °C for freeze-drying like fish body and liver samples. To assess the phosphorus content in the sludge collected from the grow-out fish in fiberglass tanks, the waste-collecting tanks were cleaned in the mornings prior to feeding the fish. Afterwards, in the evenings, the sludge from the tanks was siphoned and filtered through a mesh with a pore size of 0.1 mm for a few days at the end of the rearing period. These samples were freeze-dried, too. Experimental diets, fish bodies, liver, feces, and sludges, were ground finely with the lab grinder (GRINDMIX, GM 200, Retsch GmbH, Haan, Germany). The ground feces and diets passed through a 0.5-mm and 1.0-mm sieve, respectively. All the prepared samples were packed in airtight plastic containers and stored at − 21 °C until further lab analyses.

For proximate composition analysis, the official methods of VDLUFA (2007), which were outlined in Germany, were followed. The dry matter (DM) content was assessed by drying all the samples in an electric oven (T 12, Thermo Electron LED GmbH, Langenselbold, Germany) at 103 °C for 4 h. To determine the ash content, the dried samples were incinerated in a muffle furnace (P 330, Nabertherm GmbH, Lilienthal, Germany) at

550 °C for 3 h. The Smedes lipid extraction method from Schlechtriem et al. (2003) with small modifications was used to measure lipid contents. These parameters were analyzed in triplicate. The diets and fish bodies' gross energy (GE) were measured in duplicate with a bomb calorimeter (KV600; IKA-Werke GmbH & Co.KG, Staufen, Germany). Crude fiber (CF) contents in experimental diets and crude protein (CP = %N × 6.25) were determined according to method 6.1.3 and method 4.1.1 from VDLUFA (2007) at the Institute of Animal Nutrition and Physiology of Christian-Albrechts-University, Kiel, Germany. The amino acid profile in diets was measured by AGROLAB LUFA GmbH (Kiel, Germany) in accordance with the European Union Regulations (EC/No. 152/2009). The nitrogen-free extract (NFE) content in the diets was determined by subtracting the values of CP, CL, CF, and ash from 1000. The difference between 1000 and the ash content was considered as the organic matter (OM) content. The total phosphorous content and TiO_2 in diets and feces were measured following the methodology described by Zeller et al. (2015) at the Institute of Animal Nutrition, University of Hohenheim in Stuttgart, Germany. The water phosphorus content from each fish-rearing fiberglass tank as well as phosphorous in sediments and fish bodies were photometrically determined using PMB and VM methods, respectively (Spectroquant® test kit, Merck KGaA, Darmstadt, Germany). The pH of the diets was determined using the same probe that had been used for measuring water pH. Table 3 represents the analyzed nutritional data for experimental diets.

Calculations

Growth performance

To evaluate the growth performance of fish, the weight gain (WG) and FCR were measured for every diet as follows:

$$WG\ [g] = (final\ biomass\ weight\ [g] + mortality\ weight\ [g]) - initial\ biomass\ weight\ [g]$$

$$FCR = dry\ feed\ fed\ [g]/WG\ [g]$$

Productivity and digestibility

To determine the retention of the nutrients from the experimental diets in the fish, the nitrogen productive value (NPV), lipid productive value (LPV), organic matter productive value (OMPV), and gross energy productive value (GEPV) were measured for every diet by using nutrient productive value (NutrPV) formula.

$$NutrPV\ [\%] = [(final\ fish\ body\ nutrient\ in\ g - reference\ fish\ body\ nutrient\ in\ g) / total\ consumed\ nutrient\ in\ g] \times 100$$

Each diet's protein efficiency ratio (PER) was calculated using the formula provided by Hardy and Barrows (2002).

$$PER\ [g] = WG\ [g]/dry\ crude\ protein\ fed\ [g]$$

Table 3 Nutritional analysis of experimental diets[§] (g kg^{-1} dry matter)

Diets	Trial 1						Trial 2	
Nutritional composition	Control	20%	25%	30%	35%	40%	Control	35%
Dry matter	915.0	928.0	918.0	932.0	949.0	957.0	912.0	947.0
Gross energy [MJ kg^{-1}]	23.7	24.3	24.4	24.4	24.8	24.7	23.7	22.4
Digestible energy (DE) [MJ kg^{-1}]*	17.0	17.0	17.0	17.0	17.0	17.0	18.0	18.0
Crude protein	414.0	424.0	424.0	421.0	420.0	415.0	418.1	438.1
Digestible crude protein (DCP)*	390.7	390.5	390.2	390.5	390.5	390.2	380.2	380.2
Crude lipid	190.0	242.0	249.0	261.0	263.0	268.0	205.0	183.0
Crude fiber	189.7	94.3	123.4	130.4	134.9	173.0	61.9	41.7
Crude ash	43.0	52.0	54.0	56.0	58.0	60.0	48.0	63.0
Nitrogen-free extract (NFE)[†]	163.3	187.7	149.6	131.6	124.1	84.0	267.0	247.2
Organic matter (OM)[‡]	957.0	948.0	946.0	944.0	942.0	940.0	952.0	937.0
NFE:OM [%][††]	17.1	19.8	15.8	13.9	13.2	8.9	28.0	26.4
DCP:DE [g/MJ]	23.0	23.0	23.0	23.0	23.0	23.0	21.1	21.1
Phosphorous (total)	7.3	10.9	11.0	11.4	11.7	12.0	7.8	12.5
TiO2	5.3	5.3	5.2	5.3	5.3	5.3	5.5	5.6
pH	7.4	5.6	5.7	5.7	5.7	5.7	6.8	5.6
Amino acids								
Alanine	15.2	n.a	n.a	21.1	21.6	n.a	15.6	22.5
Arginine	20.1	n.a	n.a	26.4	26.7	n.a	19.8	27.0
Aspartic acid/asparagine	29.0	n.a	n.a	30.5	30.8	n.a	29.2	32.6
Cysteine (cystin)	1.2	n.a	n.a	12.4	12.3	n.a	1.4	13.3
Glutamic acid/glutamine	88.6	n.a	n.a	53.9	55.7	n.a	90.7	60.1
Glycine	17.9	n.a	n.a	28.5	29.2	n.a	18.0	29.6
Histidine	11.5	n.a	n.a	9.2	9.4	n.a	11.7	9.6
Isoleucine	20.2	n.a	n.a	18.3	18.6	n.a	20.4	19.1
Loucine	36.8	n.a	n.a	32.9	33.3	n.a	36.8	33.7
Lysine	30.9	n.a	n.a	24.7	24.8	n.a	31.9	27.0
Methionine (methioninsulfon)	38.5	n.a	n.a	21.3	21.3	n.a	18.5	14.3
Phenylalanine	19.3	n.a	n.a	19.2	19.0	n.a	20.4	20.4
Proline	48.4	n.a	n.a	32.2	31.6	n.a	47.9	34.1
Serine	21.4	n.a	n.a	28.3	28.3	n.a	21.9	29.5
Threonine	16.8	n.a	n.a	19.0	19.2	n.a	16.0	19.1
Tryptophan	4.8	n.a	n.a	4.4	4.5	n.a	4.6	4.5
Tyrosine	18.4	n.a	n.a	11.7	12.1	n.a	20.1	12.0
Valine	23.6	n.a	n.a	24.1	24.6	n.a	26.3	27.7

