Aquaculture 574 (2023) 739621

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Effect of feed ingredients on nutrient digestibility, waste production and physical characteristics of rainbow trout (*Oncorhynchus mykiss*) faeces.

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ARTICLE INFO

Keywords:
Rainbow trout
Alternative ingredients
Digestibility
Faecal waste production
Removal efficiency

ABSTRACT

This study assessed the effect of different dietary ingredients on the quantity and characteristics of faecal waste produced by rainbow trout (*Oncorhynchus mykiss*). Seven ingredients were tested: fish meal (FM), mussel meal (MM), poultry meal wet rendered (PM-W) or dry rendered (PM-D), insect meal (IM), single cell protein (SCP) and brewers grain protein (BGP). Eight experimental diets were formulated: a control diet (CON) being predominantly plant-based and seven test diets, which contained 70% of the CON diet and 30% of one of the test ingredients. Rainbow trout juveniles (65 g, 30 fish/tank) were fed the experimental diets at satiation for six weeks in triplicate groups. Dry matter (DM) and nutrient digestibility of diets and the test ingredients were measured. To estimate the faecal characteristics, particle size distribution (PSD) and removal efficiency of the faecal waste was determined. Nutrient digestibility of diets and ingredients differed significantly. Growth did not differ between the experimental diets, but DM digestibility was affected by the diet. Diets affected the amount of faecal waste produced, its removal efficiency (%) and the amount of non-removed faeces (g DM/kg DM feed). The highest and lowest removal efficiency was observed at FM and BGP diets, respectively. Accordingly, FM diet resulted in the lowest (37 g DM/kg DM feed), while BGP diet resulted in the highest (125 g DM/kg DM feed) amount of non-removed faeces. Additionally, it was also observed that differences in faecal removal efficiency can compensate for the variation in the quantity of faecal waste produced. Consistent with the faecal removal efficiency data, faeces PSD was also influenced by diets. FM and MM diets resulted in faeces with the lowest proportion of particles of <40 μm size, while BGP diet had the largest proportion of faecal particles of this size range. Furthermore, the effect of dietary ingredient composition was evident in the stability of faeces, with FM producing the most stable, whereas CON, BGP and SCP diets resulting in relatively unstable faecal pellets. In addition, due to differences in inclusion level of nutrients and their corresponding digestibility, the chemical composition of faeces differed between the diets. Overall, the study showed that dietary ingredient composition influences nutrient digestibility and is an important factor determining the amount of faecal waste produced, its removal efficiency, PSD, stability and composition in rainbow trout.

1. Introduction

The constrained availability of conventional feed resources implies that ingredients from diverse sources are needed for future aquafeed formulations. Consequently, new raw materials such as insect meals, single-cell proteins, by and co-products of agro and animal processing industries are being tested for their inclusion in aqua feeds (Hoerterer et al., 2022; Hua, 2021; Tacon et al., 2022). Historically, raw materials were included in feed formulations based on their nutritional properties, availability and price. However, in a production system, fish feed also forms the main source of waste, mainly in the form of faecal discharge (Kokou and Fountoulaki, 2018; Merino et al., 2007). Very few studies have attempted to explore the repercussions of changing ingredient composition of diet from the perspective of faecal solid waste

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Abbreviations: PSD, particle size distribution; NSP, non-starch polysaccharides; ADC, apparent digestibility coefficient.

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https://doi.org/10.1016/j.aquaculture.2023.739621
Received 2 February 2023; Received in revised form 15 April 2023; Accepted 23 April 2023

management.

The faecal waste generated in a production system can either be used as a nutrient source to stimulate the food web (i.e. in ponds or integrated multitrophic aquaculture (IMTA) in open water bodies) (Granada et al., 2016; Kabir et al., 2020) or has to be removed (i.e. in raceways or recirculating aquaculture system, RAS) by using different mechanical devices (van Rijn, 2013). Solid waste removal is targeted to achieve optimal water quality for the cultured fish species and reduce effluent's impact on the surrounding environment. In either scenario, it is necessary to estimate the amount of faecal waste produced and its removal efficiency. The amount of faecal waste produced depends on the digestibility of ingredients used in the dietary formulations. Removal efficiency of faecal waste is a function of its physical and chemical characteristics, such as the particle size distribution (PSD), faecal integrity (the ability of faecal particles to resist breakdown by hydrodynamic/mechanical forces in raceways or RAS) and the composition of faecal waste (Meriac et al., 2014; Schumann et al., 2022).

Rainbow trout is a significant contributor to global aquaculture, with estimated production of 959,600 t in 2020 (FAO, 2022). At present, rainbow trout is predominantly cultured in raceways, but there is strong motivation to shift towards RAS production, especially in Western Europe (Dalsgaard et al., 2013; Wind et al., 2022). Implementation of strict regulatory measures with respect to waste discharge (Dalsgaard et al., 2013; Lindland et al., 2019; van Rijn, 2013) implies that raceway and RAS production units should have an efficient solid waste removal system in place. This can be accomplished by using diets which result in lower quantities of faecal waste concomitant with improved physical characteristics, resulting in higher faecal removal efficiency, thereby lowering the amount of non-removed faecal waste remaining in the system.

One of the factors that can steer both the amount of faecal waste produced and its removal efficiency is the dietary ingredient composition. Previous studies in rainbow trout indicate that replacing fish meal-based diets with soy-based diets results in an increased amount of faecal waste and faecal particles of smaller size and lower stability (Brinker and Friedrich, 2012., Ogunkoya et al., 2006., Schumann et al., 2022). The effect of dietary ingredients on faecal waste production and its removal efficiency were also demonstrated in studies with common carp, *Cyprinus carpio* (Prabhu et al., 2019), European sea bass, *Dicentrarchus labrax* (Fountoulaki et al., 2022) and striped catfish, *Pangasianodon hypophthalmus* (Tran-Tu, 2019). However, these studies have primarily been limited to testing dietary ingredients of plant origin or the co- and by-products of plant origin. Variations in nutrient composition due to use of different ingredients can impact faecal waste characteristics implying that faecal waste production and their removal efficiency need to be investigated while using alternative ingredients. The present study, therefore, assessed the effect of dietary ingredient composition on the quantity and characteristics of faecal waste produced by rainbow trout.

2. Materials and methods

2.1. Ingredients and diets

In this experiment, seven ingredients were tested. Fish meal (FM) was used as one of the test ingredients to allow comparison of faecal waste characteristics between conventional and modern diets. Dietary ingredients tested included poultry meals differing in processing conditions: poultry meal wet rendered (PM-W) or dry rendered (PM-D); insect meal (IM), black soldier fly larvae (*Hermetia illucens*); brewers grain protein (BGP), brewery waste following centrifugation, sieving and pressing; deshelled mussel meal (MM) from blue mussel (*Mytilus edulis*) and single cell protein (SCP) from a mixed bacterial culture. The control diet (CON) was formulated using mostly plant-based ingredients (Table 1), and this basal mixture was used to create the test diets. CON diet fulfilled the known nutrient requirements for rainbow trout (NRC, 2011). Yttrium oxide was added as an inert marker in the basal mixture to determine digestibility of diets and ingredients. Seven test diets were prepared by replacing 30% of the basal mixture in the CON diet with the seven respective test ingredients, following the 70:30 ratio approach of ingredient evaluation (Bureau et al., 1999). Diets were produced by Research Diet Services (Wijk bij Duurstede, The Netherlands) by extrusion using a Clextral BC45 laboratory scale twin-screw extruder (Clextral, Firminy, France) with a 2 mm die, resulting in 3 mm sinking pellets. Throughout the experimental period, diets were stored at 4 °C. The analysed chemical composition of the test ingredients and experimental diets is provided in Table 2. Amino acid composition of the experimental diets is provided in Supplementary Table S1.

Table 1
The amounts (in g/kg) of ingredients used in the control diet.

Ingredients	Inclusion (g/kg, as is)
Wheat flour	335.8
Wheat gluten	110.0
Soy protein concentrate	110.0
Soybean meal	110.0
Pea protein	110.0
Fish oil	100.0
Fish soluble concentrate	20.0
Mono-calcium-phosphate	43.0
Chalk ($CaCO_3$)	14.0
L-Lysine	5.0
DL-Methionine	5.0
L-Threonine	2.0
Yttrium oxide	0.3
Premix[a]	15.0

Vitamins (IU or g/kg premix): thiamin, 1 g; riboflavin, 1 g; pyridoxine, 1 g; pantothenic acid, 4 g; niacin, 6.5 g; biotin, 0.02 g; cyanocobalamin, 0.017 g, folic acid, 0.33 g; ascorbic acid (as ascorbic acid phosphate), 15 g; DL-alpha tocopherol acetate, 20,000 IU; retinyl palmitate, 300,000 IU; DL-cholecalciferol, 240,000 IU; sodium menadione bisulfite (51%), 1 g; inositol, 40 g; choline, 200 g (given as choline chloride).

