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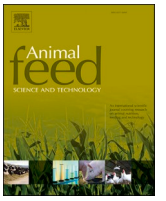

Faecal waste characteristics of yellowtail kingfish (*Seriola lalandi*) fed with pelleted and natural feed

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ABSTRACT

Yellowtail kingfish (*Seriola lalandi)* is a fast-growing fish species. One of the challenges of farming yellowtail kingfish in recirculating aquaculture systems is their poor faeces integrity, also referred to as 'diarrhoea-like' faeces. Whether diarrhoea-like faeces occur under conditions when feeding on its natural feed items or are diet induced, is unclear. This study assessed the effect of feed type (pelleted vs. natural feed) on the faecal characteristics and faecal waste production of yellowtail kingfish. Three dietary treatments were studied over a 35-d experimental period: a pelleted diet based on marine ingredients (*Marine*; open formula); an experimental pelleted feed based on the ingredient composition of a commercial kingfish feed (*Commercial Dummy*, *CD*; closed formula); and a diet composed of four, individually fed raw (unprocessed) natural ingredients and commercial dummy pellets at a ratio of 1:1 on dry matter basis (*Natural and Commercial Dummy, NCD*). The *NCD* treatment was intended to clarify whether diarrhoea-like faeces are naturally occurring in yellowtail kingfish. Each dietary treatment was tested in four tanks, which were stocked with 27 yellowtail kingfish (mean initial weight 39 g). Fish were fed to apparent satiation twice daily for 1 h. For each tank nutrient digestibility and faecal characteristics were measured. The inclusion of natural ingredients reduced the faecal waste production ($p < 0.001$). Furthermore, the faeces integrity of yellowtail kingfish fed with natural ingredients was not poor (not diarrhoea-like). At the natural treatment, fish excreted faecal pellets and short strings, which were not observed at the other treatments. Faecal waste collected from fish receiving only pelleted feed was classified as diarrhoea-like. The highest faeces removal efficiency by settling was observed at the *NCD* treatment compared to the other treatments ($p < 0.001$). Consequently, the lowest amount of non-removed faeces per feed intake ($p < 0.001$) was observed at the *NCD* treatment (62.9 g OM/kg OM FI), followed by the *Marine* (101.1 g OM/kg OM FI) and *Commercial Dummy* treatment (111.7 g OM/kg OM FI). In conclusion, this study shows the potential of dietary interventions to alter the amount and integrity of faecal waste. This offers possibilities to reduce the total suspended solid load for yellowtail kingfish farming in recirculating aquaculture systems.

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

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1. Introduction

Currently, land-based aquaculture is mainly dominated by freshwater species, but high value marine species are gaining importance (FAO, 2018). One of these marine fish species is yellowtail kingfish (*Seriola lalandi*), which has attracted attention due to its rapid growth rate and high market value (Miegel et al., 2010; Soriano et al., 2018). The yearly production of *Seriola spp.* has been around 150,000 tonnes for the past years (FAO, 2022). At global level, yellowtail kingfish is not a newly cultured fish species (Moran et al., 2009; Soriano et al., 2018), though it is relatively newly cultured in recirculating aquaculture systems (RAS) and in the Netherlands. Currently (in 2022), the production of yellowtail kingfish in the Netherlands in RAS is around 1500 tonnes and is projected to be 6000 tonnes in the coming years (EUMOFA, 2020). Commercial yellowtail kingfish farmers report that one of the challenges of farming yellowtail kingfish in RAS is the poor faeces integrity, also referred to as 'diarrhoea-like' faeces due to their instable consistency and fine faecal particles (Horstmann et al., 2023). This poor integrity makes it difficult to remove the solid faecal matter from the water, and results in high concentrations of total suspended solids (TSS) in the RAS and effluent water (Moran et al., 2009).

Removal of solids, which mainly originate from faeces and feed, is a key factor in the success of a RAS operation, because of its potential impact on water quality, animal health, system functioning, operation costs and environmental eutrophication (Amirkolaie, 2011). In commercial RAS operations, solid removal techniques are mostly based on sedimentation and/or filtration. While sedimentation efficiency is mainly determined by faecal particle size and density, filtration efficiency is mainly determined by the particle size and stability (Moccia et al., 2007; Timmons et al., 2018). When particles are too small to settle or to be captured by filtration, they

Table 1
Ingredient composition, analysed nutrient composition and physical pellet characteristics of the dietary treatments and natural feed ingredients.

	Treatment composition			Composition of natural feed ingredients			
	Marine Treatment	CD Treatment	NCD Treatment	Krill	Sand eel	Smelt	Squid
Ingredients	g/kg	g/kg	g/kg DM				
BioMar mix	-	999.8	499.9	-	-	-	-
Fish meal (LT)	684.3	-	-	-	-	-	-
Wheat	200.0	-	-	-	-	-	-
Fish oil	95.0	-	-	-	-	-	-
Taurine	10.0	-	-	-	-	-	-
Yttrium oxide	0.2	0.2	0.1	-	-	-	-
Premix[a]	15.0	-	-	-	-	-	-
Krill *(Euphausia superba)*			150.0	1000.0	-	-	-
Sand eel *(Ammodytes tobianus)*	-	-	100.0	-	1000.0	-	-
Smelt *(Atherina boyeri)*	-	-	100.0	-	-	1000.0	-
Squid *(Loligo patagonica)*	-	-	150.0	-	-	-	1000.0
Analysed nutrient content (g/kg DM)							
Dry matter (g/kg)	964	956	332[b]	158	197	221	203
Crude protein	550	588	670[b]	709	829	725	830
Crude fat	163	180	153[b]	154	71	162	79
Total carbohydrates[c]	160	149	85[b]	14	-16	20	8
Starch and sugars	144	97	53[b]	-	-	-	-
Non-starch polysaccharides[d]	16	52	32[b]	-	-	-	-
Gross energy (kJ/g DM)	22.2	23.3	23.1[b]	23.7	21.3	23.3	22.4
Crude ash	126	82	93[b]	123.0	115.6	93.4	83.5
Phosphorus	17.7	15.8	15.8[b]	13.8	21.4	17.3	12.9
Calcium	26.5	13.6	13.4[b]	15.5	19.5	19.2	1.5
Physical pellet characteristics[e]							
Hardness (kg)	5.4 ± 0.8	8.1 ± 1.1	-	-	-	-	-
Durability (g/kg)[f]	0.6 ± 0.1	0.5 ± 0.0	-	-	-	-	-
Bulk density (g/L)	616 ± 1	668 ± 1	-	-	-	-	-
Starch gelatinization degree (g/kg)	813 ± 22	709 ± 37	-	-	-	-	-
Water stability (g DM/kg DM)[g]	883 ± 0.5	845 ± 20.3	-	-	-	-	-

[a] Premix composition. Vitamins (IU or mg/kg 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 – 10000 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 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.