n.a. not analyzed*Calculated by multiplying either energy or protein digestibility coefficient for every feed ingredient from (NRC 2011) or measured digestibility coefficients from Salehi et al. (2023) in the energy or protein content of that ingredient[§]Control: casein-based semi-synthetic laboratory standard diet; 20%, 25%, 30%, 35%, and 40% are canola meal inclusion rates in the designed fishmeal-free diets[†]Calculated by subtracting crude protein, crude lipid, crude fiber, and ash from 1000[‡]Calculated by subtracting the ash content from 1000[††]Calculated by dividing NFE by OM and multiplying 100

The apparent digestibility coefficients (ADCs) of CP, CL, OM, and phosphorus availability (PhA) in the diets were estimated by calculating the ratio of the respective nutrient or phosphorus contents to the amount of marker present in the diets and feces (NRC 2011).

$$ADC_{diets} = [1 - (TiO_2\ concentration\ in\ feed)/(TiO_2\ concentration\ in\ feces) \times (nutrient\ concentration\ in\ feces)/(nutrient\ concentration\ in\ feed)] \times 100$$

The hepatosomatic index (HSI) was measured specifically for the market-size fish in the second run. Additionally, Fulton's condition factor (K) was calculated for the fish using the formula from Jobling (2001b).

$$K = (fish\ weight\ [g]/fish\ length\ [cm]^3) \times 100$$

$$HSI\ [\%] = (liver\ weight\ [g]/body\ weight\ [g]) \times 100$$

Statistical analysis

The Shapiro–Wilk test was applied to test for the normality of the mean values from each trait separately in all trials. In the first run, the Levene test was conducted to evaluate the equality of variance for the mean values of every parameter. It was also assessed whether every aquarium should be added as a random effect. Since the aquarium had an insignificant effect, a linear model without a random component was implemented following Zuur et al. (2009), and one-way ANOVA was run to compare the mean values. When normality and homogeneity of variance criteria were not met, a nonparametric Kruskal–Wallis test was conducted. Tukey's HSD and Dunn's tests were respectively carried out for parametric and nonparametric variables for recognizing the statistical pairwise differences between mean values in run one (Dinno 2015). The means in phase two were tested for homogeneity of variance with the F-test. Student's t-test was employed to identify significant differences among mean values when the data followed a normal distribution and had equal variance. In cases where these assumptions were violated, Welch's t-test was used instead. A significant level at $P < 0.05$ and R software version 3. 5. 1 from RCoreTeam (2018) were supposed and used for statistical analyses, respectively.

Results

Growth, feeding efficiency, and body indices

Neither final weight nor WG differed significantly between fish fed on various levels of CM. Moreover, for those parameters, all the experimental diets did not show any statistically significant differences compared to the casein-based diet, except for the diet

Table 4 Growth performance, feed utilization criteria and body indices of fish achieved over the period of a 56-day feeding diets[§] in aquaria (Trial 1) or tanks (Trial 2)