Minerals (g kg^{-1} premix): iron (as $FeSO_4.7H_2O$), 5 g; zinc (as $ZnSO_4.7H_2O$); 10 g; cobalt (as $CoSO_4.7H_2O$), 0.01 g; copper (as $CuSO_4.5H_2O$), 1 g; Selenium (as Na_2SeO_3), 0.02 g; manganese (as $MnSO_4.4\ H_2O$), 2 g; magnesium (as $MgSO_4.7H_2O$), 50 g; chromium (as Cr Cl3.6H2O), 0.1 g; iodine (as $CaIO_3.6H_2O$) 0.2 g.

Preservatives (g kg^{-1} premix): Anti-oxidant BHT (E300–321), 10 g; calcium propionate, 100 g.

[a] Premix composition.

2.2. Fish, rearing conditions and housing facilities

The experiment was carried out in accordance with the Dutch and European law on the use of experimental animals. The Animal Welfare Body of Wageningen University and Research (The Netherlands) classified this experiment as non-invasive and not an animal experiment according to Dutch legislation. Fish were kept and handled as per EU legislation. Juvenile rainbow trout (*Oncorhynchus mykiss*) of mixed sex were obtained from a commercial farm (Mohnen, GmbH, Stolberg, Germany), 2 weeks prior to the start of the experiment. At the start of the experiment, 720 fish with an average weight of 65 g were randomly assigned to 24 experimental tanks (30 fish per tank), resulting in 3 replicates per dietary treatment. Fish were weighed (Mettler-Toledo ICS429) at the experiment's beginning and end to determine initial and final weight and calculate growth performance. One day prior to weighing, fish were starved to allow emptying of their gastrointestinal tract to reduce discomfort during weighing and handling. Water volume in each tank was maintained at 200 L. All tanks were connected to a common RAS ensuring that inlet water quality was same for all experimental units. Briefly, the RAS was equipped with a sump, settling tank, drum filter, protein skimmer and trickling filter. Each tank was provided with air stones and the outlet was connected to swirl separators (Aqua Optima AS, column height 44 cm; diameter 24.5 cm) for the collection of faeces and quantification of the spilled feed pellets. Water flow rate to

Table 2
Analysed nutrient composition of the test ingredients and the experimental diets.

	CON	FM	MM	PM-W	PM-D	IM	SCP	BGP
Inclusion level as is basis (%)								
Test Ingredient	–	30	30	30	30	30	30	30
Basal mixture (see Table 1)	100	70	70	70	70	70	70	70
Nutrient composition ingredients (g/kg DM)								
Dry matter (DM; g/kg)		932	922	923	970	949	926	951
Crude protein		726	683	737	705	553	739	555
Crude fat		92	96	82	129	160	51	151
Total carbohydrate[a]		-10[c]	155	21	21	218	120	273
Starch + Sugars		15	95	18	22	55	36	46
Non-starch polysaccharides[b]		-25[c]	60	3	-1[c]	163	85	227
Ash		191	66	160	145	69	90	20
Phosphorus		28.6	8.8	26.1	23.9	11.7	21.7	4.3
Calcium		47.4	6.6	38.3	36.2	7.7	1.9	1.8
Magnesium		2.7	1.8	1.6	1.5	4.1	2.9	0.6
Energy (kJ/g DM)		20.5	23.2	20.7	21.8	23.3	21.6	24.8
Essential amino acids (g/kg DM)								
Arginine		41.2	31.7	38.9	44.3	26.6	44.0	26.7
Histidine		15.4	11.7	12.2	13.6	15.6	11.9	11.5
Isoleucine		26.9	27.3	23.8	23.4	22.4	29.6	23.0
Leucine		46.5	43.9	42.3	42.5	36.1	51.7	43.3
Lysine		47.0	51.8	42.9	35.9	30.4	35.5	20.6
Methionine		19.7	14.2	12.8	13.2	9.0	12.4	10.4
Phenylalanine		28.5	24.5	21.2	27.5	23.4	31.7	34.9
Threonine		27.4	30.4	19.9	23.7	21.5	34.3	19.5
Valine		32.7	29.0	31.2	32.5	30.2	40.6	29.1
Non-essential amino acids (g/kg DM)								
Alanine		42.6	31.7	51.1	42.8	33.7	51.9	23.1
Aspartic acid		60.5	69.8	55.0	49.1	47.6	64.6	36.3
Glutamic acid		83.7	82.9	77.5	77.7	56.4	82.4	128.8
Glycine		55.3	36.5	73.7	71.6	27.5	37.5	19.1
Proline		32.4	25.7	47.1	44.5	30.4	32.3	62.6
Serine		28.6	29.7	23.8	26.0	23.1	28.0	24.1
Tyrosine		21.2	21.8	16.7	20.6	30.5	25.2	20.1
Sum of amino acids[d]		610	572	590	589	464	614	533
Nutrient composition diets (g/kg DM)								
Dry matter (DM; g/kg)	933	943	963	946	948	949	936	959
Crude protein	397	496	480	502	494	447	503	447
Crude fat	151	136	142	120	142	152	118	149
Total carbohydrate[a]	370	257	303	279	266	323	295	342
Starch + Sugars	274	196	223	206	195	199	201	201
Non-starch polysaccharides[b]	96	61	80	73	71	124	94	141
Ash	83	110	75	99	99	78	84	63
Phosphorus	15.2	19.1	13.2	18.0	17.9	14.0	17.5	12.1
Calcium	15.3	24.5	12.5	22.2	21.9	12.8	11.3	11.1
Magnesium	2.7	2.7	2.5	2.4	2.3	3.2	2.8	2.1
Energy (kJ/g DM)	23.1	22.1	19.6	19.6	20.1	23.3	22.7	21.6

Notes. CON, Control; FM, Fish meal; MM, Mussel meal; PM-W, Poultry meal wet rendered; PM-D, Poultry meal dry rendered; IM, Insect meal; SCP, Single cell protein; BGP, Brewers grain protein; DM, dry matter.

[a] Total carbohydrate was calculated as 1000 – crude protein – crude fat – ash content.

[b] Non-starch polysaccharides was calculated as total carbohydrate – (starch + sugars).

[c] The negative value of carbohydrate is mainly due to an overestimation of the calculation of crude protein as 6.25 times the measured N content.

[d] Sum of amino acids is without tryptophan and cysteine (tryptophan and cysteine are destroyed during acid hydrolysis).

each experimental unit was regulated at 7 ± 0.05 L/min by using magnetic inductive flow sensor (SM 6000, IFM electronic, Essen, Germany).

Water quality parameters were monitored daily. Temperature (mean 13.9 °C range 13.7–14.4 °C) (WTW Multi 3630 IDS - FDO 925), pH (mean 7.5, range from 7.3 to 7.7), electrical conductivity (2.8 millisiemens/cm (mS/cm) ranging between 2.4 and 3.3 mS/cm) (WTW Multi 3630 IDS - Sentix 940) and dissolved oxygen (mean 8.7 mg/L, range from 7.7 to 9.9 mg/L) (WTW Multi 3630 IDS - FDO 925) were measured in the outlet water of randomly selected tanks (swirl separator connected to holding tanks) by hand held digital probes. Other water quality parameters such as TAN, total ammonia nitrogen (Merck, Aquamerck Colorimetric Ammonium test), NO_2-N (Merck Aquamerck, Colorimetric Nitrite test), NO_3-N concentrations (Merck MQuant Nitrate test strips) were maintained at <2 mg/L, < 1 mg/L, < 80 mg/L, respectively. Light intensity was set at 200 lx and photoperiod regime of 12: 12 h (light: dark) with an automatic on and off at 8:00 and 20:00 h respectively.

2.3. *Experimental procedures and sampling*

Fish were hand-fed to satiation twice a day at 9:00 and 15:00 h. Feeding was continued until voluntary feed ingestion ceased with a maximum duration of 1 h per feeding moment. The feed input was increased progressively to 12 g/kg$^{0.8}$ body weight (BW)/d during the first 2 days of the experiment to allow the fish to adapt to the diet. Fifteen minutes after feeding, uneaten/spilled feed pellets were determined by checking bottles attached to the swirl separators. Mortality was checked twice daily prior to feeding and dead fish were removed and weighed.

