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Practical low-fishmeal diets for rainbow trout (*Oncorhynchus mykiss*) reared in RAS: Effects of protein meals on fish growth, nutrient digestibility, feed physical quality, and faecal particle size

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ABSTRACT

To evaluate strategies for optimizing waste in recirculating aquaculture systems, 1020 rainbow trout (initial live weight 17.2 ± 7.50 g/fish) were distributed into 12 tanks after 21 d of acclimation and fed during 84 days with four practical diets (crude protein: 49% DM; crude fat 26% DM; gross energy: 23 MJ kg^{-1}) containing different rates of fishmeal and alternative protein meals, i.e. Diet FM (307 g kg^{-1} fishmeal, 61.2 g kg^{-1} poultry by-product meal); diet PBM (183 g kg^{-1} fishmeal, 168 g kg^{-1} poultry by-product meal); diet FeM (198 g kg^{-1} fishmeal, 61.2 g kg^{-1} poultry by-product meal, 76.5 g kg^{-1} hydrolysed feather meal); diet FeM+RM (171 g kg^{-1} fishmeal, 61.2 g kg^{-1} poultry by-product meal, 76.5 g kg^{-1} hydrolysed feather meal, 60.4 g kg^{-1} rapeseed meal). High structural integrity of extruded pellets and low oil leakage were measured in all diets, while the lowest water turbidity at 15 min after feed administration was recorded for FeM diet (2.7 vs. 12.7 mg L^{-1}; $p < 0.05$). Diets did not affect fish specific growth rate (2.16% d^{-1}). The lowest apparent digestibility of protein was measured with diet FeM (79.6%) and the highest with diet PBM (86.0%) ($p < 0.001$); apparent digestibility of lipids was higher in fish fed diets FM and PBM than the other diets (84.4% and 85.5% vs. 66.4% vs. 71.1%; $p < 0.001$). Fish fed diet PBM showed a higher percentage of retained faeces at mesh sizes 0.5–0.8 mm (33% vs. 30%; $p < 0.005$) and 0.3–0.5 mm (64% vs. 59%; $p < 0.001$) compared with the other diets. The replacement of fishmeal with poultry by-product- meal had positive effects on nutrient digestibility and faecal particle size. The replacement with hydrolysed feathers and rapeseed meals impaired nutrient digestibility but had positive implications for water turbidity.

1. Introduction

According to FAO (2020), the global supply of meat and seafood protein must be increased from about 300 to nearly 500 million tons by 2050 to feed the worldwide population. High quality and healthy fish products are expected to greatly contribute to meet this need, with aquaculture products supplying over 70% of the expected increase (EUMOFA, 2020). However, to fulfil the future demands, aquaculture must promote its sustainable development.

In fact, aquaculture production systems must abate the use of natural resources (water and soil) and control the production of wastes (Ahmed and Turchini, 2021). This requires optimization of management in all

Abbreviations: ADC, Apparent digestibility coefficient; CF, Crude fat; CP, Crude protein; CY, Carcass yield; DFI, Daily feed intake; DM, Dry matter; DORIS, Durability on a realistic test; FCR, Feed conversion ratio; FEM, Hydrolysed feather meal; FM, Fishmeal; PAPs, Processed animal proteins; PBM, Poultry by-product meal; RAS, Recirculating aquaculture system; RM, Rapeseed meal; RMSE, Root mean square error; SGR, Specific growth rate; TGC, Thermal growth coefficient.

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systems, including recirculating aquaculture systems (RAS) (Binet et al., 2020). In RAS, aquafeed is the primary external input, which has to be specifically designed in terms of nutritional and physical quality to support fast-growing and healthy fish, while maintaining good water quality by reducing the production of wastes in terms of quantity and quality of faeces and uneaten feed (Pulkkinen et al., 2021; Turchini et al., 2019).

As a matter of facts, aquaculture is still reliant on commercial aquafeed made of meal and oil from forage fish. Although improvements were made in the last decades, further research is still needed to find suitable protein and lipid sources to replace fishmeal (FM) and fish oil (Naylor et al., 2021). Besides, alternative feed inputs for the aquaculture sector will have to grow at a fast pace, while controlling costs and reducing reliance on expensive fish-based ingredients (Wang et al., 2015).

In the landscape of aquafeed formulations, several protein meals (such as processed animal proteins – PAPs – or vegetable protein meals) have been tested as alternative or supplementary protein sources to satisfy the dietary requirements of targeted farmed species (Kerama-t-Amirkolaie, 2014; Kjaer et al., 2016; Zhu et al., 2014). Among PAPs, poultry by-product meal is one of the most promising alternatives to fishmeal due to its high protein content, relatively cheap price, and broad availability (Galkanda-Arachchige et al., 2020). Along with poultry by-products, feather meal is another protein-rich and cost-effective ingredient obtainable from the poultry rendering industry (Pfeuti et al., 2019a; b). Among vegetable alternatives to fishmeal, rapeseed meal shows one of the best amino acid profiles (Collins et al., 2012) and a very competitive price (Hardy, 2010).

In most experimental trials using alternative protein meals, diets still contained high levels (40–60%) of fishmeal (Gasco et al., 2019), whereas, due to the high and steadily increasing price of fishmeal (Naylor et al., 2021), only 10–20% of this ingredient is currently included in practical diets for rainbow trout (Banjac et al., 2021; Biasato et al., 2022; Caimi et al., 2021; Chemello et al., 2020; Jannathulla et al., 2019).

In RAS, aquafeeds must also be of excellent physical quality for the most efficient handling, storage and administration (Funk et al., 2019) as well as produce large faecal particles for a rapid removal from the system (Moccia et al., 2007; Welker et al., 2021). Changes in feed ingredients can affect the technical quality of the feed, such as oil leakage, durability, and its stability in water as well as waste production (Sørensen, 2012). However, knowledge about the performance of diets based on alternative raw materials in the aquaculture environments as for waste production is still scarce (Luthada-Raswiswi, 2021).

