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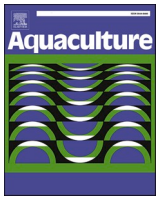

Effect of dietary protein source and ingredient grinding size on fish performance, faecal waste production and characteristics of yellowtail kingfish (*Seriola lalandi*) fed restrictively and to apparent satiation

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A R T I C L E I N F O

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A B S T R A C T

Recently, yellowtail kingfish (*Seriola lalandi*) is being cultured in recirculating aquaculture systems (RAS). Yellowtail kingfish have a poor faecal integrity, which makes the removal of faeces by traditional RAS technology difficult. Reducing the faecal waste load in RAS can be achieved by reducing the amount of faeces produced (e.g., increasing digestibility) and/or increasing the removal of faeces. This study assessed the effect of partial fish meal replacement by plant ingredients and the effect of ingredient grinding size on the amount of faecal waste produced and faecal characteristics, like faecal removal efficiency and particle size distribution (PSD), in yellowtail kingfish. This was investigated during two 35-d experiments, where fish were fed restrictively (experiment R) or to apparent satiation (experiment S). For each experiment, individual batches of four experimental diets were produced according to a 2 × 2 factorial design (protein source × ingredient grinding size). The formulas used were identical for both experiments. *FM100* diets contained only fish meal as protein source, whilst at *FM30-P70* diets approximately 70% of the fish meal were replaced by plant protein ingredients. The effect of ingredient grinding size was tested by including 40% of either a *fine* or *coarse* grinding mixture. Tanks were stocked with 20 fish and 27 fish for experiment R and experiment S, respectively. For each tank, fish performance, faecal waste production, faecal removal efficiency and faecal PSD were measured. During both experiments, ingredient grinding size did not affect the faecal removal efficiency or PSD, whilst fish fed the *fine FM30-P70* diets restrictively showed a lower faecal waste production. The inclusion of plant ingredients resulted in a lower absolute growth and higher FCR. Furthermore, fish fed the *FM30-P70* diets showed a higher faecal waste production, a smaller PSD and a lower faecal removal efficiency. This ultimately resulted in a higher amount of non-removed faeces by 58.3% and 37.1% compared to *FM100* diets for the experiment R and experiment S, respectively. In conclusion, the replacement of fish meal with plant ingredients in yellowtail kingfish diets is challenging due to the adverse effects on fish performance, faecal waste production and faecal characteristics. However, feeding yellowtail kingfish to apparent satiation partly reduced these adverse effects of plant ingredient inclusion in terms of faecal waste production and faecal characteristics. Reducing the ingredient grinding size of yellowtail kingfish diets tended to lower the faecal waste production, whilst not negatively affecting the fish performance or faecal characteristics.

1. Introduction

Yellowtail kingfish (*Seriola lalandi*) has gained attention due to its high market value and rapid growth rate (Miegel et al., 2010; Soriano et al., 2018). Yellowtail kingfish is predominantly cultured in sea cages in Spain, Mexico, Chile, Japan, Australia and New Zealand (Moran et al., 2009; Soriano et al., 2018). However, increasing efforts are being made to shift the cultivation of yellowtail kingfish in recirculating aquaculture systems (RAS) (EUMOFA, 2020). One of the advantages of using RAS is that waste water can be treated to (partly) recover the solid faecal waste

Abbreviations: NSP, non-starch polysaccharides; PSD, particle size distribution; TSS, total suspended solids..

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and remove/convert dissolved toxic waste products from the system and effluent water, allowing the reuse of water (Amirkolaie, 2011). However, the cultivation of yellowtail kingfish in RAS is challenging due to their poor faecal integrity, which is also described as 'diarrhoea-like'. The unstable faecal consistency and fine faecal particles make it difficult to remove the faecal material from the water, resulting in high concentrations of total suspended solids (TSS) in the system and effluent water (Moran et al., 2009). Due to the potential impact of TSS on animal health, system performance, operating costs and environmental eutrophication, the management of faecal waste is a key factor in the success of a RAS (Amirkolaie, 2011; Brinker et al., 2005; Brinker and Rösch, 2005; Chen et al., 1993; Fernandes and Tanner, 2008; Moran et al., 2009; Schumann et al., 2016; Unger and Brinker, 2013). In practice, waste management issues might be controlled by either lowering the amount of faecal waste excreted (by improved nutrient digestibility) or improving the faecal removal efficiency (by improved faeces integrity) (Amirkolaie, 2011; Bureau and Hua, 2010; Cho and Bureau, 1997; Kokou and Fountoulaki, 2018; Tran-Tu et al., 2018).

Seriola spp. feeds currently rely on the inclusion of fish meal (Candebat et al., 2020; Dam et al., 2019; Liu et al., 2019). Due to the increasing demand and stagnating supply of marine ingredients, a shift from marine-based to more plant-based diets took place over the last decades (Kissinger et al., 2016; Staessen et al., 2020a). For yellowtail kingfish, information on fish meal replacement by alternative ingredients is scarce. Studies with European seabass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*) reported an increased faecal waste production and reduced faecal integrity when substituting fish meal with plant protein sources (Brinker and Friedrich, 2012; Fountoulaki et al., 2022; Staessen et al., 2020a). These negative effects of plant ingredient inclusion on nutrient digestibility and thus faecal waste production were promoted when rainbow trout were fed to apparent satiation (Staessen et al., 2020a). Moreover, it was observed that in particular fat digestion was negatively affected under conditions of satiation feeding. Alterations in the digestibility of nutrients will result in changes in faecal composition, which could influence the faecal integrity (Moccia et al., 2007; Patterson and Watts, 2003; Refstie et al., 2005; Reid et al., 2009). This shows the importance of feeding level on faecal waste production and characteristics.

Another way to affect the faecal waste production and characteristics might be by altering the dietary physico-chemical properties such as ingredient grinding size (Callan et al., 2007; Kahlon et al., 2006; Staessen et al., 2020a; Tran-Tu et al., 2018). The amount of faecal waste can be altered by the ingredient grinding size as this affects nutrient digestibility (Tran-Tu et al., 2018). However, ingredient grinding size can also affect chyme viscosity (Tran-Tu et al., 2018), which may lead to altered faecal characteristics. Currently, information on the impact of ingredient grinding size, applied to produce RAS feeds, on the faecal waste production and characteristics is lacking.

This study investigated in yellowtail kingfish whether the protein source or ingredient grinding size affect the faecal waste production and characteristics, determined as faecal removal efficiency and particle size distribution (PSD). The effect of fish meal replacement was tested by formulating diets differing in their main protein source (PS): *FM100 / FM30-P70*. The effect of ingredient grinding size (GS) was investigated by diets altering partly in their ingredient grinding size: *fine / coarse*. This was obtained by using different screen sizes during milling (*fine* – 1 mm / *coarse* – 1.5 mm). To investigate the effect of feeding level, this was studied during two independent experiments: *restricted / satiation feeding*.