Parameter	Trial 1						Trial 2	
	Control	20%	25%	30%	35%	40%	Control	35%
Initial weight[†] [g]	105.4 ± 1.27	105.9 ± 0.98	103.8 ± 1.67	104.2 ± 1.59	105.0 ± 1.43	104.9 ± 1.50	5513.9 ± 13.92	5525.8 ± 3.09
Final weight[†] [g]	263.6 ± 10.70^{a}	244.6 ± 17.44^{ab}	242.0 ± 12.41^{ab}	228.0 ± 18.01^{b}	244.9 ± 13.04^{ab}	240.1 ± 5.37^{ab}	11,741.3 ± 447.67	11,581.0 ± 231.38
Mortality weight[†] [g]	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	2.4 ± 3.39	1.5 ± 2.95	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Weight gain [g]	158.3 ± 10.93^{a}	138.7 ± 16.71^{ab}	138.1 ± 11.69^{ab}	126.2 ± 15.90^{b}	141.4 ± 11.30^{ab}	135.2 ± 4.24^{ab}	6227.4 ± 452.20	6055.2 ± 230.54
Dry feed intake [g]	133.4 ± 3.07	129.3 ± 5.88	125.6 ± 3.63	124.0 ± 5.05	130.0 ± 6.1	131.1 ± 3.27	7156.5 ± 71.34*	6877.8 ± 59.00
FCR	0.84 ± 0.04	0.94 ± 0.07	0.92 ± 0.05	0.99 ± 0.09	0.92 ± 0.04	0.97 ± 0.01	1.16 ± 0.08	1.14 ± 0.04
PER	2.87 ± 0.14	2.52 ± 0.20	2.59 ± 0.15	2.41 ± 0.21	2.59 ± 2.59	2.48 ± 0.02	2.08 ± 0.14	2.01 ± 0.06
HSI [%]	2.01 ± 0.08^{a}	1.38 ± 0.14^{ab}	1.43 ± 0.07^{ab}	1.40 ± 0.06^{ab}	1.38 ± 0.06^{ab}	1.35 ± 0.07^{b}	1.66 ± 0.07	1.55 ± 0.13
K [%]	1.37 ± 0.36	1.22 ± 0.07	1.17 ± 0.03	1.19 ± 0.02	1.17 ± 0.04	1.18 ± 0.05	1.29 ± 0.02*	1.40 ± 0.02

The reported values are the mean of either four replicates ($n=4$) with their standard deviation (SD) for trial 1 or three ($n=3$) for trial 2. The means within one line not sharing a superscript letter are significantly different ($P<0.05$) for trial one *FCR* feed conversion ratio, *PER* protein efficiency ratio, *HSI* hepatosomatic index, *K* Fulton's condition factor*Values are considerably different ($P<0.05$) in trial 2 only[§]Control: casein-based semi-synthetic laboratory standard diet; 20%, 25%, 30%, 35%, and 40% are canola meal inclusion rates in the designed fishmeal-free diets[†]Average bulk weight of fish in experimental units for each treatment measured by individual weighing

Table 5 Nutrient utilization productivity criteria of fish, body analysis, and eutrophication parameters achieved over the period of a 56-day feeding trial in aquaria (Trial 1) or tanks (Trial 2)

Parameter	Trial 1 (diets[§])						Trial 2 (diets)	
	Control	20%	25%	30%	35%	40%	Control	35%
LPV [%]	81.3 ± 3.78^{a}	59.6 ± 3.16^{ab}	60.9 ± 2.34^{ab}	55.6 ± 4.95^{b}	60.5 ± 0.80^{ab}	58.5 ± 1.30^{b}	86.6 ± 4.21	90.9 ± 4.29
NPV [%]	43.4 ± 2.00^{a}	37.8 ± 2.97^{b}	38.0 ± 2.68^{ab}	36.1 ± 2.81^{b}	38.5 ± 2.59^{ab}	37.5 ± 0.27^{b}	35.4 ± 1.31	33.7 ± 0.64
OMPV [%]	36.1 ± 0.90^{a}	33.0 ± 1.93^{ab}	33.6 ± 1.99^{ab}	32.2 ± 2.97^{b}	35.1 ± 0.85^{ab}	34.2 ± 0.76^{ab}	35.1 ± 1.67	34.3 ± 1.18
GEPV [%]	433.2 ± 4.97^{a}	396.2 ± 27.50^{ab}	394.7 ± 18.40^{ab}	380.7 ± 35.49^{b}	412.8 ± 3.92^{ab}	398.2 ± 17.15^{ab}	448.6 ± 24.36	457.7 ± 18.14
PhPV [%]	71.6 ± 1.95^{a}	38.9 ± 4.54^{ab}	40.8 ± 3.83^{ab}	33.6 ± 4.05^{b}	37.4 ± 4.15^{ab}	34.6 ± 1.48^{b}	37.7 ± 2.72*	24.8 ± 3.34
Body lipid content [%]	38.2 ± 1.05^{a}	38.6 ± 0.08^{a}	39.7 ± 1.09^{ab}	39.5 ± 0.62^{ab}	40.1 ± 0.71^{ab}	40.6 ± 0.36^{b}	41.1 ± 0.59	39.7 ± 0.93
Liver lipid content [%]							12.7 ± 0.27	11.0 ± 1.18
Sludge phosphorous [g kg^{-1} DM]							9.03 ± 1.16*	22.8 ± 0.76
Water phosphorous [mg L^{-1}]							0.11 ± 0.00*	0.16 ± 0.01
Water ammonium [mg L^{-1}]							0.34 ± 0.02*	0.27 ± 0.02

The reported values are the mean of either four replicates ($n = 4$) with their standard deviation (SD) for trial 1 or three ($n = 3$) for trial 2The means within one line not sharing a superscript letter are significantly different ($P < 0.05$) for trial one *LPV* lipid productive value, *NPV* nitrogen productive value, *OMPV* organic matter productive value, *GEPV* gross energy productive value, *PhPV* phosphorous productive value*Values are considerably different ($P < 0.05$) in trial 2 only [§]Control: casein-based semi-synthetic laboratory standard diet; 20%, 25%, 30%, 35%, and 40% are canola meal inclusion rates in the designed fishmeal-free diets

containing 30% CM in the initial trial. In the second run, the results were similar to the primary run between the control diet and fish consumed 35% CM for final weight and WG. In the first run, the dry feed intake (FI) showed no differences across all feeding groups. However, in the second run, the 35% CM group exhibited a reduced dry FI compared to the control. The FCR and PER remained unchanged among either all test diets in phase one or diet containing 35% CM to the casein-based semi-purified diet. Concerning HSI, the diets with CM did not show significant differences not only within themselves, but to the control, except the one containing 40% CM. Similarly, the results for HSI were comparable to the first run between the control and extruded diet in the second run. In regard to *K*, there were no statistically meaningful changes observed across all the diets in the first run. However, in the second run, there were deviations from this pattern between the control group and the extruded diet (Table 4).