Thus, the purpose of this study was to evaluate the effect of the inclusion in feed formulation of commercially available protein meals alternative to fishmeal, i.e. poultry by-product meal, hydrolysed feather meal and rapeseed meal, on fish growth performance and digestibility of diets, the physical properties of feed pellets, and the dietary effect on the quality of faeces, with the general aim of optimizing the efficiency of waste management in RAS for rainbow trout.

2. Materials and methods

2.1. Ethics statement

The experimental procedures were conformed to the European Community Directive (No. 2010/63/EU) (EC, 2010), and authorized by the Czech Ministry of Health (No. MSMT-6744/2018–2), regarding the protection of animals used for experimental and other scientific purposes. Research staff involved in animal handling were animal specialists and veterinary practitioners.

2.2. Raw materials and diets

Poultry by-product meal (690 g kg^{-1} crude protein - CP; 99 g kg^{-1} crude fat - CF; 127 g kg^{-1} ash), hydrolysed feather meal (850 g kg^{-1} CP; 61 g kg^{-1} CF; 21 g kg^{-1} ash) and rapeseed meal (320 g kg^{-1} CP; 49 g kg^{-1} CF; 62 g kg^{-1} ash) were commercially available products, obtained according to standard practices (de Blas et al., 2019). The fishmeal used in the diets contained 660 g kg^{-1} CP, 89 g kg^{-1} CF, and 170 g kg^{-1} ash.

Poultry by-product meal, hydrolysed feather meal and rapeseed meal were used at different inclusion levels in four practical diets formulated to have a control diet (diet FM) containing 307 g kg^{-1} fishmeal and 61.2 g kg^{-1} of poultry by-product meal and three more diets in which fishmeal was reduced by 36–44% and replaced by other protein sources alone or in combination. The replacement rate in the diets containing alternative proteins was set to have isonitrogenous diets with a fishmeal content below 200 g kg^{-1} (171–198 g kg^{-1}), consistently with current commercial aquafeeds for rainbow trout. In detail, in diet PBM, fishmeal was reduced to 183 g kg^{-1} and poultry by-product meal increased till 168 g kg^{-1} (fishmeal replacement rate 40%); in diet FeM, fishmeal was partially substituted with 76.5 g kg^{-1} of hydrolysed feather meal (fishmeal replacement rate 36%); in diet FeM+RM, fishmeal was partially replaced by 76.5 g kg^{-1} of hydrolysed feather meal and 60.4 g kg^{-1} of rapeseed meal (fishmeal replacement rate 44%) (Table 1). Other protein sources were included in all diets to reach the targeted protein level: porcine haemoglobin (114–115 g kg^{-1}), soybean protein concentrate (99.5 g kg^{-1}) and other minor sources (bacterial protein, wheat gluten, hydrolysed fish protein). The inclusion level of these protein sources was constant among the diets. Wheat flour varied from 111 to 146 g kg^{-1} to adjust nutrient and energy concentration. Lipids were mainly provided by rapeseed oil (198 g kg^{-1}) and little fish oil (22.4 g kg^{-1}). The four diets

Table 1
Ingredients (g kg^{-1} as fed) and proximate composition (% DM) of the diets including different combinations of protein meals and fed rainbow trout for 84 days.

	Diet			
	FM	PBM	FeM	FeM+RM
Ingredients (g kg^{-1} as fed)				
Fishmeal from fish by-products[1] (CP 66% DM)	307	183	198	171
Poultry by-product meal[2] (CP 69% DM)	61.2	168	61.2	61.2
Hydrolysed feather meal[3] (CP 85% DM)	0	0	76.5	76.5
Rapeseed meal[4] (CP 32% DM)	0	0	0	60.4
Porcine haemoglobin	115	115	114	114
Bacterial protein meal from *C. glutamicum*	38.3	38.3	38.3	38.3
Soybean protein concentrate	99.5	99.5	99.5	99.5
Wheat flour	121	131	146	111
Vital wheat gluten	12.3	12.3	12.3	12.3
Hydrolysed fish proteins	10.3	10.3	10.3	10.3
Fish oil	22.4	22.4	22.4	22.4
Rapeseed oil	198	198	198	198
DL-Methionine	5.40	6.10	7.70	7.70
Monoammonium phosphate	0	6.20	5.40	7.70
Vitamin and mineral premix[5]	9.40	9.40	9.40	9.40
Vitamin C	0.70	0.70	0.70	0.70
Proximate composition				
Dry matter (%)	93.7	93.4	93.5	93.6
Crude fat (% DM)	25.8	26.0	25.8	25.8
Crude protein (% DM)	48.6	49.0	49.0	48.7
Ash (% DM)	7.89	6.77	5.62	5.51
Fibre (% DM)	1.97	2.47	2.53	2.53
Gross energy[6] (MJ kg^{-1})	22.8	23.0	23.1	23.1

CP: Crude protein; DM: Dry matter. [1]Fishmeal from fish by-products: crude fat 8.9%, ash 17.0%; [2]poultry by-product meal: crude fat 9.9%, ash 12.7%; [3]hydrolysed feather meal: crude fat 6.1%, ash 2.1%; [4]rapeseed meal: crude fat 4.9%, ash 6.2%. [5]Vitamin and mineral premix (quantities in 1 kg of mix): Vitamin A, 4000,000 IU; Vitamin D3, 800,000 IU; Vitamin C, 25,000 mg; Vitamin E, 15,000 mg; Inositol, 15,000 mg; Niacin, 12,000 mg; Choline chloride, 6000 mg; Calcium Pantothenate, 3000 mg; Vitamin B1, 2000 mg; Vitamin B3, 2000 mg; Vitamin B6, 1800 mg; Biotin, 100 mg; Manganese, 9000 mg; Zinc, 8000 mg; Iron, 7000 mg; Copper, 1400 mg; Cobalt, 160 mg; Iodine 120 mg; Anticaking and antioxidant + carrier, making up to 1000 g. [6]Calculated based on data of raw materials available at NaturAlleva (VRM s.r.l., Cologna Veneta, Verona, Italy).

were formulated to be isonitrogenous (CP: 49% DM) and isolipidic (CF: 26% DM), as shown in Table 1.