For quantifying the dry matter digestibility, apparent digestibility coefficient (ADC) of nutrients, amino acids (AA) and the faecal removal efficiency, faeces were collected by settling for 48 h (excluding time period during morning and afternoon feeding and collection of uneaten pellets) during week 6 (Meriac et al., 2014). Bottles attached to swirl separators were kept submerged in ice slurry. Faecal samples were pooled per tank and stored at −20 °C until further analysis. For determining faecal PSD, samples were collected in bottles attached to swirl separator after morning feeding, twice during week 6. Sample bottles were kept on ice till further analysis. A 100 g-feed subsample was pooled for each diet per week and used for feed composition analysis.

2.4. Analytical methods

Faecal samples collected for digestibility and faeces removal efficiency were dried in the oven at 70 °C until constant weight. The dried faecal samples were ground (Retsch ZM, 200) prior to the analysis. The ingredients, feed and faeces were analysed as per Elesho et al. (2021). Dry matter was estimated by drying at 103 °C until constant mass (ISO 6496, 1999). Ash was determined gravimetrically in a muffel furnace after 4-h of incineration at 550 °C (ISO 5984, 1978). The ash fraction was dissolved in concentrated sulphuric acid by autoclaving (121 °C, 20 min) to determine minerals such as phosphorus, calcium, magnesium and yttrium by inductively coupled plasma optical emission spectrometry, following Dutch analytical standards (NEN 15510:2017). The total nitrogen content was measured by the Kjeldahl-method (ISO 5983, 1997), calculating crude protein as N × 6.25 (protein conversion factor). Crude fat was determined gravimetrically using acid hydrolysis followed by extraction with petroleum-ether (Soxhlet method; ISO 6492, 1999). Gross energy was measured by bomb calorimetry (C7000; IKA®-Werke GmbH & Co. KG). Amino acids in ingredients, diets and faeces (excluding tryptophan and cysteine) were analysed by an ultra-performance liquid chromatography (UPLC, Waters Acquity, UPLC systems, Milford, MA, United States). The quantitative determination was based on the method accredited by the Nordic Committee of Food Analysis (NMKL) and as per the protocol described in detail in Belghit et al. (2019). Starch including free sugar fraction in feed and faeces were determined enzymatically using amyloglucosidase without a prior ethanol extraction for removing free sugars (Goelema et al., 1998).

The faecal PSD was measured using sieves of different mesh size (1600 μm, 850 μm, 300 μm and 40 μm). PSD was determined for undisturbed faecal waste (hereafter termed as non-stressed faeces) collected in bottles attached to swirl separator. Additionally, to test faecal stability, faecal pellets from the collection bottle were poured thrice through a 1-m-long PVC pipe with a 40-degree slope (Supplementary fig. S1). This was done to mimic the shear stress to which faecal waste may be subjected to, as they travel from fish tank to the solid removal unit such as drum filter. These faeces were termed as the stressed faeces. The particle size fractions were determined using the same method under both scenarios. Faecal waste was gently poured on a 1600 μm sieve and both the filtrate (< 1600 μm) and residue (> 1600 μm) were collected. The resulting filtrate was then passed through 850 μm, 300 μm and 40 μm sieves following the same steps. This resulted in 5 size fractions (> 1600 μm, 1600–850 μm, 850–300 μm, 300–40 μm and < 40 μm). All fractions were collected on pre-weighed 1.5 μm pore size glass filter papers (90 mm diameter, grade 696, VWR, Randor, USA) in combination with a filtration apparatus connected to a vacuum pump. Filters were stored at −20 °C until further analysis. To determine the mass of collected organic matter (OM) fractions, filters were dried and incinerated as described above for feed and faeces samples. Faecal PSD data was expressed on mass % basis for each fraction, for both non-stressed and stressed faecal waste.

2.5. Calculations

Absolute weight gain (WG, g/fish) was estimated as the difference between the average individual final (W_f) and initial (W_i) body weight per fish. Feed intake per fish per day (FI, g/fish/day) was calculated using the formula:

$$\text{FI} = (\text{total feed DM offered per day} - \text{uneaten feed DM per day})/(\text{fish number})$$

FI was summed for the whole period to obtain feed intake per fish over the entire experimental period (FI_{tot}).

Specific growth rate (SGR; %BW/d) was calculated as:

$$\text{SGR} = \left[ln\left(W_f\right) - ln\left(W_i\right) \right] \times 100/t$$

where t is the duration of trial in days.

The feed conversion ratio (FCR) was calculated as:

$$\text{FCR} = \text{FI}_{tot}\ (\text{g/fish})/\text{WG}\ (\text{g/fish})$$

The dry matter ADC of diets was calculated as follows:

$$\text{ADC}_{DM}\ (\%) = 100 \times \left[1 - \left(Y_{diet}/Y_{faeces}\right)\right]$$

where Y_{diet} and Y_{faeces} is the concentration of yttrium in diet and faeces respectively expressed on DM basis.

The ADC of AA, macronutrients and macro-minerals of diets were calculated according to the formula described by Cheng and Hardy (2002):

$$\text{ADC}\ (\%) = 100 \times \left[1 - \left(Y_{diet} \times N_{faeces}\right)/\left(Y_{faeces} \times N_{diet}\right)\right]$$

Where N_{diet} and N_{faeces} represent the nutrient percentage (g/kg DM or kJ/g DM gross energy) of the diet and faeces respectively.

ADC of dietary components in test ingredients were calculated using the following equation described by Teuling et al. (2019):

$$\text{ADC}_{\text{test ingredient}}\ (\%) = \text{ADC}_{\text{test diet}} + \left(\text{ADC}_{\text{test diet}} - \text{ADC}_{\text{CON}}\right) \times \left[(0.7 \times N_{\text{CON}})/\left(0.3 \times N_{\text{test ingredient}}\right)\right] \times 100\%$$

Where $\text{ADC}_{\text{test diet}}$ and ADC_{CON} are the apparent digestibility coefficient (%) of the dietary component in the test diet and the control diet respectively. N_{CON} and $N_{\text{test ingredient}}$ are the nutrient contents (g/kg DM) or the gross energy (KJ/g DM) in the control diet and test ingredient, respectively.

Carbohydrate content in feed, ingredients and faeces was determined by the difference as [1000 - (crude protein + fat + ash)]. NSP level is calculated as the difference between the carbohydrate and starch content.

Faecal waste production (g DM/kg DM FI) was calculated on dry matter basis as the amount of non-digested feed per kilogram feed intake as:

$$\text{Faecal waste production (g DM/kg DM FI)} = (100\% - \text{ADC}_{DM}) \times 1000$$

Faecal removal efficiency (FR, %) was estimated as the percentage of total faeces collected by settling in proportion to total faecal waste produced. In detail, this was calculated on the basis of yttrium collected in settled faeces ($Y_{removed}$, g) in relation to the amount of yttrium supplied by diet (Y_{diet}, g) as:

$$\text{FR}\ (\%) = Y_{removed}/Y_{diet} \times 100\%$$

Non-removed faeces (g DM/kg DM feed) was calculated as the difference between the total amount of faeces produced and faeces removed as:

$$\text{Non}-\text{removed faeces} = [(100\% - \text{ADC}_{\text{DM}}) \times (100\% - \text{FR})] \times 1000$$

2.6. Statistical analysis

Tanks were the experimental unit in the statistical analysis. Data on performance, feed intake, digestibility, faecal waste production, particle size distribution and faecal removal efficiency were expressed as the mean per treatment of three replicates. All measured parameters were tested for the effect of diet using one-way ANOVA assuming normal distribution. If significant ($P < 0.05$), treatment means were compared by Tukey HSD (honest significant difference) with multiple comparison with 95% level of significance. All statistical analyses were performed using SPSS statistics version 27.0 for windows (IBM Corp., Armonk, NY, USA).

3. Results

Fish Performance (Table 3): Initial body weight (65 ± 0.6 g) was similar between treatments ($P > 0.1$). Mortality was low (0.8% averaged over treatments) and unaffected by diet ($P > 0.1$). During the experiment, fish tripled in weight but final weight and weight gain were not different between the diets ($P > 0.05$). Feed intake was affected by diet ($P < 0.01$). The differences in feed intake with similar growth resulted in FCR being affected by diet ($P < 0.01$).