Nutrient utilization, fish body analysis, and eutrophication parameters

The values for nutrient utilization, fish body analysis, and eutrophication parameters are illustrated in Table 5. The test diets, which consisted of 20%, 25%, and 35% CM, showed no statistically considerable difference compared to the semi-purified casein-based diet in terms of LPV in the first run. This was followed between control and extruded feed in the second run, as well. Regarding NPV, test diets containing 25% and 35% CM represented the values of $38.0 \pm 2.68\%$ and $38.5 \pm 2.59\%$, respectively, which were comparable to the control diet ($43.4 \pm 2.00\%$) in the first phase. The NPV findings in the second run were comparatively lower than those observed in the first run, but they exhibited a similar pattern compared to control. All test diets, except for the diet containing 30% CM, did not exhibit a significant difference in comparison with the control group for both OMPV and GEPV in the first run. Likewise, these results were consistent in the second run. Calculation of phosphorous productivity in the first run showed the diets with 20%, 25%, and 35% CM had statistically identical values to the casein-based semi-purified diet; however, that parameter declined for the extruded feed compared to the control diet in the second run. Unlike other test diets, the group containing 40% CM in the first phase demonstrated a significantly higher percentage of body lipid content ($40.6 \pm 0.36\%$) compared to the control group ($38.2 \pm 1.05\%$). The observed values for body lipid content in the second run were statistically identical between the two feeding groups. The liver lipid content and eutrophication parameters were only analyzed in the second run. Except for liver lipid content which remained stable between the casein-based semi-synthetic feed and extruded one, other indicators for nutrient discharge into water were significantly different among these groups. Levels of sludge and water phosphorus were lower in tanks that received the control diet compared to those that received the extruded feed (Table 5). However, the findings for water ammonium demonstrated that the control group had a higher concentration compared to the fish-fed extruded diet. The control group and test diet had ammonium concentrations of 0.34 ± 0.02 and 0.27 ± 0.02 mg L^{-1}, respectively.

Nutrient apparent digestibility and phosphorous availability

As indicated in Table 6, CL was digested without significant differences across all treatments and the control group in both runs of trial. The values of CL ADC for practical diets were quite high (above 92%). In the first run, CP was also digested well, and the

Table 6 Nutrient apparent digestibility and phosphorous availability coefficients for experimental diets determined at the end of either a 56-day feeding period in aquaria (1st trial) or a 28-day digestibility test in aquaria (2nd trial; parallel with tanks)

Parameter	Trial 1 (diets[§])						Trial 2 (diets)	
	Control	20%	25%	30%	35%	40%	Control	35%
CL ADCs [%]	95.9 ± 0.83	94.9 ± 0.23	95.2 ± 0.38	95.2 ± 0.57	94.9 ± 0.26	95.0 ± 0.17	89.3 ± 1.88	91.8 ± 0.69
CP ADCs [%]	99.2 ± 0.08[a]	89.2 ± 0.38[ab]	90.0 ± 0.50[ab]	89.6 ± 0.48[ab]	89.5 ± 0.76[ab]	89.0 ± 0.31[b]	98.9 ± 0.04*	87.2 ± 0.29
OM ADCs [%]	82.3 ± 0.44[a]	77.9 ± 0.32[b]	78.3 ± 0.53[b]	78.5 ± 0.49[ab]	78.9 ± 0.61[ab]	78.8 ± 0.57[ab]	82.9 ± 0.94*	79.0 ± 0.44
PhA [%]	90.5 ± 0.71[a]	66.5 ± 0.74[ac]	64.4 ± 1.56[abc]	61.1 ± 0.92[abc]	59.9 ± 1.00[bc]	55.7 ± 1.37[b]	85.6 ± 1.04*	61.6 ± 2.60

The reported values are the mean of four replicates ($n=4$) with their standard deviation (SD). The means within one line not sharing a superscript letter are notably different ($P<0.05$) for trial one*CL ADCs* crude lipid apparent digestibility coefficients, *CP ADCs* crude protein apparent digestibility coefficients, *OM ADCs* organic matter apparent digestibility coefficients, *PhA* phosphorous availability*Values are considerably different ($P<0.05$) in trial 2 only[§]Control: casein-based semi-synthetic laboratory standard diet; 20%, 25%, 30%, 35%, and 40% are canola meal inclusion rates in the designed fishmeal-free diets

observed data for this macronutrient were comparable to the semi-purified casein-based diet, excluding the feeding group with 40% CM. In the second run, however, the CP of the extruded feed containing 35% CM was digested lower than that of the control group (87.2 ± 0.29% versus 98.9 ± 0.04%). The dietary inclusion of 30 to 40% CM resulted in a comparable OM ADC to the control diet in the first run; however, that nutrient was not digested as like as the casein basal diet in the second run. All test diets showed an identical PhA to the semi-purified casein-based diet with no statistical significance ($P>0.05$), except for the diets that contained 35% and 40% CM in the first run. In the second run, the extruded feed illustrated a PhA level that was nearly identical to the first run. The observed value of that parameter for that feed was 61.6 ± 2.60%, which was notably lower than the control group, 85.6 ± 1.04%.

Discussion

Growth, feeding efficiency, and body indices

Neither disease nor significant mortality was observed during the first run and, of course, the second one; therefore, it can be concluded that the growth performance parameters were accurately evaluated, and the experimental setup was correct. Except for diets containing 30% CM, all the test diets in the first run were comparable to the casein-based semi-purified diet for final weight and WG parameters. The experimental fish in the second run showed similar results for those parameters as they did in the first run. Throughout the trial, a nonsignificant mortality was observed for the 30% CM group during the primary run. This may have partially influenced the variation of final weight and WG for this group in comparison with the control, while all the other test diets recorded identical values for those parameters. In the first run, there was no remarkable change in dry FI between the diets containing CM and the control diet.