2. Materials and methods

2.1. Diets

The effect of protein source and ingredient grinding size on faecal waste production and characteristics of yellowtail kingfish was investigated during two experiments, where fish were fed restrictively (experiment R) or to apparent satiation (experiment S). Four experimental diets were formulated according to a 2 × 2 factorial design, with protein source and ingredient grinding size as factors. For each experiment, separate batches of these experimental diets were produced. The effect of the first factor, protein source, was tested by formulating diets which differed in their main protein source by replacing approximately 70% of the fishmeal by plant protein ingredients. *FM100* diets contained only fish meal as protein source (68.43%), whilst at *FM30-P70* diets approximately 70% of the fish meal was replaced by equal amounts of wheat gluten, pea protein concentrate and soy protein concentrate (Table 1). *FM30-P70* diets were supplemented with DL-methionine and taurine to fulfil the nutrient requirements regarding amino acids. Furthermore, monocalcium phosphate was added to the *FM30-P70* diets to ensure that phosphorus was not a limiting factor for growth. In all diets, a minimum of 9.5% fish oil was present to fulfil the requirements for essential fatty acids. Additional 1.78% fish oil was added to the *FM30-P70* diets in order to achieve a fat content equal to the *FM100* diets. The diet composition among experiments was similar (Table 1). The effect of the second factor, ingredient grinding size, was tested by including 40% of either a *fine* or *coarse* grinding mixture. This grinding mixture consisted of 50% fish meal and 50% wheat which were ground using either a 1 mm (*fine*) or a 1.5 mm screen (*coarse*). This procedure was applied because fine grinding of fish meal is challenging due to its fat content. The rest of each diet was ground using a 1.5 mm screen. Grinding was done by a hammermill (LHM20/16, 1.5 kW; Condux International, Mankato, United States of America). The analysed nutrient composition is given in (Table 2) and particle size distribution of diet mixture prior to extrusion and physical pellet characteristics in Table 3. The contrast in particle size distribution of the diet mixtures between the

Table 1
Diet composition of FM100 and FM30-P70 diets fed during the restricted and satiation experiment.

Protein source	FM100	FM30-P70
Ingredients (g/kg)		
Fish meal LT[a]	484.3	–
Wheat gluten[b]	–	150.0
Pea protein concentrate[c]	–	150.0
Soy protein concentrate[d]	–	150.0
Fish oil[e]	95.0	112.8
Monocalcium phosphate	–	10.0
DL-methionine	–	4.0
Taurine	5.5	8.0
Premix[f]	15.0	15.0
Yttrium oxide	0.2	0.2
Grinding mixture[g]		
Wheat	200.0	200.0
Fishmeal[a]	200.0	200.0

[a] Faroese Fish meal, minimally 71% CP LT (Köster Marine Proteins GmbH, Hamburg, Germany).
[b] Amygluten (Tereos Starch & Sweeteners, Aalst, Belgium).
[c] Pisane F0 (Cosucra, Warcoing, Belgium).
[d] Soycomil R (ADM Speciality Ingredients B.V., Amsterdam, The Netherlands).
[e] Fish oil (BioCeval GmbH & Co. KG, Cuxhaven, Germany).
[f] Premix composition. Vitamins (IU or mg/kg complete diet): Vitamin B1–15 mg; Vitamin B2–15 mg; Vitamin B6–15 mg; Vitamin B5–50 mg; Vitamin B3–150 mg; Biotine – 0.7 mg; B-12–0.05 mg; Folic acid – 3 mg; Vitamin C – 500 mg (given as ascorbic acid C, phosphate); Vitamin E – 100 IU; A-vitamin A palmitate – 10,000 IU; D-Rovimix D3–500–2500 IU; K_3 K-menadione sodium bisulphite (51%) – 15 mg; Inositol – 450 mg; Betaine – 500 mg; Choline (given as choline chloride) – 1000 mg; Anti-oxidant BHT (E300–321) – 100 mg; Calcium propionate – 1000 mg. Minerals (mg/kg complete diet); Ferric sulphate – 50 mg; Zinc sulphate – 80 mg; Cobalt sulphate – 0.2 mg; Copper sulphate – 8 mg; Sodium selenite – 0.2 mg; Manganese sulphate – 30 mg; Magnesium sulphate – 750 mg; Chromic chloride – 1 mg; Calcium iodate – 2 mg.
[g] Grinding mixture was grinded at 1 mm for *fine* diets and at 1.5 mm for *coarse* diets.

Table 2
Analysed nutrient content of the experimental diets.

Feeding level	Restricted feeding				Satiation feeding			
Protein source	FM100		FM30-P70		FM100		FM30-P70	
Ingredient grinding size	Fine	Coarse	Fine	Coarse	Fine	Coarse	Fine	Coarse
Analysed nutrient content (g/kg DM)								
Dry matter (DM, g/kg)	940.8	952.2	953.8	958.6	964.0	968.2	975.1	954.9
Crude protein	551.2	549.3	541.6	538.2	550.5	553.2	546.2	545.6
Crude fat	169.3	166.9	168.0	170.1	163.4	160.6	158.1	163.9
Total carbohydrates[a]	155.8	159.1	220.2	223.4	159.6	159.6	226.0	220.2
Starch and sugars	138.7	140.7	156.2	161.7	143.7	145.6	159.0	156.0
NSP[b]	17.0	18.4	64.0	61.7	16.0	14.0	67.1	64.2
Gross energy (kJ/g DM)	22.7	22.2	23.1	23.2	22.2	22.2	22.9	23.0
Ash	123.7	124.7	70.2	68.3	126.5	126.7	69.6	70.3
Phosphorus	16.8	16.8	11.0	10.7	17.7	18.4	11.2	11.6
Calcium	25.9	25.7	10.7	10.4	26.5	26.9	10.8	10.6

[a] Total carbohydrates content (on DM basis) was calculated as: 1000 – (crude protein + crude fat + ash).
[b] Non-starch polysaccharides (NSP) content (on DM basis) was calculated as: Total carbohydrates – (starch + sugars).

Table 3
Physical pellet characteristics and dietary particle size distribution (PSD, %; prior to extrusion) of the experimental diets.

Feeding level	Restricted feeding				Satiation feeding			
Protein source	FM100		FM30-P70		FM100		FM30-P70	
Ingredient grinding size	Fine	Coarse	Fine	Coarse	Fine	Coarse	Fine	Coarse
Physical pellet characteristics								
Hardness (kg)	5.4	5.1	6.1	5.6	5.4	6.5	6.0	6.2
Durability (%)[a]	0.1	0.1	na	0.1	0.1	0.1	0.1	0.1
Bulk density (g/L)	571	561	na	571	613	622	569	636
Gelatinization degree (%)	87.9	82.5	88.6	81.6	81.3	81.3	75.1	73.7
Dietary particle size distribution[b]								
< 40 μm	0.4	0.2	5.4	5.2	0.4	0.4	4.1	4.1
40–80 μm	8.9	5.1	38.8	34.9	4.9	1.1	37.7	33.7
80–150 μm	36.4	35.3	29.6	28.4	25.8	24.1	34.1	32.3
150–250 μm	27.3	30.3	9.2	12.2	49.8	50.7	10.7	11.6
250–315 μm	5.7	5.7	3.4	3.4	5.6	5.6	3.1	3.1
314–425 μm	7.2	6.9	4.6	4.2	6.5	5.9	3.9	3.2
425–630 μm	10.4	10.2	8.0	7.8	6.6	6.3	6.0	5.6
> 630 μm	3.7	6.3	1.1	3.9	0.3	6.0	0.4	6.3

[a] Durability expressed as feed fines (%).
[b] Calculated as: $I\%_{FM} \times P\%_{x\ \mu m} + I\%_{wheat\ gluten} \times P\%_{x\ \mu m} + I\%_{pea\ protein} \times P\%_{x\ \mu m} + I\%_{soy\ protein} \times P\%_{x\ \mu m} + I\%_{grinding\ mixture\ fine} \times P\%_{x\ \mu m} + I\%_{gringind\ mixture\ coarse} \times P\%_{x\ \mu m}$, where I% is the inclusion of each ingredient within the diet and P% the fraction (in %) of particles within each of the fractions (e.g. x – 40 μm).

fine and *coarse* diets were larger in the experiment S compared to the experiment R (Table 3).