The four diets were produced by NaturAlleva (VRM s.r.l., Cologna Veneta, Verona, Italy) as a sinking extruded pellet with a 3.0-mm diameter. All diets met the nutritional requirements for rainbow trout set out by the National Research Council (NRC, 2011).

Feed production cost of the four diets was calculated at the production plant gate based on the costs of all ingredients per each diet. Costs of labour, packaging and transport were not included.

2.3. Rearing conditions and fish

The experimental RAS was located at the Institute of Aquaculture and Protection of Waters (IAPW, Faculty of Fisheries and Protection of Waters), University of South Bohemia in Ceske Budejovice, Czech Republic. The RAS consisted of 12 rectangular white rearing tanks (volume 300 L per tank), 4 submerged biofilter tanks (volume 3500 L per tank) with bio-elements (BT 10, Ratz Aqua & Polymer Technik GmbH, Remscheid, Germany), and 4 sump tanks (volume 3500 L per tank). The RAS used filter foam (Bioakvacit PPI 10, Jezírka Banat, s.r.o., Hněvotín, Czech Republic), drum filter (AEM ECO15, DVS-FilterTechniek, Kerkrade, Netherlands), and water-gas mixer (Type 250, Ratz Aqua & Polymer Technik GmbH, Remscheid, Germany).

Tanks with fish were supplied with a water flow of 4–5 L min^{-1} by regulating pumps (DM-20,000 Vario, AquaForte, Veghel, Netherlands). Water temperature was maintained using air-conditioning (Inverter, LG, Seoul, South Korea). Photoperiod was 12L:12D with a light period between 7:00–19:00. Light intensity, measured using a light metre (DT-8809, Cem, Shenzhen, China), was 450 lx over the tanks with fish.

Water temperature, pH, and oxygen contents were daily measured using a multi-parameter probe (HI98194, Hanna Instruments, Woonsocket, Rhode Island, USA); NH_4^+, NH_3, NO_2^- concentrations were measured three times a week using LCK cuvette tests with barcode and spectrophotometer (DR 2800, Hach Lange, Loveland, Colorado, USA). During the trial, the temperature of outlet water was 15.4 ± 1.0 °C; oxygen saturation was at 93.9 ± 5.8% (morning time), 91.9 ± 5.5% (afternoon time); pH was 7.13 ± 0.38; NH_4^+ 0.95 ± 0.59 mg L^{-1}; NH_3 0.002 ± 0.004 mg L^{-1}; NO_2^- 0.52 ± 0.32 mg L^{-1}.

A total of 1020 rainbow trout (initial live weight 17.2 ± 7.50 g/fish) were purchased from a commercial farm (Vladimír Šefl Bušanovice, Bušanovice, Czech Republic) and transported after one day of fasting to IAPW facility. Prior to the experiment, fish were acclimated to the system for 21 days. Then, 85 rainbow trout per tank were randomly placed in the 12 tanks with an initial stocking density of 7.71 kg m^{-3}. The trial lasted 84 days.

Fish were fed twice a day to satiation (8:00 and 15:00) and the theoretical feed ration was calculated assessing the thermal growth coefficient (TGC) and the daily feed intake (DFI) using the following formulas (Thodesen et al., 2001):

TGC = [final body weight (g)$^{1/3}$ − initial body weight (g)$^{1/3}$] / $\sum$ Sum day temperature (°C).

DFI = 100 × daily feed intake (g) / fish body weight (calculated from the TGC).

2.4. Fish performance and in vivo recordings

At the beginning of the trial, a total of 50 fish were randomly sampled from each of the 12 tanks (600 fish in total) and fish live weight, total length and standard length were individually measured. These measurements were replicated on the days 28, 52 and 84 of the trial. For these recordings, fish were removed from their tank, placed in a separate one with oxygen supply, and anaesthetised with clove oil containing 87% eugenol (0.03 mL L^{-1} of water). They were individually photographed (DSC-HX60, Sony, Tokyo, Japan) and their biometry measured by a millimetre ruler on a scale (accuracy 0.01 g) (ImageJ programme; SKX222, Ohaus, Nänikon, Switzerland).

Finally, weight gain, specific growth rate (SGR) and feed conversion ratio (FCR) were calculated for all sampled fish on a tank basis as follows:

Weight gain (%) = [average final weight (g) – average initial weight (g)] / average initial weight (g) ×100.

SGR (% day^{-1}) = [ln (average final weight (g) – ln (average initial weight (g)] × 100 / days of trial.

FCR = weight of dry feed distributed (g) / weight gain of fish (g).

2.5. Recordings at fish slaughtering

At the end of the trial, 252 fish (21 fish per tank; 63 fish per dietary treatment) were randomly selected and anesthetized using the eugenol bath, euthanized by eugenol overdose, and then individually weighed and finally eviscerated and dissected. Weights of the carcasses and selected tissues (viscera, liver, spleen, digestive tract, and perivisceral fat) were determined to the nearest 0.0001 g (Adventurer Pro AV264C, Ohaus, Nänikon, Switzerland). Thereafter, hepatosomatic, splenic, viscerosomatic, and perivisceral fat indexes, relative gut length, relative gut mass, and carcass yield were calculated as shown below:

Viscerosomatic index (%) = viscera weight (g) / fish weight (g) × 100.