Ingredient digestibility (Table 4): Readers may refer to Supplementary Table S2 for information on ADC of macronutrients, amino acids, energy and minerals of experimental diets. Digestibility of DM, macronutrients and amino acids differed among the dietary ingredients ($P < 0.05$). ADC values of nutrients in Table 4, which are placed between brackets were excluded from the statistical analysis. This was done considering the low contribution of tested ingredients to the content of specific nutrient in experimental diets (a cut off value of ≤6% was set for this purpose) which amplifies the measurement errors in the calculated ingredient ADC values. The highest DM digestibility was recorded for MM (94.3%) followed by PM-D (87.3%), while the lowest value was found for BGP (73.9%). The MM (93.4%) displayed the highest value for crude protein ADC whereas the IM (86.0%) had the lowest. All dietary ingredients selected for this study were found to have high amino acid digestibility as evident from the ADC values >84% for all individual amino acids tested. Among ingredients tested, both poultry meal sources had similar and the lowest sum of amino acids digestibility. In concurrence with the protein digestibility data, MM also had highest sum of amino acids digestibility.

Faecal waste production, faecal removal efficiency and non-removed faeces (Fig. 1, Fig. 2, Fig. 3 and Fig. S2): Dietary ingredients affected the DM digestibility of the diets and thus total faecal waste production ($P < 0.001$) (Fig. 1). Faecal removal efficiency and the quantity of non-removed faeces were also affected by the ingredient composition of the diets ($P < 0.001$) (Fig. 2). The lowest faecal waste production was observed with MM (215 g DM/kg DM FI), being 24% lower than observed for CON diet (284 g DM/kg DM FI). In comparison to CON diet, an improvement in faecal removal efficiency was noted ($P < 0.001$) for all dietary ingredients tested except BGP (Fig. 3). High faecal waste production and low faecal removal efficiency at CON and BGP diets reflected in high amount of non-removed faecal waste (104 and 125 g DM/kg FI respectively) observed for these two diets. Overall, the amount of non-removed faecal waste exhibited up to a three-fold difference between the diets.

Faecal composition (Table 5): On dry matter basis, the composition of the faecal waste was affected ($P < 0.05$) by the ingredient composition of the diets. The smallest fraction of the faecal waste consisted of the crude fat followed by crude protein. The largest fraction of the faecal waste consisted of carbohydrates, ranging from 45% to 65% between the eight experimental diets. CON and BGP diet, which were predominantly plant-based, resulted in faeces with high carbohydrate levels, whereas it was lower in faeces of groups fed with FM and PM-based diets. Though both CON and BGP diets resulted in faeces with almost similar carbohydrate levels, the relative proportion of starch and NSP fraction in the faeces varied considerably (starch contributing only 22% of total carbohydrate in case of the BGP diet, whereas 44% in case of CON diet). Most of the carbohydrate fraction in the faeces consisted of NSP, ranging from 56% to 72%. Additionally, a substantial portion of faecal waste (ranging from 16% to 32% in BGP and FM respectively) was constituted by ash.

Particle size distribution of faecal waste (Table 6 and Plate 1*)*: From visual observation of the faecal waste collected in glass bottles (Plate 1a, representing non-stressed/undisturbed faecal waste) and upon transfer and settling in Imhoff cones (Plate 1b, representing stressed faecal waste) it was evident that there is large variability in particle sizes of the faecal waste depending on the dietary ingredients. CON and BGP diet had a larger fraction of particles of smaller size (< 40 μm) under both scenarios, while FM, MM and PM-D had greater share of particles of larger size (> 1600 μm). The other treatments appeared to be intermediate.

The PSD data (Table 6) of non-stressed and stressed faecal waste show that the faecal particles size and ability to withstand stress are affected by the ingredient composition of the diets. Under the non-stressed scenario, BGP diet had largest proportion (12.4%) of particles smaller than 40 μm and this differed ($P < 0.001$) from all other diets. The stress exposure modified the particle size distribution for all dietary groups, with FM diet resulting in the most stable faecal particles and BGP the least stable one. The proportion of faecal particles >1600 μm had a difference of up to 3-fold among diets for non-stressed faeces but increased up to 9-fold upon exposure to stress. Overall, dilution of basal diet with test ingredients lowered the proportion of smaller-sized faecal particles for all dietary ingredients tested except for the BGP under both stressed and non-stressed scenarios. Under the non-stressed scenario, CON and BGP diet had about 25% particles >1600 μm, while particles in the same size fraction constituted three times larger share i.e. about 74% particles in the case of MM diet. A significant positive correlation ($P <$

Table 3
Growth performance of rainbow trout fed to apparent satiation during the experimental period (42 days). Values are means (n = 3) and standard error of the mean (SEM).

	CON	FM	MM	PM-W	PM-D	IM	SCP	BGP	SEM	*P-value*
Initial body weight (g/fish)	65	64	66	65	65	65	65	66	0.6	ns
Final body weight (g/fish)	215	219	226	211	210	220	210	208	4.0	#
Weight gain (g/fish)	150	155	160	146	145	156	145	142	4.0	#
Feed intake (g DM/fish)	132^{ab}	128^{ab}	125^{ab}	123^{ab}	119^{a}	138^{b}	120^{a}	117^{a}	3.1	**
SGR (%/d)	2.86	2.91	2.92	2.79	2.78	2.92	2.79	2.71	0.05	#
FCR	0.88^{c}	0.83^{b}	0.79^{a}	0.85^{b}	0.82^{b}	0.89^{c}	0.83^{b}	0.82^{b}	0.006	***
Survival (%)	99	99	96	100	100	100	100	100	1.2	ns

Notes. CON, Control; FM, Fish meal; MM, Mussel meal; PM-W, Poultry meal wet rendered; PM-D, Poultry meal dry rendered; IM, Insect meal; SCP, Single cell protein; BGP, Brewers grain protein; SGR, specific growth rate; FCR, feed conversion ratio (on dry matter basis).
Means lacking a common superscript letter within a row differ significantly.
ns, not significant, $P > 0.1$; #, tendency, $P < 0.10$; **, $P < 0.01$; ***, $P < 0.001$.

Table 4

Apparent digestibility coefficient (ADC) of nutrients in ingredients fed to rainbow trout till apparent satiation during the experimental period (42 days). Values are means (n = 3) and standard error of the mean (SEM).