The diets were produced for each experiment individually (different batches) 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. After extrusion, the pellets were dried for 3 h (70 °C) and afterwards cooled to room temperature. After cooling, part of the oil (80 g/kg) in the formula was added to the experimental diets by vacuum coating (Vacuum core coater, Pegasus®-10VC, ¼ H/VV nozzle nr. 6502) at the Animal Science Group (Wageningen University and Research, Wageningen, The Netherlands). Diets were produced approximately one week prior to the start of the experiments.

2.2. Fish, rearing conditions and housing facilities

The experiments were 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 these experiments as non-invasive. Fish were kept and handled in agreement with EU-legislation. Yellowtail kingfish (*Seriola lalandi*) of mixed sex were obtained from a commercial fish farm (Kingfish Zeeland B.V., The Kingfish Company, Kats, The Netherlands). At the beginning and the end of the experiments, fish were batch weighted (Mettler-Toledo ICS429) to determine initial and final weight and growth. One day prior weighting, fish were starved. Per tank, 20 fish of 105 g and 27 fish of 39 g with were stocked for experiment R and experiment S, respectively. Tanks were connected to the same RAS (filled with artificial seawater), the latter consisting of a sump, settling tank, drum filter, protein skimmer, and trickling filter. The system's refreshment rate was adjusted to keep the NO_3-N concentration below 100 mg/L. The water flow over each tank was controlled (Magnetic-inductive flow sensor, SM 6000; ifm electroic, Essen, Germany) and kept constant at 7.0 ± 0.05 L/min. The outlet of each tank was connected to an individual swirl separator (column height 44 cm; diameter 24.5 cm; Aqua Optima AS, Pulford, United Kingdom) to quantify feed spillage after feeding and to collect faeces.

Water quality parameters were measured daily from the common outflow to ensure that the pre-set water quality parameters remained within optimum conditions for yellowtail kingfish. The pH (WTW Multi 3630 IDS - SenTix 940) was maintained within the range of 7.0 to 7.6, water temperatures were kept at 23.5 ± 0.3 °C and 23.5 ± 0.1 °C (WTW Multi 3630 IDS - FDO 925) and salinity at 35.2 ± 0.7 and 34.0 ± 1.3 ppm (WTW Multi 3630 IDS - TetraCon 925) during experiment R and S, respectively. During both experiments, the dissolved oxygen concentration in the outlet water was maintained at a level above 5.0 mg/L (WTW Multi 3630 IDS - FDO 925). Maximum allowable values for TAN (total ammonium nitrogen, NH_4-N and NH_3-N combined; Merck Aquamerck Colorimetric Ammonium test), NO_2-N (Merck Aquamerck Colorimetric Nitrite

test), NO_3-N concentrations (Merck MQuant Nitrate test strips) were < 2 mg/L, < 0.06 mg/L, < 1 mg/L, and < 100 mg/L, respectively. The photoperiod was set at 20 L:4D for the entire duration of both experiments. Light went on at 7:30 am and switched off at 3:30 am.

2.3. Experimental procedures and sampling

During both experiments, treatments were tested for 5-weeks (35 d) and randomly distributed over a total of 12 (experiment R – triplicate) and 16 tanks (experiment S – quadruplicate). During experiment R, fish were restrictively fed to maintain an equal amount of feed given per tank per day on dry matter (DM) basis for all treatments. The feeding level was set at 20 g/kg$^{0.8}$ BW/d which is approximately 80% of the predicated satiation level. Throughout the experiment, the daily amount of feed was gradually increased based on the average initial fish weight and the predicted daily growth assuming a FCR of 1 for all treatments. The daily amount of feed was divided into two equal portions, which were hand fed at 9:00 and 15:00 h. During experiment S, fish were fed twice a day at 9:00 and 15:00 h. Each feeding moment lasted maximally 1 h or was terminated earlier if fish stopped eating. During the first 3 days of each experiment, the feeding level gradually increased until the desired feeding level was reached. This allowed the fish to adapt to the diet. Fifteen minutes after feeding, the glass bottles attached to the swirl separators were checked for feed pellets to determine feed spillage. Mortality was checked twice a day before feeding.

Faeces for digestibility analysis were collected overnight for 5 days during week 5 by settling (Amirkolaie et al., 2005). Bottles, which were connected beneath the swirl separators, were submerged in ice water to minimize bacterial degradation of the sample. Faecal samples were pooled per tank and stored at −20 °C until further analysis. Faeces collection for determination of faeces removal efficiency was done at the end of the fourth week (experiment R) and end of the fifth week (experiment S). The collecting method was the same as for the faecal samples collected for digestibility purposes, expect that faecal material was collected continuous for 48 h (excluding feeding moments). Faeces collection for determination of faecal PSD was done twice weekly during the last two weeks of the experiment (3 h collection during the day after morning feeding). One sample per week was used for PSD analysis with a particle size analyser and one sample per week for PSD analysis by sieving. After collection, faeces were stored on ice until further analysis. Feed samples were taken by pooling 100 g per experimental diet per week.

2.4. Analysis

Faeces collected for digestibility and faeces removal efficiency were dried at 70 °C until constant weight (Staessen et al., 2020a). Thereafter, faeces were pooled per tank and ground (mixer mill, IKA A11 basic). Feed and faeces were analysed as described by Staessen et al. (2020a). For dry matter determination, faeces and feed were analysed gravimetrically by drying for 4 h at 103 °C until constant weight (ISO 6496, 1999). Ash was determined gravimetrically by combustion for 4 h at 550 °C in a muffle furnace (ISO 5984, 2002) until constant weight. The ash fraction was dissolved in concentrated sulphuric acid by autoclaving (121 °C, 20 min) to determine yttrium by ICP-AES (NEN 15510, 2007). Total nitrogen was determined according to Kjeldahl's method (ISO 5983-2, 2009); crude protein was calculated with a protein conversion factor of 6.25. Crude fat was determined gravimetrically using acid hydrolysis (Hydrotherm®, C. Gerhardt GmbH & Co. KG, Königswinter, Germany) followed by petroleum-ether extraction (Soxhlet method; ISO 6492, 1999). Total starch and gelatinized starch were analysed to determine the gelatinization degree of starch in the experimental diets (Nutrilab, Giessen, The Netherlands). Total starch was analysed enzymatically using amyloglucosidase after washing with 40% ethanol. Gelatinized starch was analysed according to the modified glucoamylase method described by Zhu et al. (2016). For digestibility calculations, starch content (including sugars) of pelleted diets and faeces was analysed as described above for total starch analysis, leaving out the ethanol washing step. Gross energy was measured using bomb calorimetry (C7000, IKA werke, IKA analysentechnik, Staufen, Germany).