[b] The dry matter and nutrient content of the natural treatment was calculated as: $FI\%_{commercial} \times D_{commercial} + FI\%_{Krill} \times D_{Krill} + FI\%_{Sand\ eel} \times D_{Sand\ eel} + FI\%_{Smelt} \times D_{Smelt} + FI\%_{Squid} \times D_{Squid}$, where FI% is the relative feed intake (for DM in wet weight; for nutrients in DM) of each component of the natural treatment and D the dry matter or nutrient content of each ingredient.

[c] Total carbohydrates content (on DM basis) was calculated as: 1000 – (crude protein + crude fat + ash).

[d] Non-starch polysaccharides content (on DM basis) was calculated as: Total carbohydrates – (starch + sugars).

[e] Values are means ± standard deviations.

[f] Durability expressed as g fines/kg feed.

[g] Water stability expressed as g DM remained/kg DM feed after incubation in system water for 1 h

will remain suspended in the water column (Unger and Brinker, 2013). Thus, the particles might break down or even further dissolve due to water absorption, shear forces or biological degradation, hindering their removal (Brinker et al., 2005; Unger and Brinker, 2013). Waste management issues could be controlled by reducing the faecal quantity (through improved digestion) or improving the faecal characteristics (higher and faster removal rate) (Amirkolaie, 2011).

Previous research on different fish species such as European seabass (*Dicentrarchus labrax*), rainbow trout (*Oncorhynchus mykiss*), striped catfish (*Pangasianodon hypophthalmus*) or Nile tilapia (*Oreochromis niloticus*) has shown that dietary factors such as ingredient composition, addition of binders (e.g. guar gum) or feed technology based interventions (e.g. ingredient grinding size) can influence the faecal quantity and integrity (Amirkolaie et al., 2005; Brinker et al., 2005; Fountoulaki et al., 2022; Meriac et al., 2014; Tran-Tu et al., 2018). For yellowtail kingfish, little information is available on dietary factors influencing its faecal quantity and integrity. In addition, it is not known whether the poor faeces integrity observed under commercial farming conditions is also occurring under conditions when feeding on its natural feed items. Therefore, this study aimed to clarify whether diarrhoea-like faeces occur naturally in yellowtail kingfish. This was done by comparing faecal waste characteristics and production of yellowtail kingfish fed either a pelleted or a natural feed.

2. Materials and methods

2.1. Diets

The effects of feed type on faecal waste characteristics and production of yellowtail kingfish were studied using in total three dietary treatments. The first treatment (*Marine*) served as a positive control with an open ingredient formula. The second treatment (*Commercial Dummy, CD*) was a dummy yellowtail kingfish feed based on an ingredient mixture similar to the commercially available diet used in the production by Kingfish Zeeland (Kats, The Netherlands). This mixture was a closed formula, containing the following major bulk ingredients: fish meal, soy protein concentrate, fish oil, pea protein, horse bean, wheat gluten, maize gluten, wheat, and monocalcium phosphate. The *Marine* and *CD* diet were both extruded into a 3 mm pellet. The *CD* diet was intended to mimic faecal waste characteristics and production when feeding a commercially available diet. The third treatment (*Natural and Commercial Dummy, NCD*) was intended to clarify whether diarrhoea-like faeces occur naturally in yellowtail kingfish. At the *NCD* treatment, fish were fed natural feed ingredients and *commercial dummy* pellets at a ratio of 1:1 on dry matter basis. Instead of feeding only natural feed ingredients, this ratio was chosen to reduce the risk of nutritional deficiencies (e.g., vitamin B1, EPA or DHA). Furthermore, feeding partially commercial dummy pellets, which contained yttrium oxide (Y_2O_3), enabled the measurements of apparent digestibility and faecal removal efficiency at the *NCD* treatment. The natural feed ingredients were obtained frozen (Burgers' Zoo; Arnhem, The Netherlands), thawed daily, cut in approximately 0.5 – 1 cm pieces and fed raw (unprocessed) to the fish.

The ingredient composition, analysed nutrient composition and physical pellet characteristics of the dietary treatments and the analysed nutrient composition of the natural feed ingredients are shown in Table 1. The *Marine* diet contained as major ingredients of 684.3 g/kg fish meal, 95 g/kg fish oil and 200 g/kg wheat, which was required to form proper pellets by extrusion. The *CD* diet consisted of a confidential ingredient mixture aimed to have a major ingredient composition being representative for a commercial yellowtail kingfish diet. This mixture was provided by the BioMar Group (Aarhus, Denmark), supplemented with yttrium as inert digestibility marker (0.2 g/kg) and extruded similarly as the *Marine* diet. The composition of the natural ingredients was intended to simulate a representative natural diet of yellowtail kingfish on the basis of the results from gut content analysis of *Seriola spp.* and tuna (Andaloro and Pipitone, 1997; Fielder and Heasman, 2011; Kolkovski and Sakakura, 2007; Mourente and Tocher, 2009). Therefore, the natural ingredients consisted of 300 g/kg krill (*Euphausia superbar*), 200 g/kg sand eel (*Ammodytes tobianus*), 200 g/kg smelt (*Atherina boyeri*) and 300 g/kg squid (*Loligo patagonica*) on dry matter basis. Natural ingredients were obtained from Burgers' Zoo (Arnhem, The Netherlands) as frozen blocks and stored at − 20 °C. On a daily base, natural ingredients were cut (half) frozen in 0.5 – 1 cm pieces and thawed overnight at 4 °C. To avoid system pollution due to e.g. excess water from thawing or blood, the natural ingredients were washed and sieved (850 μm mesh size) prior to individual weighing (for more details, see supplementary data). Natural ingredients were fed individually and raw (unprocessed). The pelleted diets (only *Marine* and *CD*) 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. 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 *Marine* and *CD* diets by

Table 2
Dietary particle size distribution (PSD; g/kg) of the marine and commercial diet prior to extrusion.