Hepatosomatic index (%) = liver weight (g) / fish weight (g) × 100.

Perivisceral fat index (%) = perivisceral fat weight (g) / fish weight (g) × 100.

Spleen somatic index (%) = spleen weight (g) / fish weight (g) × 100.

Relative gut length = intestine length (cm) / total length (cm).

Relative gut mass = gut weight without perivisceral fat (g) / fish weight (g).

Carcass yield (%) = carcass weight (g) / fish weight (g) × 100.

2.6. Faeces collection

The tanks were equipped with a removable chamber positioned at the bottom of rearing tank (Cho et al., 1982). It uses an upwelling wide-bore outlet tube with low flow speed that allows for the settlement and recovery of faeces. Removable chambers for faeces collection were cleaned 30 min after each feeding to avoid contamination of the faeces by feed.

Every 5 days during the 84 days of the trial, faeces were collected at 7:30 and 14:30. Faeces were siphoned out via cones, let to settle down and centrifuged (NEYA 16, REMI ELEKTROTECHNIK LTD, Vasai-Virar, India) at 4000 rpm for 10 min. Subsequently, the samples of faeces were freeze-dried (Lyophilizator ALPHA 1–4, Christ, Osterode am Harz, Germany) before the chemical and physical analyses. Freeze-dried faeces collected during the trial were merged, maintaining the traceability of the tank; three pools (one every 5 collection events) per each of the 3 tanks (replicates) of the 4 dietary treatments were sampled, for a total of 36 samples analysed.

Apparent digestibility coefficients (ADC) of diets were calculated for crude protein and crude fat, using fibre as indicator (Krontveit et al., 2014), according the following formula (Cho et al., 1982):

ADC (%) = 100 – [100 × (% indicator in feed / % indicator in faeces) × (% nutrient in faeces / % nutrient in feed)].

2.7. Chemical analysis of diets and faeces

For chemical analysis of diets, three replicates per diet were sampled (12 samples in total). The crude protein (#984.13), crude fat (#920.39), and ash (#942.05) contents of diets and faeces were analysed using AOAC methods (AOAC, 2019). The fibre content was measured following the procedure described by Reg CE 152/2009 (EC, 2009).

2.8. Physical analysis of feed pellets and faeces

Physical analyses of pellets were performed in triplicate. The water

turbidity due to the presence of suspended solids leached from the feed was measured according to Rice et al. (2017); briefly, feed samples (5 g) were added to distilled water (100 mL) in a beaker (3 beakers per treatment) and water samples were collected every 15 min to measure turbidity using a spectrophotometer (7305 Spectrophotometer, Stone, Staffordshire, UK) with a single 10 × 10 mm cuvette and set at a wavelength of 652 nm. To simulate the effect of bag storage, the oil leakage was evaluated according to Sørensen et al. (2011); the durability was assessed by a DORIS tester (Durability On a Realistic test, Akvasmart, AKVA group, Bryne, Norway) to determine the mechanical stress resistance of a feed sample (Aas et al., 2011).

On each pool of faeces collected per month and per tank, the particle size was measured according to the American Society of Agricultural and Biological Engineers (ASABE, 2008), procedure S319.4, in triplicate (i.e. 36 analysis). The determination of the faecal particle size was based on the fractionation of the sample by sieves (Endecotts, London, UK) with a defined mesh size (1.2 mm, 1.0 mm, 0.8 mm, 0.5 mm, 0.3 mm and 0.0 mm) inserted in a sieve shaker (BÜHLER, Alzenau, Germany).

2.9. Statistical analysis

Data of trout biometry and growth performance, physical analysis of feed (oil leakage, DORIS value), and faecal particle size were analysed by a one-way ANOVA using the PROC GLM of SAS (Statistical Analysis System; version 9.3, 2013, SAS Institute, Cary, NC, USA) (SAS, 2013). The model considered the diet as the main effect. Data of diet digestibility were analysed by a two-way ANOVA using the PROC GLM of SAS with diet, days of trial and their interactions as main effects. Data of water turbidity were analysed by a two-way ANOVA using the PROC MIXED of SAS with diet, feed dwell time in water and their interactions as main effects and data obtained from the same beaker were treated as repeated measures. Bonferroni's test was used to compare means. Differences among means with $p < 0.05$ were assumed to be statistically significant.

3. Results

3.1. Physical characteristics of feeds

The oil leakage in feeds ranged between 1.10% and 1.50% (Fig. 1a). The durability test showed that the shares of small and large fractures in the feed were always below 1%, with no differences between diets (Fig. 1b). Regarding the water turbidity, after 15 min, diet FeM produced less turbidity than diets FM, PBM and FeM+RM (2.74 mg L^{-1} vs. 7.02 mg L^{-1} and 13.5 mg L^{-1} and 17.8 mg L^{-1}; $p < 0.001$). After 30 min, diet FeM+RM produced lower turbidity than the other diets (23.9 mg L^{-1} vs. 30.4 mg L^{-1} and 36.4 mg L^{-1} and 40.4 mg L^{-1} in FM, PBM and FeM, respectively; $p < 0.001$). Then, from 45 min onwards, water turbidity did not differ among diets (Fig. 2).

3.2. Faecal particle size

As for faeces retained at a sieve mesh interval of 0.5–0.8 mm, the highest rate was produced by fish fed diet PBM (33%) and the lowest rate by fish fed FM diet (29%); intermediate values were recorded for diets FeM and FeM+RM (30.5%; $p < 0.05$). At a sieve mesh interval of 0.3–0.5 mm, fish fed diet PBM produced a higher percentage of retained faeces (64%) compared to the other diets (59.3%; $p < 0.001$) (Fig. 3).