PSD of the ingredient mixtures of both diets (prior to extrusion) was investigated by sieving a 50 g sub-sample through a stack of sieves (mesh sizes: 630 μm, 425 μm, 315 μm, 250 μm, 150 μm, 80 μm and 40 μm; 10 min sieving time, interval of 6 s, amplitude of 2 mm/'g'; Retsch, AS 200 control, Haan, Germany). Pellet hardness was tested using a hardness tester (KAHL Pellet Hardness Tester; AMANDUS KAHL GmbH & Co. KG, Hamburg, Germany). Durability (% feed fines) was determined by sieving a 200 g sub-sample through a sieve (1 mm mesh size; 2 min sieving time, interval of 6 s, amplitude of 2 mm/'g'; Retsch, AS 200 control, Haan, Germany). Bulk density was determined with a 1 L cylinder with slide, fall weight and filling cylinder (Biotechnion, Wageningen, The Netherlands).

Faecal PSD was analysed as a measure to determine faecal characteristics. Faecal PSD was determined by using a laser particle analyser (240 s time interval and 90% confidence interval; DIPA 2000, Donner Technologies, Or Akiva, Isreal). The particle size analyser was connected to a liquid flow controller (LFC) in combination with a mechanic stirrer (LFC-101; 150 ml/min flow speed; 20% stirrer speed, around 55 rpm). Prior to the application of the faecal material to the LFC, faeces were sieved using a screen size of 850 μm and the upper size was discarded. To correct for the upper size range of the particle size analyser (850 μm), the particle fraction above and below 850 μm was determined during the last two weeks by sieving. Therefore, collected faeces were shortly homogenized (200 rpm, 15 s, MR3000, Heidolph Instruments, Schwabach, Germany) and a sub-sample was applied to an 850 μm sieve. Both the filtrate (< 850 μm) and residue (> 850 μm) were individually collected with pre-weight 1.5 μm glass fibre filter (90 mm diameter, grade 696, VWR, Radnor, USA) using a vacuum pump. Filters were stored at −20 °C until further analysis. To determine the collected organic matter (OM) mass of the fractions < 850 μm and > 850 μm, filters were dried and combusted as described above.

2.5. Calculations and data analysis

Absolute growth (g) was calculated as the difference between the average individual initial (W_i) and final (W_f) body weight (BW; g). The absolute feed intake (FI_{abs}; g/d) was calculated as FI_{tot} / t, where FI_{tot} is the total feed intake (g DM) and t is the number of days during the experimental period. Feed intake per metabolic body weight (FI_{mbw}; g/kg$^{0.8}$/d) was calculated as FI / MBW, where MBW is the metabolic body weight (kg$^{0.8}$) which was calculated as $(W_G / 1000)^{0.8}$. The geometric mean BW (W_G; g) was calculated as $e^{((\ln Wt + \ln W0)/2)}$. Feed conversion ratio (FCR) was calculated on dry matter basis (g/g) as (FI × dmF / 1000) / (W_f – W_i), where dmF is the dry matter content of the feed (g/kg). Survival (%) was calculated as (N_f – N_i) × 100, where N_i is the number of fish at the beginning and N_f the final number of at the end of the experiment.

Apparent digestibility coefficient (ADC, %) of organic matter, crude protein, crude fat, carbohydrate, starch and gross energy were calculated according to Cheng and Hardy (2002) using yttrium as inert marker: ADC (%) = 100 × (1 – ((Y_{diet} / Y_{faeces}) × (N_{faeces} / N_{diet}))), where Y is the inert marker percentage of the diet or faeces and N is the nutrient percentage (or kJ/g gross energy) of the diet or faeces. Organic matter (g/kg DM) and total carbohydrates in feed and faeces were calculated as 1000 – ash and as 1000 – (crude protein + crude fat + ash), respectively.

Faecal waste production, faecal removal efficiency and non-removed faeces per feed intake were calculated according to Fountoulaki et al. (2022). Faecal waste production (g OM/kg FI) was determined on organic matter basis as the amount of non-digested feed per kg feed intake as (100% – ADC_{OM}) × 1000, where ADC_{OM} is the organic matter digestibility during week 5. Faeces removal efficiency (FR_{48h}, %) was calculated as the percentage of collected faeces by settling throughout 48 h continuous faeces collection in relation to the total amount of faecal

waste production. In detail, this was calculated as the amount of yttrium collected by settling ($Y_{recovered}$, g) in relation to the total amount of yttrium given via the fed (Y_{diet}, g) as $Y_{recovered} / Y_{diet} \times 100\%$. The non-removed faeces per feed intake (g OM/kg FI) was calculated as the difference between the total amount of faecal waste produced and the amount of faeces removed as $((100\% - FR_{48h}) \times (100\% - ADC_{OM})) \times 1000$, where FR_{48h} and ADC_{OM} is the faeces removal efficiency during the 48 h continuous faeces collection and ADC_{OM} the organic matter digestibility during week 5.

PSD data from the particle size analyser was obtained on volumetric basis in size classes of 1 μm (upper size class 850 μm). Data was converted into cumulative volume percentage. The upper size range was corrected by the percentage of particles greater than 850 μm. The fraction of particles > 850 μm was determined by sieving as described above according to Brinker et al. (2005).

2.6. Statistical analysis

Tanks were used as the experimental unit in all statistical analysis ($n = 12$ in experiment R; $n = 16$ in experiment S). Statistical analyses were done separately per experiment. A two-way ANOVA using a general linear model was used to investigate the effect of protein source and ingredient grinding size and their interaction. In the case of a significant interaction effect ($p < 0.05$), a Tukey HSD test (honest significant difference; 95% significance) was performed to compare treatment means. Statistical analyses were performed by using the statistical program IBM SPSS Statistics 27 (IBM, New York, United States of America).

3. Results

3.1. Experiment – Restricted feeding

Fish performance (Tables 4 and 5): Mean survival was high (97.9%) and did not differ between dietary treatments ($p > 0.05$). Initial body weight (105 g) was similar between treatments ($p > 0.05$). As intended during the restricted feeding experiment, the absolute feed intake (3.95 g DM/d) was equal among treatments. Differences were observed for final body weight, growth and FCR between the diets with different protein sources ($p < 0.05$). *FM100* diets resulted in a higher final BW and growth, and a lower FCR compared to *FM30-P70* diets.

Nutrient digestibility (Tables 6 and 7): Apparent digestibility coefficients (ADC, %) of organic matter, crude protein, crude fat, total

Table 4
Main effect of protein source on growth performance of yellowtail kingfish fed the experimental diets restrictively (3 replicates) and to apparent satiation (4 replicates) for 35 days.

	FM100	FM30-P70	SEM	PS
Restricted feeding				
Survival (%)	99	97	1.9	ns
Initial body weight (g)	105	105	0.7	ns
Final body weight (g)	276	258	0.2	***
FI_{abs} (g DM/fish/d)	4.0	4.0	0.001	–
FI_{mbw} (g DM/kg$^{0.8}$/d)	16.3	16.7	0.08	–
Growth (g/d)	4.9	4.4	0.07	***
FCR	0.81	0.91	0.013	***
Satiation feeding				
Survival (%)	100	100	0.4	ns
Initial body weight (g)	40	39	1.1	ns
Final body weight (g)	192	161	4.4	***
FI_{abs} (g DM/fish/d)	3.4	3.1	0.07	**
FI_{mbw} (g DM/kg$^{0.8}$/d)	24.0	23.5	0.20	*
Growth (g/d)	4.4	3.5	0.10	***
FCR	0.79	0.89	0.005	***

FI_{abs} – feed intake absolute; FI_{mbw} – feed intake metabolic body weight; FCR – feed conversion ratio (on DM basis); PS – protein source; GS – ingredient grinding size. Values are means and the standard error of the means (SEM); ns - not significant $p > 0.05$; * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$.