	Test ingredients								
ADC (%)	FM	MM	PM-W	PM-D	IM	SCP	BGP	SEM	*P*-value
Dry matter	85.8^{cd}	94.3^{e}	84.4^{cd}	87.3^{d}	78.0^{ab}	81.0^{bc}	73.9^{a}	1.15	***
Crude protein	90.0^{c}	93.4^{d}	87.9^{abc}	88.1^{abc}	86.0^{a}	87.1^{ab}	88.6^{bc}	0.43	***
Crude fat	85.7^{abc}	91.7b^{c}	79.1^{ab}	92.2b^{c}	97.3^{c}	73.8^{a}	93.8^{bc}	4.54	**
Total carbohydrate	(−642.4)^{x}	112.4^{c}	(422)^{x}	(407.1)^{x}	52.8^{a}	74.7^{b}	39.1^{a}	3.80	***
Starch + sugars	(587.0)^{x}	165.3	(495)^{x}	(466.5)^{x}	138.9	(279.3)^{x}	205.0	23.47	ns
NSP	(85.1)^{x}	7.7^{b}	(122.7)^{x}	(544.3)^{x}	33.5^{c}	-7.5^{a}	9.4b	3.20	***
Ash	93.8^{b}	93.4^{b}	91.2^{b}	93.7^{b}	83.0^{a}	83.4^{a}	80.3^{a}	1.50	***
Phosphorus	28.9^{ab}	56.2^{b}	26.2^{ab}	32.2^{b}	46.8^{b}	40.9^{b}	−0.6^{a}	6.72	**
Calcium	29.1^{a}	80.3^{bc}	37.3^{a}	38.9^{a}	71.2^{b}	70.3^{b}	89.1^{c}	2.35	***
Magnesium	3.6^{a}	10.6^{ab}	4.3^{a}	8.8^{ab}	8.3^{ab}	(−85.9)^{x}	69.7^{b}	12.97	*
Energy	75.6^{ab}	102.6^{c}	104.1^{c}	87.4^{bc}	56.0^{b}	27.2^{a}	104.7^{c}	4.49	***
Essential amino acids									
Arginine	97.7^{d}	97.0^{d}	93.5^{b}	91.1^{a}	96.2^{cd}	96.3^{cd}	94.4^{bc}	0.40	***
Histidine	94.6^{b}	94.2^{b}	91.7^{a}	92.3^{ab}	93.8^{ab}	92.8^{ab}	91.6^{a}	0.52	**
Isoleucine	92.4^{bc}	96.1^{d}	87.8^{a}	89.1^{ab}	93.5^{cd}	90.9^{abc}	91.5^{bc}	0.74	***
Leucine	93.7^{c}	96.7^{d}	89.1^{a}	90.2^{ab}	94.4^{cd}	92.2^{bc}	92.4^{bc}	0.61	***
Lysine	92.1b^{c}	96.8^{d}	91.2^{b}	91.2^{b}	93.7^{c}	91.6^{bc}	87.6^{a}	0.49	***
Methionine	93.6^{ab}	97.2^{c}	93.3^{ab}	92.3^{a}	95.0^{bc}	92.6^{a}	92.7^{ab}	0.48	***
Phenylalanine	93.9^{cd}	95.6^{d}	87.1^{a}	89.4^{ab}	94.4^{d}	91.0^{bc}	95.7^{d}	0.62	***
Threonine	93.9^{cd}	97.4^{d}	84.2^{a}	86.7^{ab}	92.9^{c}	92.3^{c}	88.4^{b}	0.75	***
Valine	92.3^{cd}	95.5^{d}	87.0^{a}	88.4^{ab}	92.1^{cd}	90.7^{bc}	90.4^{abc}	0.73	***
Non-essential amino acids									
Alanine	92.1^{bc}	94.6^{c}	89.7^{ab}	87.9^{a}	92.4^{bc}	90.6^{ab}	87.7^{a}	0.69	***
Aspartic acid	87.7^{abc}	95.9^{d}	83.5^{a}	84.4^{ab}	91.9^{cd}	89.1^{bc}	88.2^{abc}	0.06	***
Glutamic acid	92.6^{bc}	98.0^{d}	88.7^{a}	89.2^{a}	94.2^{c}	90.0^{ab}	93.9^{c}	0.56	***
Glycine	93.8^{b}	94.6^{b}	88.6^{a}	86.8^{a}	87.7^{a}	88.5^{a}	87.6^{a}	0.59	***
Proline	93.5^{cd}	96.2^{d}	87.1^{a}	86.4^{a}	93.1^{c}	90.3^{b}	94.4^{cd}	0.58	***
Serine	95.4^{de}	96.5^{e}	89.8^{a}	89.7^{a}	93.8^{cd}	92.4^{bc}	90.8^{ab}	0.51	***
Tyrosine	93.1^{bc}	94.8^{c}	87.4^{a}	87.5^{a}	93.2^{bc}	90.8^{ab}	93.1^{bc}	0.72	***
Sum of AA	93.0^{b}	96.3^{c}	88.5^{a}	88.5^{a}	93.1^{b}	91.1^{b}	92.1^{b}	0.52	***

Notes. FM, Fish meal; MM, Mussel meal; PM-W, Poultry meal wet rendered; PM-D, Poultry meal dry rendered; IM, Insect meal; SCP, Single cell protein; BGP, Brewers grain protein; NSP, non-starch polysaccharides; AA, Amino acids.

Means lacking a common superscript letter within a row differ significantly.

ns, not significant, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

^{X}ADC values between brackets are not included in statistical analysis since contribution of the respective nutrient from the test ingredient was <6% in the total diet.

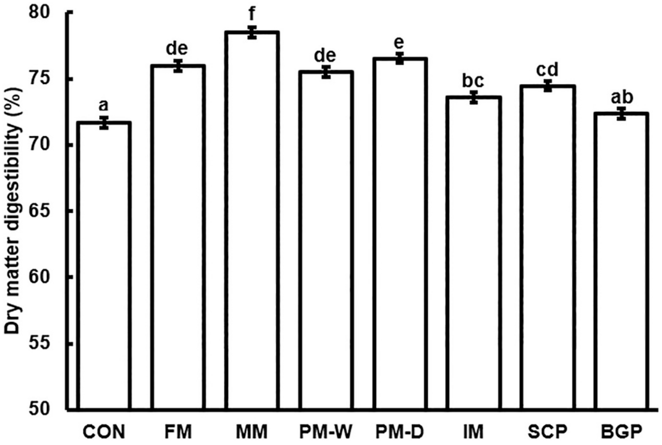

Fig. 1. Apparent digestibility coefficient (%) of dry matter (DM) in rainbow trout fed the experimental diets till apparent satiation during the experimental period (42 days).

Legend: DM ADC of diets expressed as %. CON, Control; FM, Fish meal; MM, Mussel meal; PM-W, Poultry meal wet rendered; PM-D, Poultry meal dry rendered; IM, Insect meal; SCP, Single cell protein; BGP, Brewers grain protein. Values are means ($n = 3$) and standard error of the mean (SEM). Different superscripts (lower case) labelled above the bars indicate dietary differences for dry matter digestibility ($P < 0.001$).

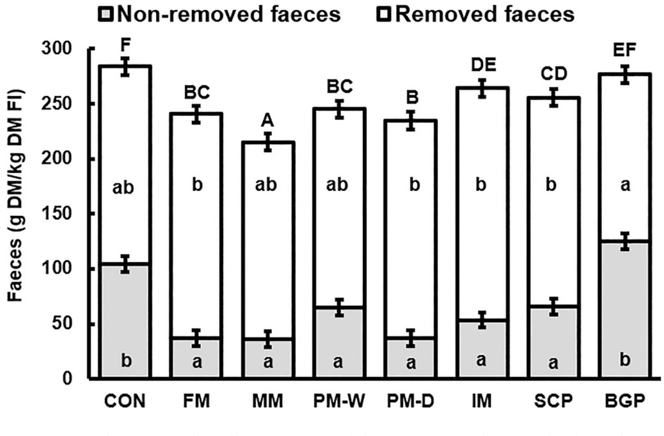

Fig. 2. Total, removed and non-removed faeces (g DM/kg DM feed intake) in rainbow trout fed the experimental diets till apparent satiation during the experimental period (42 days).

Legend: Total amount of faeces produced (entire bar), removed (white) and non-removed (grey) faeces per feed intake (g DM/kg DM feed intake). CON, Control; FM, Fish meal; MM, Mussel meal; PM-W, Poultry meal wet rendered; PM-D, Poultry meal dry rendered; IM, Insect meal; SCP, Single cell protein; BGP, Brewers grain protein. Values are means ($n = 3$) and standard error of the mean (SEM). Different superscripts (lower case) in white and grey bars indicate dietary differences for removed and non-removed faeces ($P < 0.001$). Different superscripts (upper case) labelled above the bars indicate dietary differences for total faeces produced ($P < 0.001$).

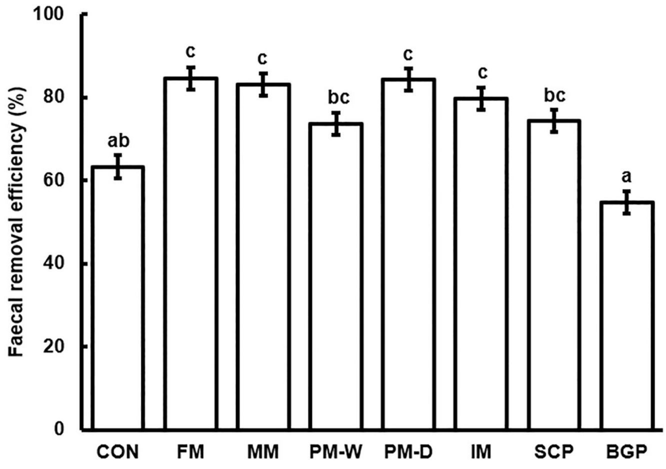

Fig. 3. Faecal removal efficiency (%) in rainbow trout fed the experimental diets till apparent satiation.
Legend: Faecal removal efficiency expressed as %. CON, Control; FM, Fish meal; MM, Mussel meal; PM-W, Poultry meal wet rendered; PM-D, Poultry meal dry rendered; IM, Insect meal; SCP, Single cell protein; BGP, Brewers grain protein. Values are means (n = 3) and standard error of the mean (SEM). Different superscripts (lower case) labelled above the bars indicate dietary differences for faecal removal efficiency (%) ($P < 0.001$).

0.001) was noted between the proportion of particles >40 μm and the faecal removal efficiency for both the non-stressed and the stressed faeces (Fig. 4). The correlation coefficient between particles >40 μm and faecal removal efficiency was almost similar in both cases ($r = 0.881$ for non-stressed faeces and $r = 0.883$ for stressed faeces) indicating that faecal waste from diets with a dominance of small-sized particles was also less stable. Correlation between particles of size >1600 μm and faecal removal efficiency was also significant ($P < 0.001$) for both stressed and non-stressed scenario (Supplementary Fig. 2).