	Marine Treatment	CD Treatment
Dietary particle size distribution (g/kg)		
< 40 μm	2	5
40 – 80 μm	2	4
80 – 150 μm	189	34
150 – 250 μm	592	612
250 – 315 μm	65	158
315 – 425 μm	75	108
425 – 630 μm	73	68
> 630 μm	2	12

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). This resulted in a dietary fat content of approximately 160 g/kg and 180 g/kg for the *Marine* and *CD* diet, respectively. Diets were produced approximately one week prior to start of the experiment and stored at 4 °C throughout the whole experimental period. It must be notified that *CD* diet was produced on an experimental scale and not in a commercial facility. Without the extrusion technique and commercial quality control performed in the commercial facility, the nutritional, physical and performance characteristics can, therefore, differ from the actual commercial product. The natural feed ingredients of the *NCD* treatment were stored at − 20 °C and required amounts were thawed each day overnight. The particle size distribution (PSD) of the dietary meals prior to extrusion is presented in Table 2.

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 experiment, fish were batch weighed (Mettler-Toledo ICS429) to determine initial and final weight and growth. Per tank, 27 fish with an average weight of 39 g were stocked. 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 count 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-pH 325) was maintained within the range of 7.0 – 7.6. Water temperature was kept at 23.5 ± 0.1 °C (Testo 110) and salinity at 34.0 ± 1.3 ppt (WTW-Multi 3430) during the experimental period. The dissolved oxygen concentration in the outlet water was maintained at a level above 5.0 mg/L (WTW-Oxi 340i). Maximum allowable values for TAN (total ammonium nitrogen; 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 20L:4D for the entire duration of the experiment.

2.3. Experimental procedures and sampling

Treatments were tested for 5 weeks (35-d) and randomly distributed in quadruplicate over a total of twelve tanks. During the first three days of the experiment, the feeding level gradually increased until apparent satiation was reached, which allowed fish to adapt to the diet. Fish were fed to apparent satiation for the remainder of the experiment. One day prior weighing, fish were starved. Feeding frequency was set at twice daily, starting at 9:00 h and 15:00 h. Fish were hand fed and the feeding duration was limited to 1 h per feeding moment. Fifteen minutes after feeding, the glass bottles attached to the swirl separators were checked for feed pellets to determine feed spillage. At the *Marine* and *CD* treatment, fish were fed to apparent satiation with the extruded pellets during the two feeding periods. At the *NCD* treatment, fish were first fed a calculated (on daily basis) amount of raw natural ingredients (krill, sand eel, smelt and squid, using a fixed ratio of 3:2:2:3 on dry matter basis as shown in Table 2). The natural ingredients were always fully finished by the fish (in about 5 min). After finishing the fixed amount of the natural ingredients, fish were fed to apparent satiation with the *CD* treatment pellets for the remaining feeding period. Feeding pellets after the natural ingredients allowed that feed intake could be quantified by counting spilled pellets. The amount of raw natural ingredients (on as is basis) to be provided for each day for each tank was calculated based on the realized total dry matter intake (natural ingredients + pellets) during the previous day for that respective tank. Therefore, the dry matter intake of each of the four natural ingredients and pelleted diet was calculated with the pre-measured dry matter values. At the *NCD* treatment, fish were fed only the CD pellets the first day. Over the next 10 days, the amount of natural ingredients was gradually increased until the targeted ratio of 1:1 on dry matter basis was reached. This was done to allow the fish to adapt to the natural feed ingredients. For more detailed information on the feeding protocol for the *NCD* treatment, please see supplementary data. Mortality was checked twice a day before feeding.

Faeces for digestibility analysis were collected overnight (16:30 h – 8:30 h) for 5 days during week 5 by settling. 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 fifth week. The collection method was the same as for the faecal samples collected for digestibility purposes, expect that faecal material was collected for two days (excluding feeding moments and feed spillage quantification). During these days, faeces were collected between 10:30 h and 15:00 h and between 16:30 h and 9:00 h. During feeding moments (9:00 h to 10:30 h and 15:30 h to 16:30 h), a different set of glass bottles was attached to the swirl separators to collect spilled feed pellets for feed intake quantification. Faeces collection for determination of faecal PSD was done twice weekly during the last two weeks of the experiment (between 10:30 h and 13:30 h collection during the day after morning feeding). One sample per week was used for PSD analysis with a particle size analyser (laser) 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, and these samples were stored at 4 °C. Feed samples of the natural ingredients were individually taken in the same manner and stored at − 20 °C until further analysis.

2.4. Analysis

Faeces collected for digestibility and faeces removal efficiency were dried at 70 °C. Faeces were pooled per tank and ground (mixer mill, IKA A11 basic). Natural ingredients were homogenized by grinding in a meat mincer (4.5 mm die; TW-R70, Feuma Gastromaschinen GmbH, Gößnitz, Germany). 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 as $N \times 6.25$. Crude fat was determined gravimetrically using acid hydrolysis in combination with 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 (without sugars) 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 (g fines/kg feed) 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/'gg; Retsch, AS 200 control, Haan, Germany). Bulk density was determined with a 1 litre cylinder with slide, fall weight and filling cylinder (Biotechnion, Wageningen, The Netherlands). Water stability (g DM remained/kg DM feed) was analysed according to Baeverfjord et al. (2006). Diet samples were weighed into 1 mm sieves. Sieves were placed in a moving water bath (24 °C, 75 rpm; SW23, JULABO GmbH, Seelbach, Germany) with system water (34 ppt) for 1 h. Afterwards, the diet samples were dried at 103 °C for DM quantification.