Table 5
Main effect of ingredient grinding size on growth performance of yellowtail kingfish fed the experimental diets restrictively (3 replicates) and to apparent satiation (4 replicates) for 35 days.

	Fine	Coarse	SEM	GS
Restricted feeding				
Survival (%)	97	99	1.9	ns
Initial body weight (g)	105	105	0.7	ns
Final body weight (g)	269	266	0.2	ns
FI_{abs} (g DM/fish/d)	4.0	4.0	0.001	–
FI_{mbw} (g DM/kg$^{0.8}$/d)	16.4	16.6	0.08	–
Growth (g/d)	4.7	4.6	0.07	ns
FCR	0.85	0.86	0.013	ns
Satiation feeding				
Survival (%)	100	100	0.4	ns
Initial body weight (g)	39	40	1.1	ns
Final body weight (g)	175	178	4.4	ns
FI_{abs} (g DM/fish/d)	3.2	3.3	0.07	ns
FI_{mbw} (g DM/kg$^{0.8}$/d)	23.8	23.7	0.20	ns
Growth (g/d)	3.9	4.0	0.10	ns
FCR	0.84	0.83	0.005	#

FI_{abs} – feed intake absolute; FI_{mbw} – feed intake metabolic body weight; FCR – feed conversion ratio (on DM basis); PS – protein source; GS – ingredient grinding size. Values are means and the standard error of the means (SEM); ns - not significant $p > 0.1$; # – tendency $p < 0.1$.

Table 6
Main effect of protein source on apparent digestibility coefficient (ADC, %) of yellowtail kingfish fed the experimental diets restrictively (3 replicates) and to apparent satiation (4 replicates) for 35 days.

	FM100	FM30-P70	SEM	PS
Restricted feeding				
Organic matter	84.7	73.8	0.97	***
Crude protein	93.2	90.7	0.54	**
Crude fat	90.3	74.4	1.27	***
Total carbohydrates	48.9	32.4	2.60	***
Starch and sugars	80.1	72.3	1.37	***
Energy	87.5	77.4	0.93	***
Satiation feeding				
Organic matter	82.1	75.9	0.68	***
Crude protein	91.6	92.0	0.32	ns
Crude fat	89.4	83.1	0.99	***
Total carbohydrates	41.5	31.1	1.92	***
Starch and sugars	79.5	68.6	1.14	***
Energy	84.8	79.6	0.55	***

PS – protein source; GS – ingredient grinding size. Values are means and the standard error of the means (SEM); ns - not significant $p > 0.05$; ** - $p < 0.01$; *** - $p < 0.001$.

Table 7
Main effect of ingredient grinding size on apparent digestibility coefficient (ADC, %) of yellowtail kingfish fed the experimental diets restrictively (3 replicates) and to apparent satiation (4 replicates) for 35 days.

	Fine	Coarse	SEM	GS
Restricted feeding				
Organic matter	80.3	78.2	0.97	#
Crude protein	92.1	91.7	0.54	ns
Crude fat	83.1	81.6	1.27	ns
Total carbohydrates	43.6	37.8	2.60	#
Starch and sugars	77.7	74.6	1.37	ns
Energy	83.2	81.7	0.93	ns
Satiation feeding				
Organic matter	79.7	78.3	0.68	#
Crude protein	91.8	91.9	0.32	ns
Crude fat	86.6	85.9	0.99	ns
Total carbohydrates	40.0	32.6	1.92	**
Starch and sugars	76.3	71.8	1.14	**
Energy	82.8	81.6	0.55	*

PS – protein source; GS – ingredient grinding size. Values are means and the standard error of the means (SEM); ns - not significant $p > 0.1$; # – tendency $p < 0.1$; * - $p < 0.05$; ** - $p < 0.01$.

carbohydrates, starch and energy were affected by the protein source ($p < 0.01$), being higher in fish fed the *FM100* diets than the *FM30-P70* diets. A tendency for a higher organic matter ($p = 0.064$) and total carbohydrate ADC ($p = 0.054$) was observed for *fine* diets in comparison to the *coarse* diets. In restrictively fed fish, ADC of all nutrients was unaffected by the interaction effect of protein source and ingredient grinding size (supplementary data).

Faecal waste production and characteristics: The total amount of faecal waste production, faecal removal efficiency and the amount of non-removed faeces were affected by dietary protein source ($p < 0.05$; Figs. 1, 2 and 3). The amount of faecal waste production of fish receiving the *FM100* diets (153.4 g OM/kg FI) was 41.4% lower compared to fish receiving the *FM30-P70* diets (261.9 g OM/kg FI; $p < 0.001$). *Fine* ingredient grinding showed a tendency ($p = 0.064$) for a reduced faecal waste production compared to *course* ingredient grinding. Fish fed the *FM100* diets had higher faeces removal efficiency of 45.4% compared to 23.4% for fish fed the *FM30-P70* diets ($p < 0.001$), which is a 94.5% higher removal efficiency at the *FM100* diets. No ingredient grinding effect, nor an interaction effect was observed on faeces removal efficiency ($p > 0.05$). Consequently, *FM100* diets showed a lower amount of non-removed faeces (83.8 g OM/kg FI) by 58.3% compared to the *FM30-P70* diets (201.2 g OM/kg FI). Faecal PSD was significantly ($p < 0.001$) affected by the protein source (Table 8, $p < 0.001$). Fish fed *FM30-P70* diets excreted larger amounts of small faecal particles compared to fish fed *FM100* diets ($p < 0.001$).

3.2. Experiment – Satiation feeding

Fish performance (Tables 4 and 5): Mean survival was high (99.8%) and did not differ between dietary treatments ($p > 0.05$). Initial body weight (39 g) was similar between treatments ($p > 0.05$). The feed intake was higher for fish fed the *FM100* diets compared to fish fed the *FM30-P70* diets ($p < 0.01$). Differences were observed for final body weight, growth and FCR between the diets with different protein sources ($p < 0.05$). *FM100* diets resulted in a higher final BW and growth, and a lower FCR compared to the *FM30-P70* diets. None of the performance parameters was affected by ingredient grinding size or interaction effect ($p > 0.05$).

Nutrient digestibility (Tables 6 and 7): Apparent digestibility coefficients (ADC, %) of organic matter, crude fat, total carbohydrates, starch and energy were affected by the dietary protein source ($p < 0.001$), being higher in fish fed the *FM100* diets than the *FM30-P70* diets. *Fine* ingredient grinding had a positive effect on the starch, energy, total carbohydrate ADC ($p < 0.05$) and tended to improve organic matter ADC ($p = 0.063$) compared to the *coarse* ingredient grinding. Only starch ADC was affected by the interaction effect ($p < 0.01$, supplementary data). *Fine* ingredient grinding resulted in an increased starch ADC for *FM100* diets, whilst ingredient grinding size did not affect the starch ADC of *FM30-P70* diets (supplementary data).