4. Discussion

In this study, we set out to evaluate the impact of dietary ingredients of diverse origins on faecal waste production and characteristics in rainbow trout. Though a statistically significant difference was observed between the diets in FCR, ADC of nutrients in ingredients and diets, and a tendency for weight gain, these results are discussed briefly, since the focus of the study is on faecal waste production and its characteristics.

4.1. Ingredient digestibility and faecal waste production

Reducing waste generation and ensuring proper treatment is a major challenge to the sustainable expansion of the aquaculture industry (Naylor et al., 2021). Highly digestible feeds maximise production and reduce waste discharge from aquaculture production systems (Bureau and Hua, 2010). Digestibility of feed is a function of nutrient composition, which varies in response to the ingredients used in formulation (Glencross, 2020; Prabhu et al., 2019). In this study, digestibility of macronutrients and amino acids in ingredients estimated stands in line with previously reported digestibility values for IM (Gasco et al., 2022;

Table 5
Nutrient composition (g/kg DM) of faecal matter of rainbow trout fed the experimental diets till apparent satiation during the experimental period (42 days). Values are means ($n = 3$) and standard error of the mean (SEM).

	CON	FM	MM	PM-W	PM-D	IM	SCP	BGP	SEM	*P*-value
Crude protein	94^{a}	169^{d}	149^{bc}	186^{e}	190^{e}	161^{cd}	187^{e}	138^{b}	2.8	***
Crude fat	59^{ab}	66a^{b}	69^{b}	63^{ab}	61^{ab}	48^{a}	60^{ab}	51^{ab}	4.0	*
Total carbohydrate	653^{f}	450^{a}	570^{d}	469^{b}	467^{ab}	605^{e}	543^{c}	652^{f}	3.5	***
Starch + sugar	285^{d}	142^{a}	178^{bc}	147^{ab}	131^{ab}	180^{c}	145^{a}	146^{ab}	6.5	***
NSP	368^{b}	308^{a}	392^{bc}	322^{a}	336^{a}	425^{d}	399^{cd}	506^{e}	5.9	***
Ash	194^{b}	315^{e}	212^{c}	282^{d}	282^{d}	186^{b}	210^{c}	159^{a}	2.6	***

Notes. CON, Control; FM, Fish meal; MM, Mussel meal; PM-W, Poultry meal wet rendered; PM-D, Poultry meal dry rendered; IM, Insect meal; SCP, Single cell protein; BGP, Brewers grain protein; NSP, non-starch polysaccharides.
Means lacking a common superscript letter within a row differ significantly.
*, $P < 0.05$; ***, $P < 0.001$.

Table 6
Particle size distribution (PSD) of non-stressed faeces (no mechanical stress) and stressed faeces (with mechanical stress) obtained by filtration in mass percentage of rainbow trout fed the experimental diets till apparent satiation during the experimental period (42 days). Values are means ($n = 3$) and standard error of the mean (SEM).

	CON	FM	MM	PM-W	PM-D	IM	SCP	BGP	SEM	*P*-value
Non-stressed faeces										
< 40 μm	6.7^{a}	3.6^{a}	3.2^{a}	4.9^{a}	2.8^{a}	4.9^{a}	5.2^{a}	12.4^{b}	0.81	***
40–300 μm	25.3^{bc}	15.7^{ab}	9.1^{a}	16.4^{ab}	10.8^{a}	18.1^{ab}	23.2^{bc}	31.1^{c}	2.44	***
300–850 μm	25.6^{c}	14.8^{abc}	9.0^{a}	18.1^{abc}	13.1^{ab}	18.9^{abc}	21.9^{bc}	23.1^{bc}	2.53	**
850–1600 μm	16.3^{b}	8.0^{ab}	4.7^{a}	8.7^{ab}	6.1^{a}	11.0^{ab}	10.3^{ab}	8.5^{ab}	1.92	*
> 1600 μm	26.2^{a}	57.8^{bc}	74.1^{c}	51.8^{abc}	67.2^{bc}	47.1^{abc}	39.4^{ab}	25.0^{a}	6.34	***
Stressed faeces										
< 40 μm	14.2^{c}	5.8^{a}	6.6^{ab}	8.3^{ab}	8.0^{ab}	9.5^{ab}	10.2^{b}	20.2^{d}	0.75	***
40–300 μm	35.4^{cd}	15.7^{a}	19.1^{ab}	28.3^{bc}	24.4^{abc}	25.7^{abc}	33.6^{cd}	41.0^{d}	2.52	***
300–850 μm	27.9^{bc}	18.5^{a}	22.8^{abc}	25.5^{abc}	25.1^{abc}	23.9^{abc}	30.0^{c}	21.3^{ab}	1.75	**
850–1600 μm	17.3	12.7	16.8	17.0	15.4	17.1	16.5	11.7	2.84	ns
> 1600 μm	5.2^{a}	47.3^{c}	34.6^{bc}	21.0^{abc}	27.0^{abc}	23.8^{abc}	9.7^{ab}	5.7^{a}	5.50	***

Notes. CON, Control; FM, Fish meal; MM, Mussel meal; PM-W, Poultry meal wet rendered; PM-D, Poultry meal dry rendered; IM, Insect meal; SCP, Single cell protein; BGP, Brewers grain protein.
Means lacking a common superscript letter within a row differ significantly.
ns, not significant, $P > 0.05$; *, $P < 0.05$, **, $P < 0.01$; ***, $P < 0.001$.

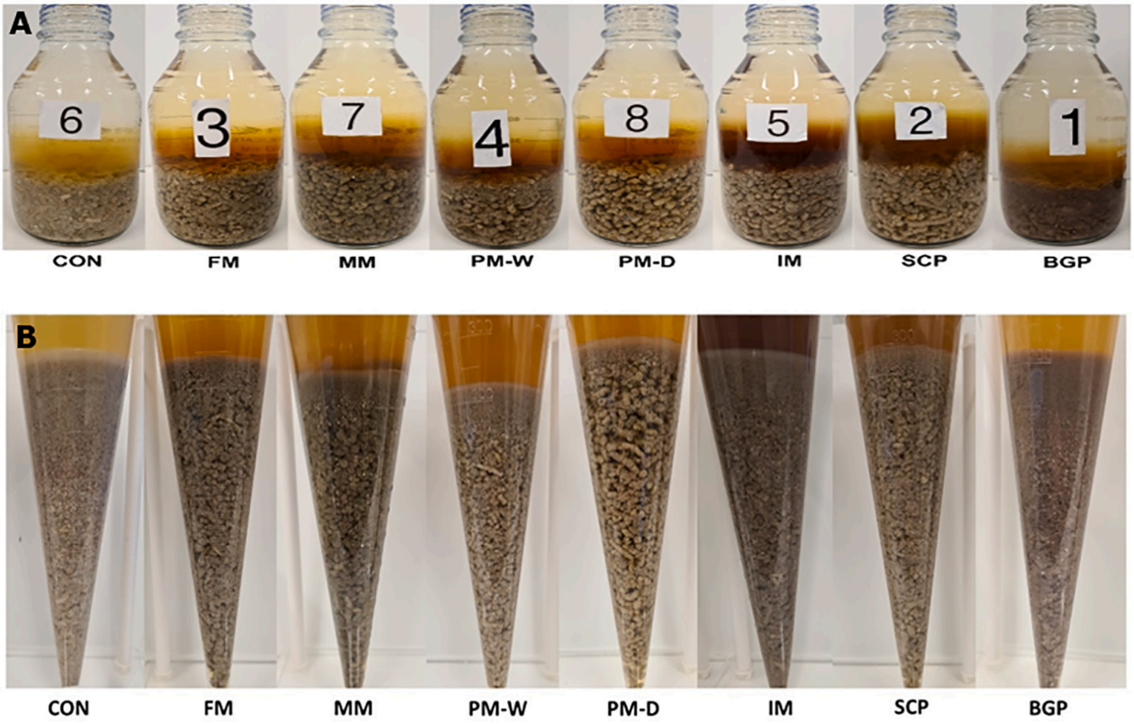

Plate 1. Appearance of rainbow trout faeces collected overnight in bottles (A) and after being transferred from the bottles to see-through Imhoff cones (B).
Legend: Each bottle/cone represents faecal waste collected from one treatment of each experimental diet. CON, Control; FM, Fish meal; MM, Mussel meal; PM-W, Poultry meal wet rendered; PM-D, Poultry meal dry rendered; IM, Insect meal; SCP, Single cell protein; BGP, Brewers grain protein.