However, in the second run, that parameter was significantly lower ($P < 0.05$) for the extruded diet rather than the casein-based diet. Rainbow trout typically consume feed in order to meet their energy needs (Lee and Putnam 1973). The findings in the first run for dry FI aligned with Morales et al. (1994), approving that the FI can be influenced by dietary energy. They discovered a noteworthy inverse correlation between food intake and energy content of feed. In the initial run, where the gross energy remained almost stable across all diets, the fish demonstrated an identical feed consumption pattern. The recent findings from Qian et al. (2024) also suggested that supplementing rainbow trout feed with plant protein sources had a minimal effect on FI. In the subsequent run, there was a noticeable difference in dry FI between the groups. Some rest feed was observed in rearing control tanks which could be attributed to the higher gross energy content (23.7 MJ kg^{-1} DM) compared to the extruded feed (22.4 MJ kg^{-1} DM), and it is possible that the FI used to calculate the FCR was overestimated, as the leftover feed was not collected from the control tanks. Furthermore, this rest feed may have resulted in higher levels of ammonium in the water of control tanks compared to that in the test group (Table 5), negatively impacting the FI even though the observed ammonium levels are significantly lower than the sublethal amounts in salmonids (Fivelstad et al. 1993). However, Thurston et al. (1981) found that larger rainbow trout were more susceptible to toxic levels of ammonia compared to smaller fish. In this study, the inclusion rate of PPCon, BM, and PBM was almost fixed in all practical test diets but CM concentration increased at the expense of FeM. The maximum content of CM and FeM in test diets was 40% and 20%, respectively. The experimental fish readily consumed all test diets and demonstrated comparable FCR and mortality outcomes to those fed with semi-purified casein-based diet. This confirms that the diets were well accepted by rainbow trout.

The combination of terrestrial protein sources and synthetic amino acids used in formulating the test diets was as utilizable as casein and gelatin in terms of supporting the growth in rainbow trout since PER remained stable among all diets during the first and second runs. These diets provided excellent protein quality that met the nutritional requirements of the fish. This finding aligns with previous research where a mixture of BM, PBM, FeM, and CM, with inclusion rates of 10%, 22%, 10%, and 10% respectively, as the sole dietary protein components, resulted in a comparable PER to that of a casein-based semi-synthetic control diet in the same fish species (Salehi et al. 2024). Lu et al. (2015) observed an indistinguishable difference between the control diet containing fish meal and a test diet with completely terrestrial protein sources for PER, too. That fish meal–free test diet was formulated with PBM, FeM, and BM as well as some other plant protein ingredients with inclusion rates of 21%, 5%, 9%, and 22%, respectively. The liver weight in relation to the fish body (HSI) had a consistent stability between the control diet and all experimental diets in both runs, except for the diet with 40% CM in the first run. The fish exhibited a HSI of $2.0 \pm 0.08\%$ and $1.4 \pm 0.07\%$ for the control and the test diet, respectively. There are various argumentations about the factors affecting the size of the liver in fish. Certain researchers have stated that there is a negative relationship between dietary phosphorus and HSI. This implies that consuming feeds lacking sufficient phosphorus content may result in an elevation of liver weight and lipid accumulation in that organ (Zhang et al. 2023). This outcome was not approved in the present study with rainbow trout since a statistically non-significant difference was observed for the liver lipid content between the control and test diet in the second run despite lower dietary total phosphorous content (g kg^{-1} DM) in the control (7.8) versus test group (12.5). In rainbow trout, it is not simple to definitively conclude that higher values for HSI indicate greater lipid deposition specifically in hepatocytes, particularly when diets are compared with almost the same energy content since the liver is

not the primary lipid accumulation site in this fish species compared to marine species (Guillaume et al. 2001). NFE, in the context of proximate nutrient analysis, refers to both simple and complex sugars, soluble polysaccharides such as starch and water-soluble vitamins (Hardy and Barrows 2002). Therefore, this can be concluded that the control diet had a greater carbohydrate content rather than the test diet containing 40% canola meal since the NFE:OM ratio was quite higher in the former than latter diet, 17.1% versus 8.9%, in the initial run. According to Webster and Lim (2002), carbohydrates can transform into glycogen and be stored in the liver and muscle for energy requirements; hence, the control diet could lead to an enlargement of the liver size. *K* did not alter significantly between diets in the first run; however, this parameter was higher for the extruded feed ($1.4 \pm 0.02\%$) than the pelleted casein-based synthetic diet ($1.3 \pm 0.02\%$). This indicates that the feeding condition was well enough which resulted in a higher weight at a specific length as mentioned by Jobling (2001b).

The findings of this study indicate that rainbow trout can achieve satisfactory growth using feeds that do not contain fish meal, but instead include poultry protein sources and CM. The maximum inclusion rate for CM in these feeds was determined to be 40%. This finding is in contrast with previous studies. Hernández et al. (2013) noted that the addition of CM (also referred to as rapeseed cake) as a protein source at a level of 30%, alongside fish meal, comprising approximately 45% of the diet, damaged the growth performance of rainbow trout. The researchers hypothesized that the presence of antinutritional factors in the plant ingredients might be responsible for the observed reduction in growth. Nevertheless, the study did not identify a significant difference in terms of *K* and HSI between the control diet, which solely contained fish meal as the protein source, and that test group, Thiessen et al. (2003) found similar observations in their study for 20% CM (plus 34% fish meal) in rainbow trout. It appears that recently developed cultivars of canola can be included in rainbow trout diets at percentages of even 40% in combination with other poultry protein sources without health disorders and detrimental effects on FCR and PER.