Faecal waste production and characteristics: The total amount of faecal waste production, faecal removal efficiency and the amount of non-removed faeces were affected by dietary protein source ($p < 0.05$; Figs. 1, 2 and 3). The amount of faecal waste production of fish receiving the *FM100* diets (179.3 g OM/kg FI) was 25.7% lower compared to fish receiving the *FM30-P70* diets (241.3 g OM/kg FI; $p < 0.001$). *Fine* ingredient grinding showed a tendency ($p = 0.063$) of a reduced faecal waste production compared to *coarse* ingredient grinding ($p < 0.1$). Fish fed the *FM100* diets had higher faeces removal efficiency of 46.9%

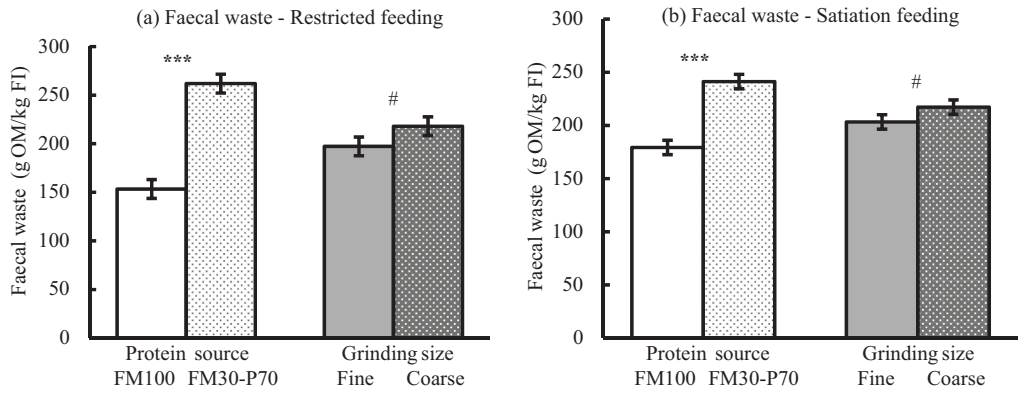

Fig. 1. Main effects of protein source and ingredient griding size on faecal waste per feed intake (g OM/kg feed intake) of yellowtail kingfish during (a) restricted feeding and (b) satiation feeding; OM - organic matter; FI - feed intake; error bars indicate standard error of means; # - tendency $p < 0.1$; *** - $p < 0.001$.

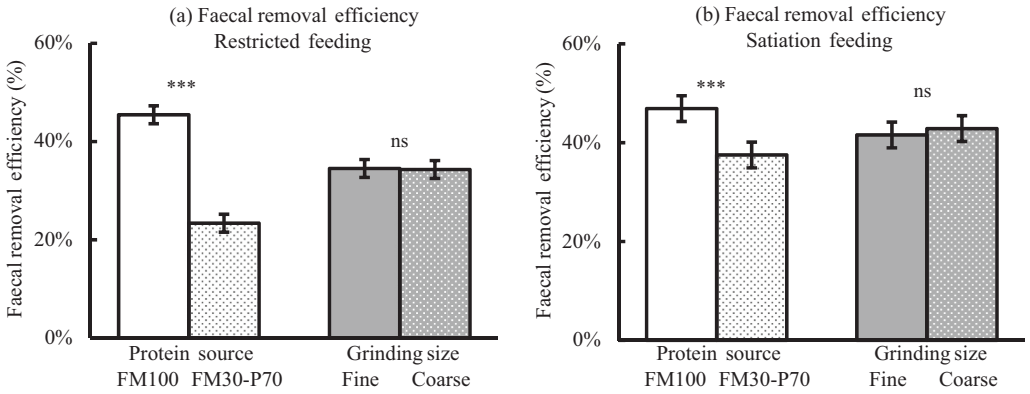

Fig. 2. Main effects of protein source and ingredient grinding size on faeces removal efficiency (%) of yellowtail kingfish during (a) restricted feeding and (b) satiation feeding; error bars indicate standard error of means; ns – not significant $p > 0.05$; *** – $p < 0.001$.

Table 8
Main effect of protein source on faecal particle size distribution (%, PSD) of yellowtail kingfish fed the experimental diets restrictively (3 replicates) and to apparent satiation (4 replicates) for 35 days.

	FM100	FM30-P70	SEM	PS
Restricted feeding				
< 40 μm	0.2	0.5	0.08	***
40–100 μm	2.7	5.2	0.25	***
100–250 μm	14.1	25.6	1.55	***
250–850 μm	19.0	30.0	0.93	***
> 850 μm	64.0	38.7	2.42	***
Satiation feeding				
< 40 μm	0.4	0.5	0.04	**
40–100 μm	5.3	6.2	0.41	#
100–250 μm	25.9	29.1	1.52	#
250–850 μm	32.4	34.9	1.58	ns
> 850 μm	36.0	29.3	3.30	#

Values are means and the standard error of the means (SEM); ns - not significant $p > 0.1$; # – tendency $p < 0.1$; ** - $p < 0.01$; *** - $p < 0.001$.

Table 9
Main effect of ingredient grinding size on faecal particle size distribution (%, PSD) of yellowtail kingfish fed the experimental diets restrictively (3 replicates) and to apparent satiation (4 replicates) for 35 days.

	Fine	Coarse	SEM	GS
Restricted feeding				
< 40 μm	0.3	0.3	0.08	ns
40–100 μm	3.9	3.9	0.25	ns
100–250 μm	20.0	19.7	1.55	ns
250–850 μm	24.8	24.2	0.93	ns
> 850 μm	50.9	51.9	2.42	ns
Satiation feeding				
< 40 μm	0.5	0.5	0.04	ns
40–100 μm	5.9	5.6	0.41	ns
100–250 μm	28.5	26.5	1.52	ns
250–850 μm	35.2	32.1	1.58	#
> 850 μm	30.0	35.3	3.30	ns

Values are means and the standard error of the means (SEM); ns - not significant $p > 0.1$; # – tendency $p < 0.1$.

compared to 37.5% for fish fed the *FM30-P70* diets ($p < 0.001$), which is a 25.0% higher removal efficiency at the *FM100* diets. Ingredient grinding size nor the interaction effect affected faeces removal efficiency ($p > 0.05$). Consequently, *FM100* diets (95.3 g OM/kg FI) resulted in a reduced amount of non-removed faeces by 37.1% compared to the *FM30-P70* diets (151.4 g OM/kg FI). Faecal PSD at the fraction < 40 μm was significantly (Table 8, $p < 0.01$) affected by the protein source. Compared to the other diets, a larger number of particles were observed in the fraction 250–850 μm for fish fed the *fine FM30-P70* diet (Table 9, PS × GS; $p < 0.05$).

4. Discussion

4.1. Fish performance and nutrient digestibility

In the current study, the effect of dietary protein source, ingredient grinding size and feeding level on fish performance, faecal waste production and characteristics of yellowtail kingfish were investigated. Diets were comparable in crude protein and fat content. Carbohydrate content, in particular non-starch polysaccharides (NSP), was higher in *FM30-P70* diets.