Faecal PSD was analysed as a measure to determine faeces 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, Israel). 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 fraction of particles greater than 850 μm, the particle fraction above and below 850 μm was determined during the last two weeks by manual 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. The sub-sampling was necessary to ensure that the representable 3 h faeces sampling does not result in excessive clogging of the filter material as 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) by 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). Specific growth rate (SGR; %/d) was calculated as $(\ln W_t - \ln W_0) / t \times 100\%$, where W_0 and W_t are the initial and final BW (g) for each feeding period respectively, and t is the number of days during the experimental period. Thermal growth coefficient (TGC) was calculated as $((W_t^{1/3} - W_0^{1/3}) / T \times t) \times 1000$, where T is the daily average water temperature and t is the number of days during the experimental period. The absolute feed intake (FI_{abs}; g/d) was calculated as FI_{tot} / t, where FI_{tot} is the total feed intake per fish (g DM or g WW). 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 $e^{((\ln Wt + \ln W0)/2)}$. Digestible energy intake (FI_{DE}; kJ/d) was calculated as $FI_{abs} \times N_{diet} \times ADC_E / 1000$, where N_{diet} is the energy content (kJ/g gross energy) of the diet and ADC_E the digestibility coefficient (g/kg) of energy. Feed conversion ratio (FCR) was calculated on dry matter basis (g/g) as (FI × dmF / 1000) / $(W_t - W_0)$, where dmF is the dry matter content of the feed (g/kg). Survival (%) was calculated as $(N_t - N_0) \times 100$, where N_0 is the number of fish at the beginning and N_t the final number of at the end of the experiment.

Apparent digestibility coefficient (ADC, g/kg) of organic matter, crude protein, crude fat, carbohydrate, starch + sugars and gross energy were calculated according to Cheng and Hardy (2002) using yttrium as inert marker; $ADC\ (g/kg) = 1000 \times (1 - ((Y_{diet} / Y_{faeces}) \times (N_{faeces} / N_{diet})))$, 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. The ingredient ADC (ADC_{ti}, g/kg) of the natural feed ingredients (combined) was calculated as $ADC_{nt} + (ADC_{nt} - ADC_{cd}) \times (I_{com} \times D_{com} \times DM_{com} / I_{ing} \times D_{ing} \times DM_{ing})$, where ADC_{nt} is the ADC of the *NCD* treatment (g/kg), ADC_{cd} is the ADC of the *CD* diet (g/kg), I_{com} and I_{ing} are the inclusion of the *CD* diet and natural ingredients in the *NCD* treatment, D_{com} is the nutrient percentage (or kJ/g gross energy) of the *CD* diet, D_{ing} is the nutrient percentage (or kJ/g gross energy) of the natural ingredients, and DM_{com} and DM_{ing} are the dry matter percentage of the *CD* diet and natural ingredients, respectively (Bureau and Hua, 2006). The calculated ingredient ADC values for the natural ingredients were made on the assumption that the amount of faeces originated from the natural feed ingredients and the *CD* pellets were identical. 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 OM FI) was determined on organic matter basis as the amount of non-digested feed per kilogram organic matter feed intake as (1000 – ADC_{OM}), where ADC_{OM} is the organic matter digestibility during week 5. Faeces removal efficiency (FR) was calculated as the amount (g OM/kg faecal OM) of collected faeces by settling during the two-day continuous faeces collection per kilogram produced organic matter faeces. 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 feed (Y_{diet}, g) as $Y_{recovered}$ / Y_{diet} × 1000. The non-removed faeces per organic matter feed intake (g OM/kg OM FI) was calculated as ((1000 – FR) × (1000 – ADC_{OM})) / 1000, where FR is the faeces removal efficiency (g OM/kg faecal OM) during the two-day continuous faeces collection and ADC_{OM} the organic matter digestibility (g/kg) during week 5. Faecal waste production and non-removed faeces per feed intake on g OM/kg DM FI are provided in the supplementary data.

PSD data from the particle size analyser was obtained on volumetric percentages in size classes of 1 μm (upper size class 850 μm). Data was converted into cumulative volume amounts (g/kg faecal material). The upper size range was corrected by the amount (g/kg faecal material) 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 (n = 12) were considered as experimental units. Data were expressed as the mean per quadruplicate. Normality of data was assumed. One-way ANOVA was used to investigate the effect of treatment. In the case of a significant treatment 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. Fish performance

Fish performance data is presented in Table 3. Survival was high (99.7%) and unaffected by treatments. Initial body weight (39 g) did not differ ($p > 0.05$) between dietary treatments. Dietary treatments influenced feed intake, final body weight, growth and FCR ($p < 0.05$). Feed intake, both on dry matter (DM) and wet weight (WW) basis differed between treatments. Absolute feed intake on DM basis (FI_{absDM}) was higher for the *CD* treatment compared to the *Marine* treatment ($p < 0.05$). FI_{absDM} at the *NCD* treatment was intermediate to FI_{absDM} at the *Marine* and *CD* treatment ($p > 0.05$). Fish receiving the *NCD* treatment had the highest feed intake on WW basis (FI_{absWW}), being 199.0% and 182.1% higher compared to FI_{absWW} of fish receiving the *Marine* and *CD* treatment, respectively. Metabolic feed intake (FI_{mbw}) was highest for the commercial treatment. No significant differences were observed for the digestible energy intake (FI_{DE}) between the *NCD* and *CD* treatment. Growth and thermal growth coefficient (TGC) were influenced by dietary treatment ($p < 0.001$), being highest in fish at the *NCD* treatment and lowest at the *Marine* treatment. The *NCD* treatment (FCR 0.69) had the lowest FCR on DM basis ($p < 0.001$), followed by the *CD* (FCR 0.77) and the *Marine* treatment (FCR 0.79).

Table 3
Fish performance of yellowtail kingfish fed the dietary treatments to apparent satiation (4 replicates) for 35 days.