Nutrient utilization, fish body analysis, and eutrophication parameters

Nutrient retention is a parameter that can be determined for specific nutrients in the entire body of a fish over a defined period of time. This calculation allows for the assessment of nutrient deposition and availability within the fish's body (Hardy and Barrows 2002). Therefore, LPV, NPV, OMPV, GEPV, and PhPV were calculated in both the first and the second runs to determine the productivity of experimental feeds for those nutrients and phosphorous (Table 5). The transfer of lipid, protein, organic matter, and gross energy from the feed to the fish body tissues was identical across all test diets, which concluded unique weight gain among them. The reflection of nutrient utilization in weight gain was also observed by Nyadjeu et al. (2022) in Nile tilapia. Feeding diets containing 25% and 35% CM were statistically not different from the casein-based semi-purified diet for all the parameters measured for nutrient and phosphorous retention in the first run; however, PhPV for 35% CM diet did not remain comparable to the control group in the second run. The observed NPV for the control group in the first run was $43.4 \pm 2.00\%$ which was identical to $43.5 \pm 5.10\%$ observed for that casein-based semi-purified diet in rainbow trout in the recently published study (Salehi et al. 2024). Protein retention values can be influenced by various determinants, including the use of high-fat feeds, precise formulation to align dietary amino acid levels with requirements, and incorporation of high-quality protein feed ingredients. This parameter is also taken into account as an essential factor

when developing environmentally friendly diets (Li et al. 2000). The NPV for the casein-basal diet was quite high, particularly in younger fish in the first run. This high productivity value was expected due to the inclusion of highly digestible and processed feed ingredients in the control; likewise, diets containing 25% and 35% CM revealed comparable results to that semi-purified diet. By incorporating nonprotein energy sources such as digestible carbohydrates, and especially lipids into the diet of farmed fish, it is possible to enhance protein/amino acid retention by preventing the breakdown of these valuable nutrients for energy purposes, thus maximizing their utilization as the sparing effect. This can also aid in the reduction of nonfecal nitrogen load in farm discharge (Bureau et al. 2002). OMPV was premium in fish ingested control diets in the first and second runs; likewise, GEPV was a reflection of OMPV in both runs. All test diets, containing practical feed components, demonstrated comparable consequences to the semi-purified casein-based diet for those parameters except for the diet with 30% CM. Biochemical oxygen demand (BOD) is defined as the quantity of oxygen that bacteria need to decompose OM. It serves as a primary indicator for assessing and managing pollution in natural water bodies (Owsley 2000). Hence, the diet containing 35% CM can be considered a valuable formulation in preserving the water quality of fish ponds by limiting the amount of OM present in the water.

Phosphorus retention was notably high in fish that were fed with casein-based semi-purified diets. The PhPV for control diets in the first and the second runs were 71.6 ± 1.95% and 37.7 ± 2.72%, respectively. With respect to this parameter, the practical test diets with 20%, 25%, and 35% CM were not statistically different from the control diet in the first run; however, the extruded feed exhibited a lower retention value for phosphorous (24.8 ± 3.34%) rather than the pelleted control group in the first run. The phosphorus retention data obtained in the present study exhibited higher values compared to 19–22% that was reported by Burel et al. (2000) in the same fish species. In their study, rainbow trout were fed two different CMs (referred to as rapeseed by the authors) at levels of 30% and 50% of the diet, along with Norwegian herring meal. Sugiura (1998) described phosphorous retention in a fish's body can be influenced crucially by the concentration and availability of that element. As the dietary concentration of phosphorus decreases, there is an increase in the absorption of phosphorus. Fish have a regulatory mechanism to control the absorption of phosphorus from their intestines. They have the ability to absorb dietary phosphorus up to the level required by their bodies, while any excess phosphorus is eliminated. The excretion of excess insoluble phosphorus salts primarily occurs through feces when the soluble source of phosphorus is excreted via the urinary route. This is reflected in the current study. Overall phosphorous content enhanced parallel with CM incorporation rate (Table 3) which was higher than the recommended phosphorous level for rainbow trout based on NRC (2011) recommendation, 0.7% of dry complete feed. Moreover, plant sources contain a substantial amount of phytic phosphorus, which is approximately 40–60% bioavailable to fish (Guillaume et al. 2001). In various salmonids' diets, a phosphorus retention rate of 14–22% was observed, suggesting roughly 80% of the dietary phosphorus discharged into the water in a soluble form and solids (Ketola and Harland 1993). For the pelleted 35% CM diet, the retained phosphorus was observed to be 37.4 ± 4.15% in the first run, while in the second run, the extruded version showed a value of 24.8 ± 3.34%. These observations suggest that dietary phosphorus was utilized more efficiently in small fish in contrast to large fish. Currently, the feed for commercial-size fish is formulated based on data obtained from juvenile fish reared in laboratory settings. However, it is important to recognize that as animals grow, their nutrient requirements generally decrease due to a decrease in growth rate. This leads to a drop in the utilization of dietary

nutrients, including phosphorous, for growth but an increase in nutrient allocation towards maintenance. Notably, a significant portion of these nutrients can be recycled within the animal's body (Sugiura 1998). Therefore, in order to ensure optimal growth performance and minimize surplus phosphorous effluent in commercial-size rainbow trout production, it is crucial to take into account the minimum dietary phosphorous requirements when formulating diets for large fish as well as considering the availability of this element in each feed component used to formulate diet.