3.3. Apparent digestibility coefficients of nutrients in the diets

The ADC of protein were different among the four diets ($p < 0.001$) with the lowest value associated to diet FeM (79.6%) and the highest value to diet PBM (86.0%) (Table 2). Moreover, ADC of protein significantly increased over time ($p < 0.001$), from 80.3% at 28 days of trial to 86.6% at 84 days of trial (Table 2). Significant interaction ($p < 0.001$)

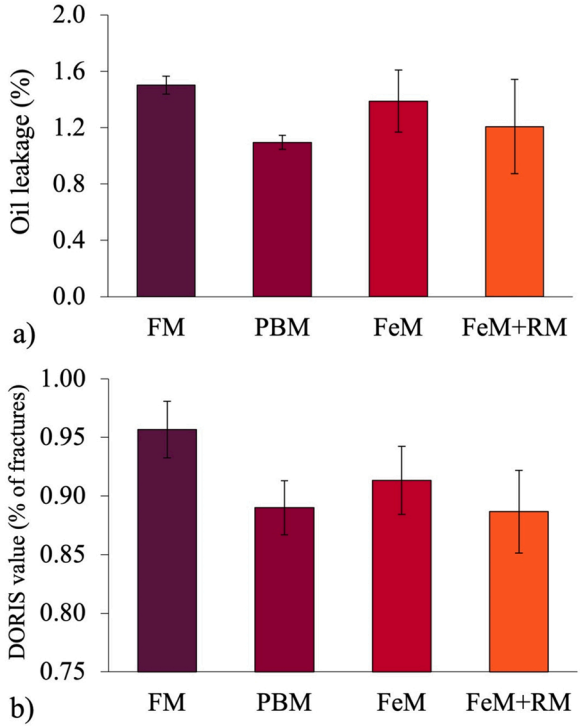

Fig. 1. Oil leakage (a) and DORIS value (b) of diets including different combinations of protein meals and fed rainbow trout for 84 days. Data are represented as means ± standard error of the mean. No statistical differences were found among the diets.

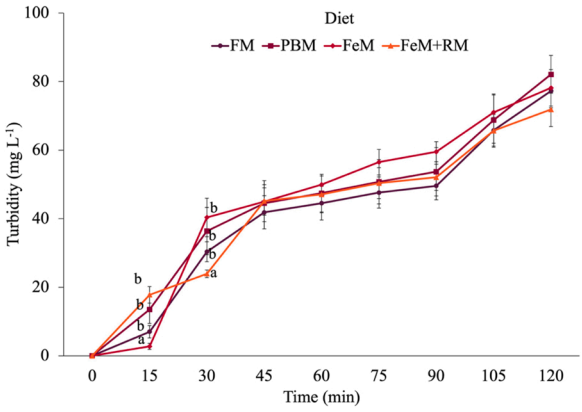

Fig. 2. Effect of diets including different combinations of protein meals on the water turbidity measured every 15 min. Data are represented as means ± standard error of the mean. Different letters above bars represent significant differences ($p < 0.001$) between means.

between diet and days of trial were observed, with the lowest protein ADC associated to diet FeM at 28 days of trial (75.2%), while the highest one to diet PBM at 84 days of trial (89.4%) (Fig. 4a). The highest increase in ADC of protein over time was associated to diet FeM (+10.8% from 28 days to 84 days of trial), while the lowest one to diet FM (+5.44% from 28 days to 84 days of trial).

The ADC of lipids was higher in diets FM and PBM than in diets FeM and FeM+RM (84.4% and 85.5% vs. 71.1% vs. 66.4%; $p < 0.001$). Moreover, ADC of lipids significantly increased over time ($p < 0.001$) from 69.7% at 28 days of trial to 83.0% at 84 days of trial (Table 2). A significant interaction ($p < 0.001$) between diet and days of trial was observed, with the lowest lipid digestibility associated to diet FeM+RM at 28 days of trial (54.6%), while the highest one to diet FM at 84 days of

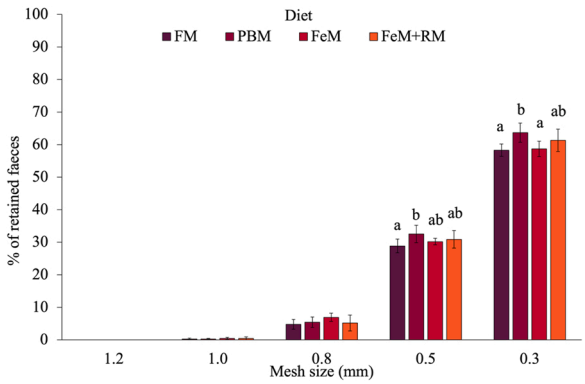

Fig. 3. Percentage of dried faeces retained from different sieve mesh sizes (mm) and obtained from rainbow trout fed for 84 days diets including different combinations of protein meals. Different letters above bars represent significant differences ($p < 0.05$ for 0.5 mm of mesh size and $p < 0.001$ for 0.3 mm of mesh size) between means.

trial (92.2%) (Figure 4b). The highest increase in lipid digestibility over time was observed in diet FeM+RM (+42.5% from 28 days to 84 days of trial), while the lowest one in diet FeM (+7.94% from 28 days to 84 days of trial).

3.4. Fish growth performance, survival rate and somatic indexes

Diets did not affect fish final live weight (191 g, on average), daily weight gain (1.71 g d^{-1}), specific growth rate (2.16% d^{-1}), feed conversion ratio (1.05), feed intake (1.65 g DM $fish^{-1}$ day^{-1}), and fish survival (97.7%) (Table 3).