	Marine Treatment	CD Treatment	NCD Treatment	SEM	p-value
Survival (%)	100	99	100	0.5	ns
Initial body weight (g)	40	38	38	1.1	ns
Final body weight (g)	191^{a}	202^{b}	217^{c}	2.1	***
FI_{absDM} (g DM/d)	3.4^{a}	3.6^{b}	3.5^{ab}	0.03	*
FI Pellet (g DM/d)	3.4	3.6	1.9	-	-
FI Krill (g DM/d)	-	-	0.6	-	-
FI Sand eel (g DM/d)	-	-	0.3	-	-
FI Smelt (g DM/d)	-	-	0.3	-	-
FI Squid (g DM/d)	-	-	0.4	-	-
FI_{absWW} (g WW/d)	3.6^{a}	3.8^{a}	10.7^{b}	0.06	***
FI_{mbw} (g DM/$kg^{0.8}$/d)	24.2^{a}	25.4^{b}	24.1^{a}	0.20	**
FI_{DE} (kJ/d)	65.4^{a}	70.5^{b}	72.9^{b}	0.72	***
Growth (g/d)	4.3^{a}	4.7^{b}	5.1^{c}	0.05	***
SGR (%/d)	4.5^{a}	4.8^{b}	5.0^{b}	0.07	**
TGC	2.86^{a}	3.05^{b}	3.22^{c}	0.030	***
FCR	0.79^{a}	0.77^{b}	0.69^{c}	0.005	***

FIabsDM – dry matter feed intake absolute; FIabsWW – wet weight feed intake absolute; FImbw – feed intake metabolic body weight; FIDE – digestible energy intake; SGR – specific growth rate; TGC – thermal growth coefficient; FCR – feed conversion ratio (on DM basis). Values are means and the standard error of the means (SEM); means within the same row not sharing a common letter are significantly different ($p < 0.05$); ns - not significant; * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$.

3.2. Digestibility

Apparent digestibility coefficients (ADC, g/kg) of organic matter, crude protein, crude fat, total carbohydrates, starch and energy were different ($p < 0.01$) between treatments (Table 4). Highest organic matter, crude protein, fat, starch and energy digestibility were observed at the *NCD* treatment ($p < 0.01$). Lowest organic matter, crude protein and energy digestibilities were observed at the *CD* treatment ($p < 0.01$). Due to partial inclusion of the pelleted *CD* diet in the *NCD* treatment, it was possible to estimate the ingredient digestibility of the natural ingredients by using the *CD* treatment as a reference diet. The organic matter, crude protein, fat and energy digestibility of the natural ingredients was 943 g/kg, 942 g/kg, 1038 g/kg and 975 g/kg, respectively.

3.3. Faecal characteristics and removal efficiency

The faecal waste collected overnight for the three treatments is shown in Image 1. It can be observed that faeces at the *Marine* and *CD* (Image 1, bottle #6 and bottle #5) treatments had a diarrhoea-like appearance and were not different between these treatments. Notably, this diarrhoea-like faeces appeared from the moment of faecal egestion and was not a matter of faecal disintegration over a period of time in the bottles. The faeces at the *NCD* treatment showed two distinct layers in the collection bottles (Image 1, bottle #4). The bottom layer looked identical to the diarrhoea-like faeces egested by the fish like in the other treatments. In contrast, the upper layer consisted of distinctive faecal pellets and short strings, with different coloration. Notably, this was only observed during the overnight collection and not during the day collection between the morning and afternoon feeding for fish receiving the *NCD* treatment.

The total amount of faecal waste, faecal removal efficiency and the amount of non-removed faeces were different ($p < 0.001$) between treatments (Fig. 1 and Fig. 2). Fish receiving the *NCD* treatment (130.9 g OM/kg OM FI) had a lower amount of faecal waste produced by around 22.6% and 32.3% compared to fish receiving the *Marine* (169.1 g OM/kg OM FI) and *CD* treatment (193.4 g OM/kg OM FI), respectively ($p < 0.001$). Faeces removal efficiency of fish receiving the *NCD* treatment (520 g/kg faecal OM) was 29.0% and 23.1% higher compared to fish receiving the *Marine* (403 g/kg faecal OM) and *CD* treatment (422 g/kg faecal OM), respectively ($p < 0.001$). Ultimately, this resulted in the lowest amount of non-removed faeces for the *NCD* treatment (62.9 g OM/kg OM FI) compared to the *Marine* (101.0 g OM/kg OM FI) and *CD* treatment (111.7 g OM/kg OM FI) ($p < 0.001$). In other words, the amount of non-removed faeces (g OM/kg OM FI) at the *NCD* treatment was 37.7% and 43.7% lower compared to that at the *Marine* and *CD* treatment, respectively.

Faecal PSD (sampling: 3 h continuous collection after morning feeding) was different ($p < 0.05$) between dietary treatments (Table 5). Compared to the *Marine* and *CD* treatment, the *NCD* treatment had higher percentages of particles within the fractions < 250 μm ($p < 0.01$). No differences were observed for the fraction 250 – 850 μm ($p > 0.05$). The *NCD* treatment had numerically the lowest percentage of particles > 850 μm compared to the *CD* treatment ($p < 0.05$). Overall, smallest particles were observed for the *NCD* treatment.

4. Discussion

4.1. Fish performance and nutrient digestibility

Although the feed intake on dry matter basis was highest at the CD treatment, the feed intake (on as is basis) of yellowtail kingfish receiving the *NCD* treatment was approximately three times the feed intake of fish receiving the *CD* treatment (Table 3). This indicates that feed intake of yellowtail kingfish fed only a pelleted diet was not limited by stomach volume. On the other hand, the digestible energy intake was equal at the *NCD* and *CD* treatment. According to Jobling (1983), it is assumed that feed intake is not regulated by stomach volume but by digestible energy intake. In the current study, the fish receiving the *NCD* treatment compensated the lower nutrient density of the natural feed ingredients, which was due to the higher moisture content, with a higher FI_{WW}. Saravanan et al. (2013) showed that feed intake can also depend on oxygen use per unit of feed. In our study, the concentration of non-protein energy sources (carbohydrates, especially starch) differed among dietary treatments. The highest starch intake was observed for the *Marine* treatment, followed by the *CD* and *NCD* treatment (Table 1); the reverse observations were made for digestible energy intake (Table 3).

Table 4
Apparent digestibility coefficient (ADC, g/kg) of yellowtail kingfish fed the dietary treatments to apparent satiation (4 replicates) for 35 days.