In the initial run, the lipid content in the bodies of fish that consumed pelleted practical test diets containing 20–35% CM was recognized to be the same as the values observed in fish fed a casein-based semi-purified diet. However, the body lipid content in the fish fed with a diet containing 40% CM was higher than both the control and 20% CM groups. During the subsequent run, experimental fish that consumed extruded feed consisting of 35% CM exhibited a similar pattern to that observed in the first run. These observations are in contrast to the study of Qian et al. (2024), which mentioned a reverse relationship between fish body lipid accumulation and the inclusion rate of canola (referred to as rapeseed by the authors) meal. However, a high positive correlation between feed lipid content and body lipid accretion was observed in the first ($r=0.64$, $P=4.5e^{-05}$) and second ($r=0.77$, $P=0.049$) runs of this study. This was also recognized by Reinitz and Hitzel (1980). They realized the percentage of dietary lipids was the primary factor influencing the body composition of rainbow trout. As the dietary lipid content increased, there was a decrease in the percentage of whole-body protein and moisture, while the percentage of whole-body fat increased. Such correlation was observed by Pongmaneerat and Watanabe (1993a) and Belghit et al. (2019), as well. The lipid composition in the liver was only assessed in the second run of the trial, and no considerable difference was recognized between the casein-based semi-purified diet and the extruded feed containing 35% CM (Table 5). A systematic meta-analysis was conducted to investigate the replacement of fish meal with plant protein sources in carnivorous fish species. The findings exhibited that the majority of plant protein sources had a neutral effect on liver lipid accumulation, with the exception of cottonseed, which enhanced notably the liver lipid content (Qian et al. 2024). Previous studies indicated that liver lipid content can be affected by various factors. These factors include dietary imbalances in amino acids (Lee and Wales 1973), the composition of dietary saturated and unsaturated fatty acids (Robaina et al. 1997) as well as insufficient intake of essential fatty acids (Guillaume et al. 2001). Therefore, it can be concluded that the extruded fish-meal–free diet was appropriately balanced in terms of essential amino acids, fatty acids, and gross energy. The liver lipid levels observed in this study were in agreement with the findings of Meng et al. (2022). They noted that rainbow trout can maintain hepatic lipid balance when consuming dietary lipid levels between 150 and 300 g kg^{-1} by managing lipid absorption and changing energy sources between glycolysis and fatty acid β-oxidation.

The phosphorous content in the sludge and water from the tanks, belonging to extruded feed were significantly greater than the control group in the second run (Table 5). The control diet, which consisted of highly processed protein sources like casein, was expected to produce a lower phosphorus effluent compared to the extruded feed. This difference may be attributed not only to the chemical form of phosphorous but also to its lower content in the control group. Phosphoric acid exists as an integral component of phosphoproteins in casein (McDonald et al. 2011), which can be highly available for rainbow trout. In contrast to phosphorous, ammonia level was recognized as greater in the control tanks than test diet. The produced amounts of effluents vary markedly among all types of aquaculture production systems. Typically, those systems situated in open waters, such as cages, as well as

flow-through raceway systems which discharge continuously, tend to produce more effluent compared to recirculating or pond systems. Aquaculture effluents can potentially cause detrimental environmental effects due to their dissolved and suspended nutrient loads. Phosphorus (P) and nitrogen (N) are considered the primary components of aquaculture effluent for their significant environmental implications. The main source of these materials from aquaculture discharges is fish feed. A significant portion of the feed will ultimately go to waste in the form of fish excretion products, unused feed, and feces. The concentration and availability of P in the feed play a notable role in the amount of excreted P. Nitrogenous products, particularly ammonia, are synthetized through protein breakdown. Both the quality and quantity of dietary protein, as well as the protein-to-energy ratio of the diet, affect nitrogen retention and excretion (Chen and Fornshell 2000). Despite the strong correlation between the amount of provided dry feed and the presence of dissolved ammonia, the relationship was found to be statistically insignificant ($r = 0.72$, $P = 0.11$). Therefore, it can be assumed that higher levels of dissolved ammonia in the control tanks can be resulted from the uneaten feed which was observed during the rearing period for the control casein–based group in contrast to the extruded feed. In accordance with Chen et al. (2024), feed can lead to a significant effluent of nitrogen in aquaculture systems.

Nutrient apparent digestibility and phosphorous availability

In all experimental phases, the CL of test diets was digested as high as that in the control casein–based diets. The observed CL ADC for the fish meal–free test diets ranged from 92 to 95%, which aligns with recently studied fish-meal–free diets in rainbow trout. The reported CL ADC was 92.3% for that diet which was formulated with 42% terrestrial animal protein sources (10% poultry BM, 22% PBM, 10% FeM) and 10% CM (Salehi et al. 2024). The increasing inclusion rate of CM in the present investigation did not damage lipid digestibility, which was in line with the study of Staessen et al. (2020). They recognized fat digestibility in plant-based diets for rainbow trout can be negatively affected by non-starch polysaccharide (NSP) and high feeding rates. Based on the results of the present study, it can be concluded that the included amounts of CM and the feeding rates used have resulted in an efficient CL ADC. Despite the variations in total lipid content among the diets being studied, the digestion of CL was statistically identical across the feeding groups, which was in accordance with Pongmaneerat and Watanabe (1993b). They found CL ADC above 91%, while the dietary CL ranged from 20.2 to 25.7% of DM in the feed. This suggests that using identical fat and oil sources may lead to comparable results in terms of CL ADC. In general, lipids, especially polyunsaturated fatty acids, are known to have high digestibility in fish. However, factors such as low water temperature, high saturation level, and longer carbon chain can decrease the digestibility of lipids (Guillaume et al. 2001).