Fish viscerosomatic index (24.6% on average) and carcass yield (75.4%) did not differ among the four diets (Table 4). Hepatosomatic index was lower in fish diet FeM+RM compared to those fed diet FeM (–11.4%; $p < 0.05$), with intermediate values in fish fed diets FM and PBM, while perivisceral fat index was similar among the diets (4.39%). Spleen somatic index was lower in fish fed diet FeM+RM compared to those fed diets FM and PBM (–24.1%; $p < 0.05$). Regarding gut, relative gut length was higher in fish fed diet FeM+RM than in those fed the other diets (+15.6%; $p < 0.01$), whereas relative gut mass was lower in fish fed diet FM compared to the other fish (–15.6%; $p < 0.01$) (Table 4).

3.5. Production costs of the diets

The highest production cost per ton of diet was measured for diet FM followed by diet PBM, diet FeM and diet FeM+RM (from 921 to 862, 844 and 821 € ton^{-1}) (Table 5). When costs were expressed per kg of produced fish, the highest values were recorded for diet FM (0.95 € kg^{-1}) and the lowest for diet FeM+RM (0.87 € kg^{-1}), with diet PBM and diet FeM showing intermediate and similar values (0.91 and 0.90 € kg^{-1}) (Table 5).

4. Discussion

In this study, we evaluated four practical, market-ready diets for rainbow trout characterized by decreasing levels of fishmeal (from 307 to 171 g kg^{-1}; replacement rate 36–44%) and increasing alternative protein meals such as poultry by-product meal (from 61.2 to 168 g kg^{-1}), hydrolysed feather meal (from 0 to 76.5 g kg^{-1}), and rapeseed meal (from 0 to 60.4 g kg^{-1}).

As for PAPs, the moderate inclusion (168 g kg^{-1}) of poultry by-product meal (diet PBM) did not impair trout growth and FCR where poultry by-product meal can be largely included (up to 590 g kg^{-1}) as protein source in rainbow trout diets without affecting growth and feed conversion ratio (Keramat-Amirkolaie et al., 2014; Parés-Sierra et al., 2014; Yanik et al., 2003). In our study, the inclusion of 76.5 g kg^{-1} of hydrolysed feather meal with 61.2 g kg^{-1} of poultry by-product meal and 198 g kg^{-1} of fishmeal (diet FeM) did not affect rainbow trout growth performance and feed conversion ratio while reducing aquafeed production costs by 8% (Table 5). In fact, feather meal composition and digestibility remain extremely variable (Pfeuti et al., 2019b) and a blend of hydrolysed feather meal with other protein meals is suggested to better balance dietary amino acid profile (Yu, 2019). Based on our results and previous literature (Bureau et al., 2000), hydrolysed feather meal could be effectively incorporated from 76.5 to 150 g kg^{-1} in low-fishmeal ($\leq$ 200 g kg^{-1}) diets for juvenile rainbow trout, whereas higher inclusion levels seem to be possible only through additional enzymatic pre-treatments of the feathers and amino acid supplementation (Pfeuti et al., 2019a; b).

As for vegetable alternatives, our study suggests that a low inclusion of rapeseed meal in combination with animal protein meals can be successfully applied in low-fishmeal diets for rainbow trout juveniles. Previous studies using low-fishmeal diets showed that growth impaired in trout fed diets with 50 g $kg^{-1}$of rapeseed meal and 205 g $kg^{-1}$of fishmeal (Burel et al., 2000), or diets with 75 g $kg^{-1}$of rapeseed meal in combination with other vegetable proteins and 160 g kg^{-1} of fishmeal (Alami-Durante et al., 2010), and with 500 g kg^{-1} of rapeseed meal and 245 g kg^{-1} of fishmeal (Burel et al., 2001).

Indeed, we measured the highest protein digestibility in the diet with the highest inclusion of poultry by-products (diet PBM). High digestibility of poultry by-product meal has already been reported in studies on rainbow trout (Badillo et al., 2014; Galkanda-Arachchige et al., 2020), with ADC of protein comparable to those reported for fishmeal, ranging from 69% to 96% (Sealey et al., 2015), while a reduction in protein digestibility was observed when diets included rather high levels (300–450 g kg^{-1}) of poultry by-product meal (Alexis et al., 1985; Keramat-Amirkolaie et al., 2014).

The practical diets tested in the present study containing feather meal showed ADC of protein similar to that of previous studies on feather meal-rich diet for rainbow trout (75–87%) (Bureau et al., 1999, 2000), but lower values compared to diets based on fishmeal and poultry by-product meals. This confirms that the inclusion of hydrolysed feather meal in rainbow trout diets has to be carefully evaluated in terms of protein and amino acid digestibility (Bureau et al., 1999; Cheng and Hardy, 2002; Cho et al., 1982) and further research is needed to find pre-treatment methods to improve the nutrient availability of the feathers (Pfeuti et al., 2019a; b).

On the other hand, scant information is available about lipid digestibility of PAPs-rich diets for rainbow trout (Bureau et al., 1999, 2000). In other species such as Nile tilapia (*Oreochromis niloticus*) (Tran-Ngoc et al., 2019) and orange-spotted grouper (*Epinephelus*

Table 2
Effect of diet and days of trial on apparent digestibility coefficients of protein and lipids of diets including different combinations of protein meals and fed rainbow trout for 84 days.

	Diet (D)				Days of trial (T)			*p*-value			RMSE
	FM	PBM	FeM	FeM+RM	28	52	84	D	T	D×T	
Protein	84.7^{c}	86.0^{d}	79.6^{a}	83.4^{b}	80.3^{a}	83.3^{b}	86.6^{c}	< 0.001	< 0.001	< 0.001	1.25
Lipids	84.4^{c}	85.5^{c}	71.1^{b}	66.4^{a}	69.7^{a}	77.8^{b}	83.0^{c}	< 0.001	< 0.001	< 0.001	2.22

RMSE: Root mean square error. Different superscript letters represent significant differences between means.