	Marine Treatment	CD Treatment	NCD Treatment	SEM	p-value
ADC diets (g/kg)					
Organic matter	831[b]	807[a]	869[c]	4.3	***
Crude protein	916[b]	907[a]	926[c]	2.3	**
Crude fat	902[a]	894[a]	947[b]	5.7	***
Total carbohydrates	463[b]	304[a]	274[a]	10.6	***
Starch and sugars	839[a]	824[a]	911[b]	10.7	***
Energy	856[b]	838[a]	901[c]	3.4	***

ADC – apparent digestibility coefficient. Values are means and the standard error of the means (SEM); means within the same row not sharing a common letter are significantly different ($p < 0.05$); ** - $p < 0.01$; *** - $p < 0.001$.

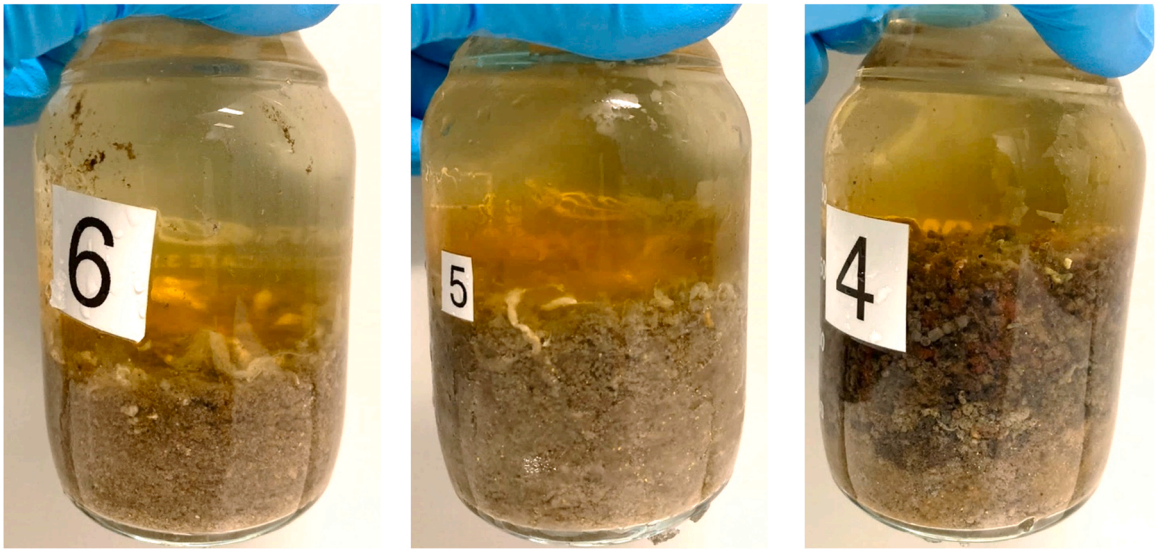

Image 1. Overnight collected faeces (each collection bottle represents one replicate per treatment) of yellowtail kingfish fed the Marine (bottle #6), CD (bottle #5) and NCD treatment (bottle #4) to apparent satiation for 35 days.

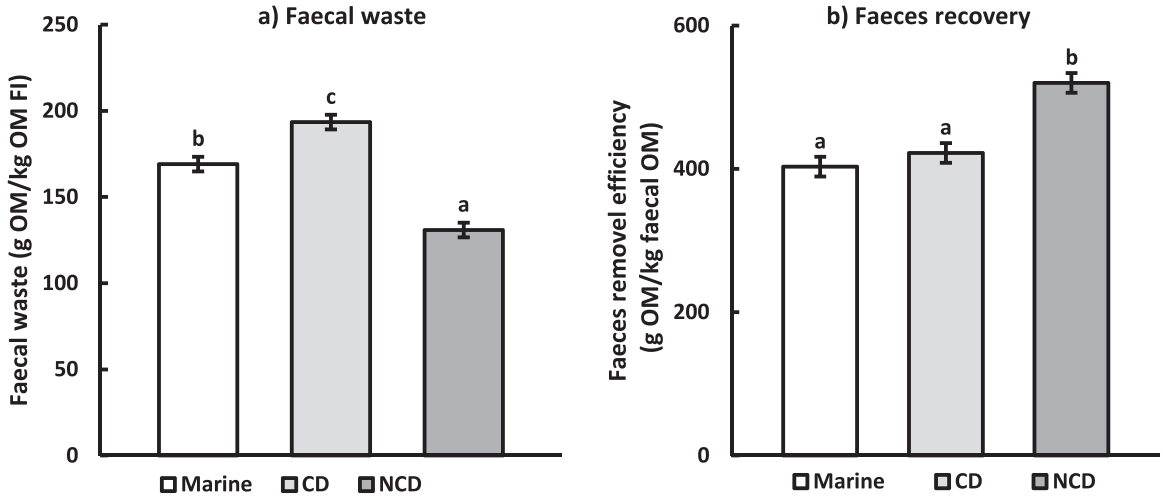

Fig. 1. a) Amount of faecal waste produced in g OM/kg OM FI and b) faeces removal efficiency by settling in g OM/kg faecal OM of yellowtail kingfish fed the dietary treatments to apparent satiation (4 replicates) for 35 days; amount of faecal waste produced and faeces removal efficiency by settling were affected by treatment ($p < 0.001$); OM – organic matter; FI – feed intake; bars are means; error bars indicate standard error of means; bars not sharing a common letter are significantly different ($p < 0.05$).

This is in line with previous studies by Saravanan et al., (2013, 2012), which showed an increase in oxygen use when fish were fed starch-rich diets, ultimately resulting in a lower feed intake. Compared to the conclusions of Jobling (1983) and Saravanan et al., (2013, 2012), opposite findings were made by Booth et al. (2013). In their study, neither an increase in carbohydrate (and starch) inclusion nor an increase in energy content of the diet led to a lower metabolic feed intake. Therefore, it is not clear whether dietary oxygen use plays a role in feed intake regulation in yellowtail kingfish. In summary, it is not possible to clearly determine which factors steered feed intake during our experiment, but our study shows that stomach volume was not limiting feed intake when feeding pelleted feeds. Therefore, it may be interesting to investigate factors that influence feed intake in yellowtail kingfish.