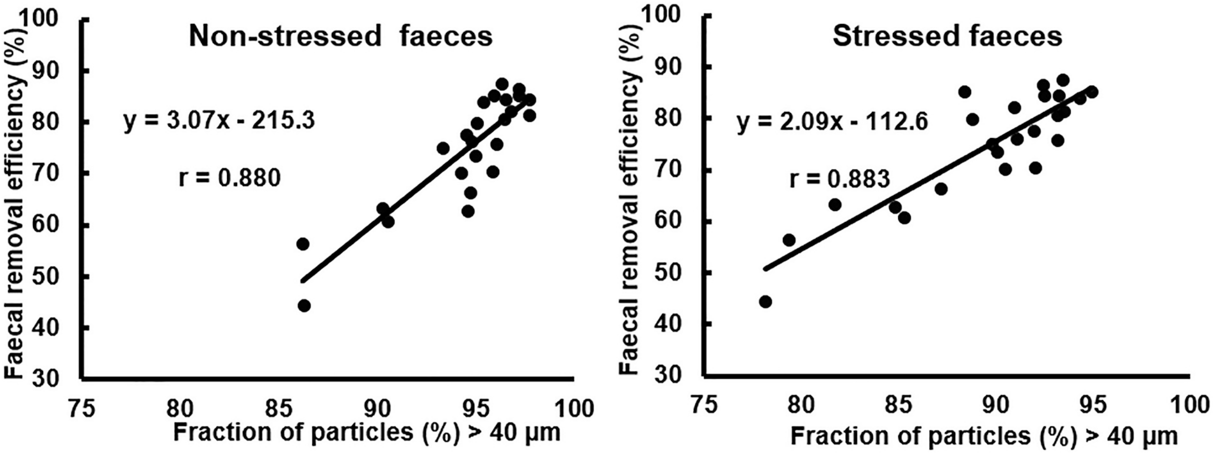

Fig. 4. Correlation between fraction of particles (%) in size >40 μm and faecal removal efficiency (%) for non-stressed and stressed faeces.
Legend: X axis represents the fraction of particles (%) > 40 μm, while Y axis represents the faecal removal efficiency (%) recorded for each experimental unit. Correlation was significant at $P < 0.001$.

Eggink et al., 2022), MM (Langeland et al., 2016), BGP (Zaretabar et al., 2021), PM (Bureau et al., 1999; Cheng and Hardy, 2002) and SCP (Aas et al., 2006; Rajesh et al., 2022) in rainbow trout or other closely related fish species. Low protein digestibility for insect meal, as observed in this study, could be ascribed to the ability of chitin to sequester proteins (Piccolo et al., 2017; Weththasinghe et al., 2022) or to reduce the activity of brush border proteases (Belghit et al., 2018). Similarly, low CP digestibility in SCP could be linked to the inability of digestive enzymes to penetrate the bacterial cell wall and membranes (Aas et al., 2006). The effect of variation in nutrient digestibility in response to dietary ingredient composition exhibited its effect on DM digestibility and consequently the amount of faecal waste produced varied with diets containing different test ingredients. The presence of high carbohydrate levels could explain the low DM digestibility in CON, BGP and IM diets. Unlike previous studies where >90% starch ADC has been reported for rainbow trout (Groot et al., 2021; Krogdahl et al., 2004; Staessen et al., 2020), digestibility of starch in this study was around 80%. Compared to the CON diet, decline in starch content of all the diets was observed due to dilution of the basal mixture with the test ingredients. This might explain the improvement in starch digestibility values and lowering of the waste produced for all the experimental diets compared to CON diet. As expected, NSP were not digested (except for in the case of IM diet) and thus excreted as faecal waste (Glencross et al., 2012; Groot et al., 2021). NSP in the case of IM occurs mainly in the form of chitin and its digestibility can be attributed to the presence of chitinase activity in rainbow trout based on earlier reports (Eggink et al., 2022; Lindsay et al., 1984). It is good to realise that, chitin is biologically and chemically distinct from the plant based NSP, however due to the methodological approach adopted to estimate NSP in this study, it is shown as part of NSP fraction. Additionally, the variability in inclusion levels, digestibility and absorption of other nutrients (protein, amnio acids, lipids and minerals) due to factors such as accessibility by digestive enzymes, interference with nutrient absorption processes, complex formation etc., might have influenced the overall DM digestibility, and consequently led to differences in the faecal waste production between diets.

Traditionally, diet-based strategies to reduce faecal waste production are aimed at improving the feed conversion ratio (Gatlin III et al., 2007). However, our study shows that this may not always be an effective strategy. Despite finding no difference in FCR values ($P > 0.1$) between PM-D and BGP, we observed 18% higher faecal waste production in BGP (277 g DM/kg DM FI) compared to PM-D (235 g DM/kg DM FI) fed fish. This deviation in the relationship between FCR and faecal waste production was influenced by the macronutrient composition of the diet and the variable utilisation efficiency of digestible energy (contributed by various macronutrients in the diet) for growth/maintenance (Groot et al., 2021; Schrama et al., 2018). Since the estimation of FCR also includes a metabolisation input (energy cost of metabolising nutrients), dry matter digestibility serves as a more precise metric for estimating faecal waste production than FCR.

4.2. Faecal particle size distribution

The PSD of faeces is an important factor determining waste removal in production systems by sedimentation and/or filtration. The high proportion of small-sized faecal particles in CON and BGP diets (Table 6) in the current study is consistent with previous reports of reduced faecal quality in rainbow trout fed plant protein-based diets (Schumann et al., 2022; Welker et al., 2021). In line with our findings, these authors also report increased share of large-sized faecal particles with the inclusion of raw materials of animal origin, such as poultry meal, blood meal and fish meal in the diet. Small faecal particles by virtue of having large surface area-to-volume ratio have higher leaching potential than the larger particles, and therefore increase the load of dissolved organic matter in the system. Additionally, small-sized faecal particles increase bacterial load in the system by acting as a substrate for the multiplication of heterotrophic bacteria (Pedersen et al., 2017). Larger faecal particle size with ingredients of animal origin such as PM, MM, IM, SCP and MM are likely to be removed more efficiently (by settling/screen filtration) resulting in reduced faecal waste load on the system and also on the environment when discharged.

Notably, the non-removed portion of faecal waste has greater proportion of particles of size <40 μm. This information on faecal PSD is important for RAS as most commercial operations use drum filter screens of mesh size ≥40 μm. Consequently, particles <40 μm size will accumulate in the system and become a source of suspended and dissolved solid waste, when no foam fractionation or granular filters are used. This increase in the proportion of smaller size particles has practical implications such as increasing the frequency and volume of backwashing (when using drum filter), increasing operational costs for energy and instrumentation, and simultaneously reducing the performance of biofilters and UV systems. Further, data on faecal PSD generated in this study could be used as critical input in quantifying the impact of solid waste released via effluent water on the surrounding environment. Smaller-sized faecal particles will settle slowly and drift for longer distances, increasing the area over which the impact of solid waste from aquaculture operations would extend (Cromey et al., 2009; Magill et al., 2006).

The comparison of faecal PSD under stressed and non-stressed scenarios provided information on the cohesiveness of particles contained in faecal pellets and hence their ability to resist breakdown by shearing forces in tanks, pumps or pipes (McMillan et al., 2003). From the PSD data under stressed and non-stressed scenarios, we can infer that FM resulted in the most stable faecal pellets, while BGP and SCP resulted in relatively less stable faecal pellets. Lower faecal stability based on rheological measurements in diets of plant origin have also been reported earlier in rainbow trout (Brinker and Friedrich, 2012; Schumann et al., 2022). The exact reasons for differences in faeces stability is unknown, but some studies have suggested the possible role of water content and viscosity of faeces (Brinker and Friedrich, 2012; Bureau and Hua, 2010; Francis et al., 2001). Additionally, using different methods for processing various raw materials can bring changes in functional properties such as gelling strength and water-holding capacity of proteins (Ma et al., 2022) thereby affecting the cohesiveness of faecal pellets (Schumann et al., 2022). Difference between the proportion of faecal particles <40 μm was larger between the two extremes (FM, lowest and BGP, highest) in stressed than in non-stressed scenarios. This can be related to the fact that when faeces are exposed to mechanical stress, faeces of all size ranges can contribute to size class <40 μm, thereby amplifying the variability at this size class. Faeces breakdown might also happen in a practical setting when faeces are exposed to shearing force upon discharge as they travel from the fish tank to the solid removal units. A solution to this issue can lie in segregating larger faecal particles from smaller ones close to the culture tank and directing the waste stream dominated by small-sized faecal particles alone to the drum filter or other filtration devices.