Plant ingredient inclusion resulted in reduced feed intake when fish were fed to apparent satiation. Similar results were observed in a study with rainbow trout by Staessen et al. (2020a). Literature suggests that feed intake is regulated by digestible energy intake (Houlihan et al., 2001; Jobling, 1983). This was not the case in the current study, as fish in the *FM30-P70* treatments had a lower digestible energy intake. It is likely that the lower feed intake of yellowtail kingfish, fed the *FM30-P70* diets, is related to the lower palatability of plant ingredients (Houlihan et al., 2001; Kokou and Fountoulaki, 2018; Sinha et al., 2011). Plant protein sources are generally inferior to fish meal in terms of palatability (Houlihan et al., 2001; Sinha et al., 2011) which can be related to the presence of antinutritional factors (ANF). ANF, such as saponins, tannins, protease inhibitors, lectins, phytates and NSP, can negatively affect feed intake (Galkanda-Arachchige et al., 2019; Houlihan et al., 2001; Kokou and Fountoulaki, 2018; Krogdahl et al., 2010; Sinha et al., 2011). For example, saponins which are present in soy and pea products, are known to have a bitter taste (Francis et al., 2001; Houlihan et al., 2001; Kokou and Fountoulaki, 2018; Krogdahl et al., 2010).

During both experiments, fat digestibilities of the *FM100* diets were comparable to those reported by Candebat et al. (2020) and Liu et al. (2019), whereas higher crude protein digestibilities were observed in the current study. These results contrast with the lower crude protein and fat digestibility observed by Dam et al. (2019), Pirozzi et al. (2019), and Booth and Pirozzi (2021). Factors that may allude to the differences in nutrient digestibility between studies are for instance; differences in faecal collection method, water temperature, and other environmental conditions (Amirkolaie et al., 2006; Dam et al., 2019; Pirozzi et al., 2019). In the current study, the inclusion of plant ingredients were associated with higher carbohydrate inclusion. This negatively affected nutrient digestibility, in particular fat digestibility, which is consistent with results in rainbow trout (Staessen et al., 2020a). However, the negative effect was greater in the current study with yellowtail kingfish than in the study with rainbow trout. This suggests that yellowtail kingfish are more sensitive to carbohydrates compared to other fish species (Booth et al., 2013; Maas et al., 2019; Staessen et al., 2020a). The overall negative effects of *FM30-P70* diets on nutrient digestibility are expected due to the higher intake of NSP. NSP are considered as

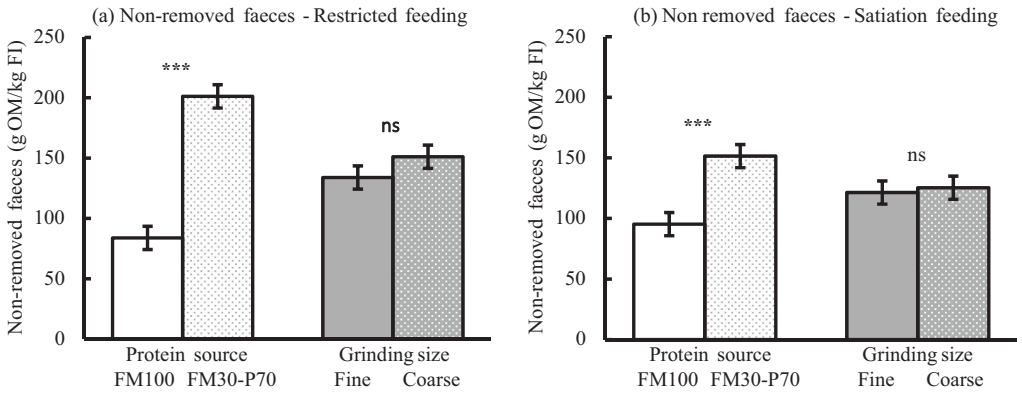

Fig. 3. Main effects of protein source and ingredient grinding size on non-removed faeces per feed intake (g OM/kg feed intake) during (a) restricted feeding and (b) satiation feeding; OM - organic matter; FI - feed intake; error bars indicate standard error of means; ns – not significant $p > 0.1$; *** - $p < 0.001$.

indigestible carbohydrates that can act as ANF (Maas et al., 2020a; Sinha et al., 2011). Literature shows that increasing levels of NSP negatively affect the digesta viscosity, resulting in a reduced interaction of enzymes with the substrate and adversely affect gut morphology and physiology (Maas et al., 2020b; Refstie et al., 1999; Sinha et al., 2011). Moreover, NSP are known to have the potential to bind bile acids in the gastrointestinal tract. Because NSP are largely indigestible by fish, this can result in an increasing faecal bile acid loss, as shown in rainbow trout (Staessen et al., 2020b). Fat digestion is largely dependent on bile acids, the loss of which is expected to result in the lower fat digestion for the *FM30-P70* diets (Kortner et al., 2013; Sinha et al., 2011; Staessen et al., 2020a, 2020b). Furthermore, *FM100* diets are expected to have contained greater amounts of bile acids and their precursors cholesterol and taurine (all present in fish meal), whereas they were absent in the plant ingredients (Kortner et al., 2013; Staessen et al., 2020a, 2020b).

Another factor that could have resulted in lower nutrient digestibility of fish receiving *FM30-P70* diets is the amount of undigested starch in the gastrointestinal tract. Fish fed *FM30-P70* diets had a greater starch intake and lower starch digestibility. According to the literature, starch can induce osmotic imbalances and fermentation processes in the gastrointestinal tract, which can affect nutrient digestion (Amirkolaie et al., 2006; Booth et al., 2013; Hung et al., 1990; Kokou and Fountoulaki, 2018; Refstie et al., 2005; Sinha et al., 2011; van Barneveld, 1999). Comparing the nutrient digestibilities of *FM100* diets among experiments, restrictive feeding resulted in higher nutrient digestibility. An increasing feeding level shortens the gut transit time (Bromley, 1994), which ultimately leads to lower nutrient digestibility (Hung et al., 1990; Miegel et al., 2010; Staessen et al., 2020a). However, contradictory results were observed for *FM30-P70* diets among experiments, where restrictive feeding resulted in a lower nutrient digestibility, especially for fat. This can be explained by the findings of Hung et al. (1990), who showed that disaccharides (breakdown products of starch) have a greater negative effect on osmolality and water retention in the distal intestine of white sturgeon (*Acipenser transmontanus*) compared to starch. Accordingly, it is expected that fish fed the *FM30-P70* diets restrictively had a slower gut transit rate, which in combination with the higher gelatinisation degree resulted in greater breakdown of starch into mono- and disaccharides, whereas this was not reflected in the starch digestibility data (starch ADC was analysed as starch, including sugars) (Hung et al., 1990; Miegel et al., 2010). In this case, the hypothesised greater breakdown of starch would be expected to increase the negative effects on nutrient digestibility of fish fed the *FM30-P70* diets restrictively, whereas this was not the case for *FM100* diets (Hung et al., 1990). This suggests that yellowtail kingfish are less tolerant of mono- and disaccharides compared to starch. However, since no clear evidence is presented, this remains only a hypothesis. Overall, it is hypothesised that the higher dietary carbohydrate intake, especially NSP, is responsible for the poorer nutrient digestibility of yellowtail kingfish when fed *FM30-P70* diets.