The observed CP ADC for the casein-based semi-purified diets was approximately 99% in both runs, which was quite high. All the test diets, except for the one containing 40% CM, demonstrated comparable digestibility values for that macronutrient (above 89%) rather than the control group in the first run. The high observed ADC for CP in the test diets was predictable because all the protein sources applied to formulate the practical diets were known to be highly digestible for rainbow trout in contrast to typical raw materials (Salehi et al. 2023). The decreased ADC for CP in the diet containing 40% CM can be attributed to the potential higher levels of fiber and phytate. According to Jobling (1981), Mwachireya et al. (1999), and Guillaume et al. (2001), the presence of non-digestible

carbohydrates derived from plants had an adverse impact on the efficiency of protein digestion and absorption. Even though the quantity of phytate was not specifically measured in the diets examined in this study, it seems that the amount of this anti-nutrient increased in proportion to the inclusion rate of CM. In the second run, the extruded diet with 35% CM exhibited approximately two units lower CP ADC (87.2%) than the level observed in the first run for the pelleted feed (89.5%). As the plant proteins are commonly surrounded by starch, heating has the potential to decrease the digestibility of proteins and the availability of amino acids through the interaction between amino acids and reducing sugars by Maillard browning, or through the reaction between amino acids and aldehydes found in oxidized lipids (Sugiura and Hardy 2000; Belitz et al. 2008; NRC 2011). Moreover, heating may result in lower protein solubility as mentioned by Arndt et al. (1999) in salmonids.

The OM content of the semi-purified casein basal diet was digested similarly in both the first and second runs, with values of $82.3 \pm 0.44\%$ and $82.9 \pm 0.94\%$, respectively. In the first run, the values observed for diets containing 30–40% CM were comparable to those of the control diet. In the second run, the OM ADC for the diet with 35% CM was almost identical to that of the first run, but it was noticed statistically different from the control feed. The stepwise increase of CM in the experimental diets seems to raise the proportion of CL in the OM content, resulting in a positive impact on OM ADC. The recognized high digestibility value of OM for diets with 30–40% CM is crucial from the point of environmental pollution concerns. Organic decomposition consumes oxygen and has the potential to lower the concentration of dissolved oxygen in water as mentioned earlier. Consequently, higher OM ADC in feeds can lead to more dissolved oxygen and less effluent treatment effort in aquaculture production systems.

With respect to the availability of phosphorous, all diets containing CM, other than the one consisting of 20% CM, demonstrated comparable values. In the initial run, there was a decreasing trend in PhA as CM content increased gradually. This implies that diets with 35% and 40% CM had significantly lower PhA compared to the casein-based semi-purified diet. In the subsequent run, the PhA somewhat improved with extrusion ($61.6 \pm 2.60\%$) compared to the pellet type ($59.9 \pm 1.00\%$) used in the first run, but it still remained lower than the control feed ($85.6 \pm 1.04\%$). Thiessen et al. (2003) suggested the effect of extrusion on the enhancement of phytate degradation in juvenile rainbow trout. The observations of PhA in the first run support the hypothesis proposed by Sugiura (1998) regarding the inverse correlation between dietary phosphorus and its availability in fish, which was also observed in the current work ($r = -0.89$, $P = 6.7\ e^{-09}$). The findings of the present study are also in agreement with Burel et al. (2000) since a lower PhA was observed by increasing the incorporation of canola meal (referred to as rapeseed meal by the authors) in the diets of juvenile rainbow trout at the level of 50% CM, containing 41 µmol/g DM glucosinolates in particular. In addition, fish and other monogastric animals cannot digest phytate-P, which serves as the primary storage form of phosphorus in seeds (NRC 2011). As a result, the increased incorporation of CM led to a decrease in the availability of phosphorus for rainbow trout in this study. The influence of fiber type on nutrient digestibility and mineral availability in rainbow trout can be inferred from the current study. The casein-based semi-synthetic control diet used in this study was characterized by a high content of crude fiber, which mostly contained purified cellulose. Interestingly, this diet yielded relatively high values for nutrient digestibility and phosphorous availability in studied fish, which is in accordance with previous studies. Hansen and Storebakken (2007) found that the inclusion of cellulose at levels up to 150 g kg^{-1} did not adversely affect the ADC of the main nutrients in rainbow trout. In Nile tilapia, the addition of pure cellulose did not affect digesta viscosity, growth, and digestibility of CP and starch. Furthermore, it enhanced feces recovery (Amirkolaie et al. 2005). These findings suggest that

purified cellulose may not have a negative correlation with nutrient digestibility and can be at levels of up to 19% of dry feed for rainbow trout. However, further investigation into the influence of various non-digestible carbohydrates and fibers on nutrient digestibility and mineral availability in rainbow trout is still necessary for future studies.

Conclusion

The assessment of five formulated diets with terrestrial protein sources demonstrated that 35% CM with approximately 32% highly digestible poultry by-products can yield comparable growth performance, nutrient utilization productivity, body indices, and nutrient apparent digestibility with casein-based semi-purified diet in rainbow trout. Since the availability of phosphorus from plant protein sources is restricted for rainbow trout, it is essential to consider the minimum dietary phosphorus requirements when formulating diets for the grow-out period. Additionally, the availability value of this element in every feed component incorporated into the diet must be taken into account.

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Author contributions Hamed Salehi designed and managed the study, carried out trials, gathered data, coordinated lab activities, analyzed data, and drafted the manuscript. Stefan Reiser supervised the experimental work and provided inputs for the manuscript. Ulfert Focken designed and supervised the project, provided funding, and made contributions to the manuscript.

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Data availability All the data used in the manuscript can be accessed upon request.

Declarations

Ethics approval The experimental procedures were carried out in accordance with the European directive 2010/63/EU regarding the protection of animals used for scientific purposes.

Conflict of interest The authors declare no competing interests.

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