4.3. Faecal removal efficiency

Overall, a high removal efficiency of ≥75% was recorded with all the diets. The high removal efficiency, as observed in this experiment, is in line with previous observations using settling tanks for rainbow trout (~75%; Meriac et al., 2014), common carp (~75%; Prabhu et al., 2019), tilapia (~70%, Amirkolaie et al., 2005) and European seabass (~70%, Fountoulaki et al., 2022). On the contrary, lower faecal removal rates were reported in the case of pangasius (~36%, Tran-Tu, 2019) and yellowtail kingfish (~38%, Horstmann et al., 2023), indicating species-specific differences in faecal characteristics. This has implications for the system design (level of stress to which faecal waste can be subjected) and the solid removal system (drum filter vs. settling or combination of both, additional installation of protein skimmer and/or granular filter) to be used, as the most optimal choice would differ with fish species. We found a lower faecal removal efficiency in the CON and BGP diets, compared to the FM, PM-D, MM and SCP diets. In accordance with our findings, the ingredient composition of the diets was reported to affect faecal removal efficiency in other fish species such as seabass (Fountoulaki et al., 2022), common carp (Prabhu et al., 2019), and pangasius (Tran-Tu, 2019). However, in the above-mentioned studies, the removal efficiency was largely tested with ingredients of plant origin with varying characteristics, such as type of NSP. Findings were contrasting in different fish species but were reported to be influenced by variations in the level and types of NSP between the dietary ingredients. A similar role of the NSP component in the diet is also possible in this study since the BGP diet, which had the highest dietary NSP content, also resulted in the lowest removal efficiency. Due to a lack of data on the removal efficiency with dietary ingredients of animal or microbial origin for rainbow trout or other fish species, a further comparison was not possible. Overall, our study suggested that alternate animal-based dietary ingredients (PM, MM, IM) respond similar to fish meal-based diet in their impact on faecal solid waste removal.

Since the settling of faecal waste in a swirl separator increases with an increase in size and density of faecal particles, it is expected that diets resulting in smaller-sized faecal waste will yield lower removal efficiency. This was evident with the low removal efficiency obtained in the case of CON and BGP diets which also had a greater proportion of particles <40 μm. This suggests that faecal PSD is a good predictor of removal efficiency by settling in the case of rainbow trout. Magill et al. (2006) also found a positive correlation between settling velocity and faecal particle size for European sea bass and gilthead sea bream (*Sparus aurata*). Substituting fish meal with plant protein sources in rainbow trout increased the proportion of small-sized particles in faeces (Brinker and Friedrich, 2012). A reduced faecal density and sinking velocity with an increase in soybean meal proportion in the rainbow trout diet has also been reported (Ogunkoya et al., 2006). However, in contrast to our findings, for Atlantic salmon and pangasius, the variation in settling velocity and removal efficiency could not be explained by differences in the PSD of faecal waste (Chen et al., 1999; Tran-Tu, 2019). This might be related to the fact that a pre-selected and hence non-representative sample was used to measure particle size or settling velocity in those studies. For example, Chen et al., 1999 used particles in size range of 4 to 6.8 mm for measuring the settling velocity, despite the fact the majority of the faeces is constituted by particles of size <4 mm. Overall, findings of our study indicate that settling units can be successfully used to remove a large share of faecal waste in the case of rainbow trout, and this efficiency would differ with the ingredient composition of the diet.

The chemical composition of the faeces in relation to the ingredient composition of the diet has been reported only in a few fish species such as common carp (Prabhu et al., 2019) and European seabass (Fountoulaki et al., 2022). Consistent with the observation in the studies mentioned above, we also observed that the composition of the faeces strongly varied in response to the ingredient composition of the diet. The faecal composition might affect removal efficiency by influencing the faecal density and PSD. In this study, the high ash content in faecal waste from FM, PM and MM might have positively influenced faecal removal efficiency by increasing the density of faecal pellets. On the contrary, the high NSP level in the diet and consequently in the faecal waste of BGP might be the possible reason for the low removal efficiency through its aforementioned impact on PSD. An increased supply of indigestible NSP to the posterior gut increases the substrate availability for fermentation by the gut microbiota. It was suggested that gas produced by microbial fermentation may get trapped in the faecal strand and negatively affect the binding, resulting in smaller-sized faecal particles (Amirkolaie et al., 2006). Further, reduced gut transit time with high insoluble NSP content of diet can impact the faecal quality by interfering with the water reabsorption process in the mid and hindgut (McRorie Jr, 2019). Additionally, NSP may induce intestinal inflammation and enhance gut permeability resulting in high water content in the faeces as reported in the case of largemouth seabass (Liu et al., 2022). Nevertheless, the relationship between the faecal removal efficiency and diet/faecal NSP content should be analysed with caution as evident by the high removal efficiency in the current study of the IM despite of having high NSP level. Thus, it is good to consider that the impact of diet/faecal waste NSP level on the removal efficiency in different fish species may differ depending on various factors such as the species, type of NSP (insoluble vs soluble), origin (plant-derived or animal-derived), viscosity and dosage etc. (Amirkolaie et al., 2005; Brinker, 2007; Fountoulaki et al., 2022; Prabhu et al., 2019; Tran-Tu et al., 2020).

The composition of the faecal waste, apart from impacting the faecal characteristics, also holds relevance in the context of the circular economy approach. The composition of the faeces, such as the proportion of bioavailable carbon and the level of ash, would determine its valorisation by using them as a substrate in a denitrification reactor (Meriac et al., 2014), for growing insect biomass (Schmitt et al., 2019), bio-gas production (Suhr et al., 2015) or as manure in agricultural farms (Radziemska et al., 2019).

In the present work, the low DM digestibility coupled with the low faecal removal efficiency resulted in a higher amount of non-removed faecal waste in CON and BGP-fed groups. This conforms with the previous findings in rainbow trout, where high suspended solid load was noted in systems receiving a grain-based diet compared to a fish meal-based diet (Davidson et al., 2013). Even though diets other than CON and BGP, produced significantly different amounts of faecal waste, the amount of non-removed faeces with those diets were similar. This indicates that differences in faecal removal efficiency can compensate for the variation in the quantity of faecal waste produced. Taken together, these findings highlight the importance of diet-induced changes in the digestibility, physical characteristics and chemical composition of faecal particles and their implications for solid waste management.

5. Conclusion

The ingredient composition of the diet altered the amount, removal efficiency and particle size distribution of the rainbow trout faecal waste. Further, the ingredient composition of the diet impacted the ability of the faecal waste to withstand mechanical stress. Thus, the dietary ingredient composition can be used to steer faecal waste production and its removal efficiency. Regarding the specific dietary ingredients tested in this study, BGP and CON resulted in higher faecal waste production and faecal pellets of smaller size and lower stability, increasing the quantity of non-removed faecal waste. Alternative dietary ingredients such as insect meal, mussel meal and poultry meal resulted in faecal waste of equally good characteristics as that of fish meal-based diet and hence can be considered as a suitable raw material source for diet formulations from system and waste management perspective in aquaculture. Single cell protein though resulted in relatively unstable faecal pellets, this did not reflect in the amount of non-removed faecal waste.

CRediT authorship contribution statement

Satya Prakash: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **Roel M. Maas:** Conceptualization, Methodology, Validation, Writing – review & editing. **Peter-Melvin M.M. Fransen:** Methodology, Formal analysis, Investigation. **Fotini Kokou:** Conceptualization, Writing – review & editing. **Johan W. Schrama:** Conceptualization, Methodology, Validation, Writing – review & editing, Supervision, Funding acquisition, Project administration. **Antony J. Prabhu Philip:** Conceptualization, Methodology, Validation, Writing – review & editing, Supervision, Funding acquisition, Project administration.

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.

Acknowledgement

The authors acknowledge the technical assistance given by the staff of the aquaculture research facility (in particular Menno ter Veld and Wian Nusselder). We would like to thank Ronald Booms, Samara Hutting, Tino Leffering and Peter Horstmann for their support during filtration and lab analysis. First author acknowledges the Indian Council of Agricultural Research, New Delhi, India for providing a PhD Scholarship under Netaji Subhas-ICAR International Fellowship 2019-2020. This study is a contribution to the strategic cooperation between Aquaculture and Fisheries Group, Wageningen University, The Netherlands and Feed and Nutrition group, Institute of Marine Research, Norway (grant #, 15699). Additional funding for this research was obtained from Protix B.V., Darling Ingredients International Rendering and Specialties B.V., and Duynie Group.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

org/10.1016/j.aquaculture.2023.739621.

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