Little information is available on the starch digestibility in yellowtail kingfish. Despite the high gelatinization degree of the pelleted diets (Table 1), starch digestibility varied between 824 g/kg and 911 g/kg. This indicates that yellowtail kingfish are less well able to digest starch, similar to Atlantic salmon (*Salmo salar*) (Hemre et al., 1995; Krogdahl et al., 2004). Studies with rainbow trout, European sea bass and especially omnivorous or herbivorous fish species such as Nile tilapia report starch digestibilities of over 980 g/kg (Burel et al., 2000; Krogdahl et al., 2004; Maas et al., 2019; Peres and Oliva-Teles, 2002). This is probably related to differences in enzyme activity (in particular α-amylase activity) in the gastrointestinal tract of fish species (which is low in yellowtail kingfish) (Chen et al.,

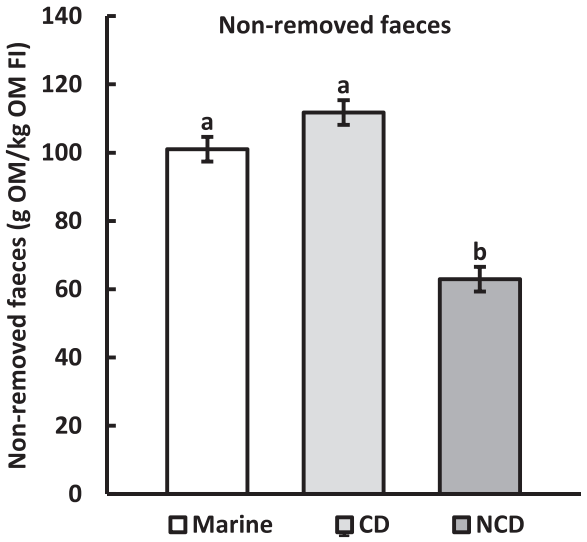

Fig. 2. Amount of non-removed faeces by settling in g OM/kg OM FI of yellowtail kingfish fed the dietary treatments to apparent satiation (4 replicates) for 35 days; amount of non-removed faeces by settling was affect by treatment ($p < 0.001$); OM – organic matter; FI – feed intake; bars are means; error bars indicate standard error of means; bars not sharing a common letter are significantly different ($p < 0.05$).

Table 5
Faecal particle size distribution (PSD; g/kg) of yellowtail kingfish fed the dietary treatments to apparent satiation (4 replicates) for 35 days (faeces continuous collected for 3 h after morning feeding).

	Marine Treatment	CD Treatment	NCD Treatment	SEM	p-value
Faecal particle size distribution (g/kg)					
< 40 μm	4^{a}	3^{a}	6^{b}	0.4	**
40 – 100 μm	53^{a}	61^{a}	85^{b}	4.9	**
100 – 250 μm	256^{a}	313^{a}	390^{b}	16.8	**
250 – 850 μm	320	344	332	21.5	ns
> 850 μm	367^{b}	280^{ab}	187^{a}	37.9	*

Values are means and the standard error of the means (SEM); means within the same row not sharing a common letter are significantly different ($p < 0.05$); ns - not significant; * - $p < 0.05$; ** - $p < 0.01$.

2006; Kaushik et al., 2022) or due to the relatively short gut length of yellowtail kingfish (Pirozzi et al., 2019). Furthermore, the inclusion of natural ingredients, associated with a reduced dietary starch intake, resulted in an increased starch digestibility (Table 4). This is in line with a study with Atlantic salmon where an increased starch intake reduced starch digestibility (Krogdahl et al., 2004). On the other hand, assuming that increasing starch intake reduces starch digestibility, one would expect a lower starch digestibility for the *Marine* treatment than for the *CD* treatment (Table 1, Table 4). However, starch digestibility was equal at *Marine* and *CD* treatment, which might be related to the higher degree of gelatinisation of the *Marine* treatment. Moreover, it could also be that the amylopectin to amylose ratio was different among treatments, which could ultimately reflect in the digestibility of starch (Glencross et al., 2012; Kaushik et al., 2022). In summary, the current study shows that starch is poorly digested by yellowtail kingfish. It may be worthwhile to investigate the factors affecting starch digestibility, particularly the effects on α-amylase activity, secretion and biosynthesis in yellowtail kingfish.

When averaged over both pelleted diets, fat and crude protein digestibility were 898 g/kg and 912 g/kg, respectively. Comparable fat digestibilities are reported for yellowtail kingfish in literature, while crude protein digestibility tended to be higher in the current study (Candebat et al., 2020; Liu et al., 2019). This high crude protein digestibility might be related to the high inclusion level of high-quality LT fish meal. When comparing the dietary treatments of our study, the highest organic matter, crude protein, fat and energy digestibility (Table 4) were observed for the *NCD* treatment. This can be attributed to the inclusion of the natural ingredients, as the organic matter, crude protein, fat and energy digestibility of the natural ingredients was 943 g/kg, 942 g/kg, 1038 g/kg and 975 g/kg, respectively. Fish receiving the *NCD* treatment consumed fewer non-starch polysaccharides (NSP) compared to fish receiving the *CD* treatment (Table 1). NSP are also known to be anti-nutritional factors and literature shows that NSP can lead to impaired digestibility and absorption of nutrients due to an alteration of the gut physiology and its functions (Maas et al., 2019; Sinha et al., 2011). For example, increased intake of NSP can impair the effectiveness of enzymes due to an increased viscosity of chyme in the gastrointestinal tract. This reduces the diffusion rate of digestive enzymes and their interaction with the intestinal mucosal surface (Maas et al., 2019; Sinha et al., 2011; Tran-Tu et al., 2018). In addition, fish receiving the *NCD* treatment had a reduced starch intake