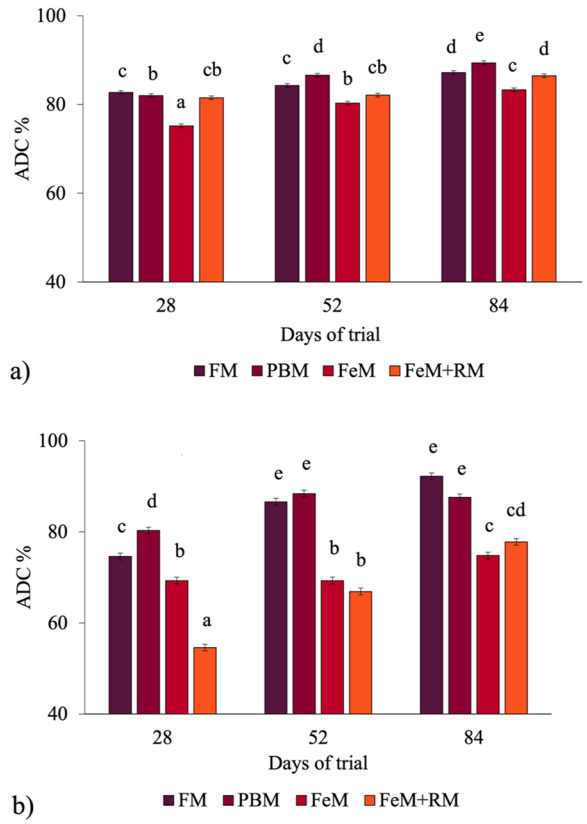

Fig. 4. Protein (a) and lipid (b) apparent digestibility coefficients (ADC) of the diets including different combinations of protein meals at different days of trial (significant interaction diets x days of trial). Data are represented as means ± standard error of the mean. Different letters above bars represent significant differences ($p < 0.001$) between means.

Table 3
Growth performance of rainbow trout fed diets including different combinations of protein meals for 84 days.

	Diets				*p*-value	RMSE
	FM	PBM	FeM	FeM+RM		
Fish per treatment (n)	255	255	255	255		
Tanks (n)	3	3	3	3		
Initial weight (g)	26.9	27.5	27.2	26.7	0.38	4.15
Final weight (g)	199	191	189	186	0.08	36.4
Daily weight gain (g d^{-1})	1.72	1.73	1.69	1.70	0.96	0.10
Specific growth rate (% d^{-1})	2.20	2.17	2.14	2.12	0.13	0.04
Feed conversion ratio	1.03	1.05	1.07	1.06	0.72	0.04
Feed intake (g DM $fish^{-1}$ day^{-1})	1.64	1.64	1.66	1.64	0.25	0.02
Survival (%)	99.1	97.7	94.9	99.1	0.10	2.00

RMSE: Root mean square error. Different superscript letters represent significant differences between means.

coioides) (He et al., 2022), ADC of lipids were significantly lower with hydrolysed feather meal-rich diets compared to fishmeal-based ones. In our trial, diets PBM and FM showed good ADC values for lipids (85% on average), whereas ADC of feather-meal-containing diets were fairly below 80% (66.4–71.1%) consistently with literature (Hua and Bureau, 2009).

Compared to fishmeal, alternative protein ingredients could affect nutrient utilisation, energy metabolism and allocation, besides body composition (Hoerterer et al., 2022; Glencross et al., 2020). For instance, the inclusion of vegetable proteins in rainbow trout diets has

Table 4
Somatic indices and carcass yield of rainbow trout fed diets including different combinations of protein meals for 84 days.

	Diets				*p*-value	RMSE
	FM	PBM	FeM	FeM+RM		
Viscerosomatic index (%)	22.8	24.1	24.6	26.9	0.10	5.31
Hepatosomatic index (%)	1.64^{ab}	1.57^{ab}	1.75^{b}	1.55^{a}	0.03	0.23
Perivisceral fat index (%)	4.38	3.93	4.31	4.95	0.06	1.21
Spleen somatic index (%)	0.14^{b}	0.15^{b}	0.13^{ab}	0.11^{a}	0.03	0.04
Relative gut length	0.59^{a}	0.59^{a}	0.61^{a}	0.69^{b}	< 0.001	0.07
Relative gut mass	0.09^{a}	0.11^{b}	0.11^{b}	0.10^{b}	< 0.01	0.02
Carcass yield (%)	77.2	75.9	75.3	73.1	0.10	5.31

RMSE: Root mean square error. Different superscript letters represent significant differences between means.

Table 5
Production costs of the diets including different combinations of protein meals.

	Diets			
	FM	PBM	FeM	FeM+RM
Feed production cost[a] (€ ton^{-1})	921	862	844	821
Feed cost to produce 1 kg of fish (€ kg^{-1})	0.95	0.91	0.90	0.87
Change with respect to control diet (FM) (%)		-8	-10	-11

[a] Feed production cost at production plant gate: costs of labour, packaging and transport are not included.

been found to impact on the hepatic content of glycogen and lipids (Krogdahl et al., 2005). In our study, fish fed the diet containing hydrolysed feather meal showed the highest hepatosomatic index, whereas those fed diets including both hydrolysed feather and rapeseed meals displayed the lowest one. On one hand, an increase of hepatosomatic index was attributed either to a suboptimal amino acid profile of the vegetable proteins that induced fat deposition in the liver or to an impairment of starch metabolism (Gaylord and Barrows, 2009; Kaiser et al., 2022; Yao et al., 2019); on the other hand, a reduction of the hepatosomatic index, as observed in our study, might be related to a decreased availability of nutrients with a consequent depletion of the hepatic deposits of glycogen and lipids used to maintain metabolism (Kaiser et al., 2022).