A positive trend for *fine* ingredient grinding on organic matter digestibility was observed during both experiments. Literature on the effect of ingredient grinding size on nutrient digestibility is conflicting (Callan et al., 2007; Moreira et al., 2009; Sveier et al., 1999; Tran-Tu et al., 2018; Zhu et al., 2001). Studies on pigs have shown a positive trend of *fine* ingredient grinding on nutrient digestibility (Callan et al., 2007; Moreira et al., 2009), whilst in studies on various fish species this trend was absent or reversed (Sveier et al., 1999; Tran-Tu et al., 2018; Zhu et al., 2001). The low contrast in grinding size (only 40% of the total diet was ground differently) could explain the absence of a significant effect for the factor ingredient grinding size. The positive trend of *fine* ingredient grinding on organic matter digestibility could be explained by a decrease in dietary viscosity and an increased particle surface area, which ultimately improves the mixing of the chyme and the effectiveness of endogenous enzymes (Callan et al., 2007; Sinha et al., 2011; Tran-Tu et al., 2018).

Besides the desired ingredient grinding size contrast among *fine* and *coarse* diets, an unintended ingredient grinding size contrast occurred within the factor protein source, as *FM30-P70* diets had a smaller ingredient grinding size compared to *FM100* diets. It could be that this unintended ingredient grinding size contrast positively affected the nutrient digestibility of the *FM30-P70* diets compared to the *FM100* diets. Since higher nutrient digestibilities were observed for the *FM100* diets, and the effect on nutrient digestibility was greater for the factor protein source than for the factor ingredient grinding size, this unintended ingredient grinding size contrast is unlikely to have had a decisive influence on the results.

4.2. Faecal waste production and characteristics

One of the challenges of farming yellowtail kingfish in RAS is controlling the amount of TSS in the system and discharge water. In practice, both the amount of faecal waste produced and faecal removal efficiency affect the amount of TSS (Amirkolaie, 2011; Bureau and Hua, 2010; Cho and Bureau, 1997; Kokou and Fountoulaki, 2018; Tran-Tu et al., 2018). Both *FM100* diets and *fine* ingredient grinding (tended to) had a positive effect on organic matter digestibility, although the effect being greater for the former. As faecal waste production follows the amount of non-digested feed (Kokou and Fountoulaki, 2018), the *FM100* and *fine* (Tendency) diets showed resulted in a lower amount of faecal waste production.

Another important step in waste management is the efficient removal of faecal waste. Faecal characteristics of yellowtail kingfish were assessed as faecal removal efficiency and PSD, both measurements being in line with each other. Regarding the effect of ingredient grinding size, it was hypothesised that *coarse* ingredient grinding would result in higher chyme viscosity, thus improving faecal integrity (Tran-Tu et al., 2018). In the current study, no effect of ingredient grinding size on faecal integrity was observed. In terms of protein source, average faeces removal efficiency over both experiments were 46.2% and 30.4% for *FM100* and *FM30-P70* diets, respectively. Thus, the inclusion of plant ingredients resulted in lower faecal integrity compared to *FM100* diets. This is in line with findings of Brinker and Friedrich (2012), who observed lower faeces integrity of rainbow trout when fish meal was entirely replaced by plant protein ingredients. However, in a study with European seabass, reduced faeces removal efficiency was only observed when fish meal was replaced with field peas or feather meal, whilst no effects were observed when fish meal was replaced with sunflower cake, wheat distillers grain, soy protein concentrate or corn gluten meal (Fountoulaki et al., 2022).

The lower faecal removal efficiency of *FM30-P70* diets can be explained by a treatment effect regarding the faecal waste composition (Patterson and Watts, 2003; Reid et al., 2009). Fish receiving the *FM30-P70* diets had higher carbohydrate intake and lower digestibility, resulting in a higher proportion of undigested carbohydrates in the gastrointestinal tract and ultimately in the faeces. Moccia et al. (2007) and Reid et al. (2009) concluded that faecal composition does not significantly influence faecal characteristics. Different findings were obtained by Patterson and Watts (2003), who found that the main source of non-removed faecal particles are derived from the indigestible cellulose fraction and gelatinized starches.

Another explanation could be the aforementioned effect of greater carbohydrate inclusion on osmolality and bacterial fermentation processes (Amirkolaie et al., 2006; Booth et al., 2013; Furuichi and Yone, 1981; Hung et al., 1990; Kokou and Fountoulaki, 2018; Refstie et al., 1999; Shimeno et al., 1977; Sinha et al., 2011; van Barneveld, 1999). Fermentation processes can lead to gas production, the entrapment of which in faeces can lead to poor faecal integrity (diarrhoea) (Hung et al., 1990; Kokou and Fountoulaki, 2018; Refstie et al., 1999; Sinha et al., 2011; van Barneveld, 1999). When comparing the experiments among each other, similar faecal removal efficiencies were observed for *FM100* diets. However, lower faeces removal efficiencies were observed in fish fed the *FM30-P70* diets restrictively than in fish fed the *FM30-P70* diets

to apparent satiation. This can be due to the aforementioned hypothesised greater presence of mono- and disaccharides for fish fed the *FM30-P70* diet restrictively (Hung et al., 1990; Miegel et al., 2010), which in turn has greater implications for faecal integrity (Hung et al., 1990). However, as literature is scarce on the effect of different carbohydrate types on faecal characteristics, in particular osmolality, water reabsorption and bacterial fermentation, this remains only as an observation. In summary, the inclusion of *plant* ingredients reduced faeces removal efficiency. This could be due to both differences in faecal composition as well as the negative effects of carbohydrates (especially mono- and disaccharides) of the *plant* ingredients. It might be worthwhile to investigate the effect of dietary carbohydrate inclusion and type, on faecal characteristics.

Both the amount of faecal waste production and faecal removal efficiency are important factors influencing waste management in RAS as they determine the amount of non-removed faeces (Amirkolaie, 2011; Bureau and Hua, 2010; Kokou and Fountoulaki, 2018; Tran-Tu et al., 2018). Ingredient grinding size did not affect the amount of non-removed faeces. However, the inclusion of plant protein ingredients negatively affected the amount of non-removed faeces. In particular, it became clear that the inclusion of plant ingredients increased the amount of non-removed faeces, due to both a lower nutrient digestibility and faecal removal efficiency. In practice, a higher amount of non-removed faeces would result in higher concentrations of TSS in the system water, potentially impairing animal health and system performance, whilst increasing the operation costs and ultimately contribute to environmental eutrophication when discharged into natural waters (Amirkolaie, 2011; Brinker et al., 2005; Brinker and Rösch, 2005; Chen et al., 1993; Fernandes and Tanner, 2008; Schumann et al., 2016; Unger and Brinker, 2013).

5. Conclusion

Replacing fish meal with plant ingredients in yellowtail kingfish diets remains a challenge due to the negative effects on fish performance, faecal waste production and faecal characteristics (faecal particle size distribution and faecal removal efficiency). However, when feeding yellowtail kingfish to apparent satiation, these negative effects of plant protein ingredients on faecal waste production and faecal characteristics were partially reduced. Reducing the ingredient grinding size of yellowtail kingfish diets showed a tendency towards reduced faecal waste production without affecting fish performance and faecal removal efficiency.

CRediT authorship contribution statement

Peter Horstmann: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **Roel M. Maas:** Conceptualization, Methodology, Data curation, Writing – review & editing. **Xander V. de Boer:** Investigation, Data curation, Writing – review & editing. **Theodorus M.B. de Jong:** Investigation, Data curation, Writing – review & editing. **Thomas W.O. Staessen:** Conceptualization, Writing – review & editing, Funding acquisition, Project administration. **Fotini Kokou:** Conceptualization, Writing – review & editing. **Johan W. Schrama:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2022.738875.

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