As for the physical quality, feed pellets for salmonids have to contain up to 40% of lipids and tolerate high levels of mechanical and thermal stress during the transportation to the farm and during storage (Chaabani et al., 2020; Dethlefsen et al., 2017). Oil leakage will eventually interfere with the nutritional quality of pellets and if oil is released in the tanks, it can pollute pipes and biological filters and small particles may be stuck in the system (Chaabani et al., 2020). Oil leakage rates ranged from 1.2% and 4.7% in rainbow trout and Atlantic salmon feeds with different lipid (Chaabani et al., 2020) and protein (Banjac et al., 2021) sources, which are in line with our findings (1.30% on average). While the increase in the dietary protein content is recognized to lead to a more compact structure of the feed, resulting in an increased durability (Banjac et al., 2021), few information is available about the effects of protein sources. Based on literature, the inclusion of soybean led either to an increase (Hussain et al., 2022) or to a decrease (Samuelsen et al., 2022) of feed durability compared to a fishmeal-based diet, whereas the only two studies regarding PAPs evaluated the effects of the inclusion of black soldier fly meal or paste (Irungu et al., 2018; Weththasinghe et al., 2021) or cricket meal (Irungu et al., 2018) in low-fishmeal salmonid feeds, showing no notable impacts of the protein source on pellet structural integrity. In our study, the protein source did not impact on feed durability and all pellets showed a high durability with a very low

amount (0.9%, on average) of fractures produced after being submitted to the test, in line with previous studies on salmonid feeds (0.4–3.7%) (Banjac et al., 2021; Irungu et al., 2018; Weththasinghe et al., 2021).

In a close environment like RAS, suspended and dissolved particles deriving from uneaten feed affect turbidity of water and thus light penetration (Becke et al., 2020; Schumann and Brinker, 2020). The leftover feed in RAS is normally conveyed, filtered, and eliminated from the system in less than one hour, therefore the first minutes after feed administration are crucial (Dalsgaard et al., 2013). Our results suggest that the lower turbidity in water produced after 15 min by diet FeM and after other 15 min by diet FeM+RM might be a consequence of the hydrolysation process of feather meal, that might have improved the adhesiveness of the meal and the bounding with the other feed components (Adhikari et al., 2018; Piazza and Garcia, 2010, 2016), leading to more water-stable feed.

Finally, in RAS, the formation of large faecal particles is desirable, as they are more rapidly and easily removed from the system than smaller ones (Brinker, 2009; Unger and Brinker, 2013). Overall, compared with previous studies on rainbow trout (Welker et al., 2018, 2020, 2021), we found a similar proportion of fine particles in faeces (58–64%), but a lower percentage of mid-large faecal particles (5–7% vs. 21–38%). These differences might be likely related to the different methods used to measure faecal size, with laser diffraction (Welker et al., 2018) or microscopic analysis (Welker et al., 2020, 2021) being more accurate than using the percentage of retained faeces at different sieve mash sizes.

As for faeces particle size, in previous studies, changes were observed with the replacement of fishmeal with soybean meal and sunflower meal in diets for rainbow trout (Unger and Brinker, 2013; Welker et al., 2020, 2021). These effects were associated to the presence of antinutritional factors (i.e., protease inhibitors, phytic acid, oligosaccharides) in plant sources (Welker et al., 2021), which reduce nutrient digestibility and cause diarrhoea that decreases faecal stability (Welker et al., 2020). On the other hand, diets mainly containing animal proteins such as fishmeal and poultry by-product meal likely produce greater amounts of large and mid-size faecal particles, which is likely related to the higher digestibility of these sources compared to plant-based ones (Schumann and Brinker, 2020). In our study, the diet PBM, containing poultry by-product meal and fishmeal as main protein sources, showed the highest protein digestibility and was associated to a higher size of faeces particles.

5. Conclusion

This study provides new insights into the inclusion of alternative protein meals, such as poultry by-product meal, hydrolysed feather meal and rapeseed meal, in diets for rainbow trout reared in RAS.

Under our conditions, the partial substitution of fishmeal from fish by-products with alternative protein meals did not affect trout growth performance and feed conversion ratio. The digestibility of protein resulted satisfactory (>80%) for all diets, whereas improvement for lipid digestibility is desirable in diet containing hydrolysed feather meal and rapeseed meal.

The change of the protein sources did not affect the physical quality of the feeds, which showed a high structural integrity and a low oil leakage. The inclusion of hydrolysed feather meal reduced water turbidity during the first thirty minutes after feed administration and the inclusion of poultry by-product meal induced the production of larger particles of faeces, which are considered positively in view of the reduction and collection of wastes in a RAS system.

A comprehensive evaluation of aquafeeds, by considering not only their nutritional aspects but also their physical characteristics and effects on waste production together with economic impacts, would be paramount for future feed formulations, especially when designed for sustainable, highly controlled, and resource-efficient environments like RAS.

CRediT authorship contribution statement

Fabio Brambilla, Vlastimil Stejskal: Conceptualization, Project administration, Supervision. **Cecilia Fanizza, Francesco Bordignon, Vlastimil Stejskal, Angela Trocino**: Data curation. **Markéta Dvořáková Prokešová, Mahyar Zare, Hung Quang Tran, Vlastimil Stejskal, Cecilia Fanizza**: Formal analysis. **Fabio Brambilla**: Funding. **Cecilia Fanizza, Markéta Dvořáková Prokešová, Mahyar Zare, Hung Quang Tran**: Investigation. **Fabio Brambilla, Vlastimil Stejskal, Angela Trocino**: Methodology. **Cecilia Fanizza, Francesco Bordignon, Angela Trocino**: Writing – original draft. All authors: Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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