and showed a higher starch digestibility, both ultimately resulting in lower amounts of undigested starch. Literature suggests that high levels of undigested starch can negatively affect fat and protein digestion (Hemre et al., 2002, 1995). Research with Atlantic salmon by Hemre et al. (1995) and Krogdahl et al. (2004) have shown that larger amounts of ingested and undigested starch can negatively affect nutrient digestion. In contrast, in studies with rainbow trout and Nile tilapia, increasing amounts of starch in the diet did not negatively affect the digestion of other nutrients (Amirkolaie et al., 2006; Krogdahl et al., 2004). It follows that fish species which are less able to digest starch due to low amylase activity and a relative short gut length, like yellowtail kingfish and Atlantic salmon, seem to be more susceptible to negative effects of starch (Chen et al., 2006, 2019). The negative effects of starch may be related to the fact that undigested starch can behave like dietary fibre in the gastrointestinal tract (Hemre et al., 1995). Accordingly, the pelleted treatments are likely to have negatively affected intestinal physiology and morphology, reduced the mixing of enzymes and chyme, and consequently impaired nutrition digestion and absorption (Amirkolaie et al., 2006; Sinha et al., 2011). In addition, starch and NSP can alter gut microflora, induce microbial fermentation processes and lead to osmotic imbalances, negatively affecting the digestibility of nutrients (Booth et al., 2013; Hung et al., 1990; Kokou and Fountoulaki, 2018; Refstie et al., 2005; Sinha et al., 2011; van Barneveld, 1999). When comparing the pelleted diets (similar dietary carbohydrate content), it seems that the inclusion of NSP has a greater impact on nutrient digestibility than the inclusion of starch. However, as mentioned above, this could also be related to difference in the type of the ingredients included in the *CD* diet.

With regard to the *NCD* treatment, the fat digestibility of the natural ingredients was 1038 g/kg. Thus, the inclusion of natural ingredients had a positive effect on the fat digestibility of the pellet feed within the *NCD* treatment and ultimately resulted in the highest observed fat digestibility among treatments (Table 4). This is partly expected due to the reduced intake of NSP (Leenhouwers et al., 2007; Refstie et al., 1999; Sinha et al., 2011). Fat digestion is largely dependent on bile acids, while NSP are known to increase bile acid secretion and its deconjugation (Kortner et al., 2013; Sinha et al., 2011; Staessen et al., 2020a, 2020b). Thus, the reduced intake of NSP may have ultimately reduced the loss of bile acids and therefore increased fat digestion. On the other hand, it is likely that the natural ingredients contained taurine, cholesterol and bile acids (Kortner et al., 2013; Staessen et al., 2020a, 2020b). Taurine and cholesterol are precursors in the bile acid metabolism, which are converted to bile acids in the liver (Kortner et al., 2013). Contrary to the improved fat digestibility in fish fed the *NCD* treatment, is the intake of chitin (contained in krill and squid) (Abdel-Ghany and Salem, 2020; Olsen et al., 2006; Ringø et al., 2012; Teng et al., 2001; Yoshitomi and Nagano, 2012). Chitin is referred to as dietary fibre (Shahidi et al., 1999), which is known to promote the excretion of bile acids and their deconjugation, ultimately leading to lower fat absorption (Abdel-Ghany and Salem, 2020; Muzzarelli, 1996; Shahidi et al., 1999).

In terms of feed properties, the *NCD* treatment differed in processing (thawed, raw and unprocessed) and moisture content (Table 1). According to literature, it is not expected that the higher nutrient digestibility of the *NCD* treatment can be assigned to the moisture content of the natural ingredients (Lee et al., 2000; Oehme et al., 2014). This was as well shown by a previous experiment with yellowtail kingfish in the same research facility, where an increase in dietary moisture content from 50 g/kg to 150 g/kg did not affect nutrient digestibilities (unpublished data). However, it is expected that unprocessed feed ingredients could lead to increased crude protein and fat digestibilities because processing (such as rendering prior to feed production or extrusion) can cause reactions like racemization of amino acids, loss of amino acids, protein cross-linking, formation of disulfide bonds and Maillard reactions (Deng et al., 2005; Jasour et al., 2018; Mai et al., 2022, 1991). In addition, extrusion processes could also denature active enzymes (Singh et al., 2007). In summary, it is suggested that the inclusion of natural ingredients resulted in an improved crude protein and fat digestibility of the *NCD* treatment due to a dilution of carbohydrates (in particular starch), the absence of processing reactions and the supplementation of emulsifiers or their precursors.

4.2. Faecal waste production and characteristics

Waste management is a key factor concerning the success of a RAS operation. In practical management of RAS, important factors to control the amount of TSS in the system are the amount of faecal waste produced and the faecal removal efficiency (Amirkolaie, 2011; Bureau and Hua, 2010; Kokou and Fountoulaki, 2018; Tran-Tu et al., 2018). During the current study, the *NCD* treatment resulted in a higher digestibility of organic matter, ultimately resulting in a lower faecal waste production by 22.6% and 32.3% (Fig. 1a) compared to the *Marine* and *CD* treatment, respectively.

Another important step in waste management is the efficient and fast removal of solid waste. Our study revealed that the faeces characteristics of yellowtail kingfish receiving natural feed items are not of poor integrity (not diarrhoea-like). Visually, the formation of faecal pellets was observed for the *NCD* treatment (Image 1), whereas the faecal waste of the *Marine* and *CD* treatment was described as diarrhoea-like. The formation of faecal pellets could be explained by the presence of natural binders and structural agents in the natural ingredients such as chitin, keratin or collagen (Joy et al., 2017; Shahidi et al., 1999; Sharma and Gupta, 2016). Chitin and its deacetylated form chitosan are present in the exoskeleton of krill and structural components of squid (Abdel-Ghany and Salem, 2020; Ringø et al., 2012; Teng et al., 2001). In food science, chitin and chitosan are known to act as a thickening, water binding and stabilizing agents (Muzzarelli, 1996; Shahidi et al., 1999). Collagen, present in fibrous tissues such as ligaments, skin, bones, tendons, cartilages, corneas, and blood vessels, and keratin, mainly present in epithelial cells of higher vertebrates, are both structural proteins which are difficult to digest (Joy et al., 2017; Kersanté et al., 2021; Lall and Dumas, 2015; Sharma and Gupta, 2016). Literature from non-nutritional research suggests that both collagen and keratin can provide a matrix-structure (Joy et al., 2017; Wang et al., 2016). This could have resulted in the faecal pelletisation during the current study. Differences in moisture content are not expected to have resulted in faecal pelleting of the natural ingredients. During a previous experiment, increasing dietary moisture content did not positively affect the faecal removal efficiency (unpublished data). However, this could be due to the small differences in moisture content (50 g/kg vs. 150 g/kg) compared to the large differences in moisture content (50 g/kg vs. >775 g/kg) when feeding raw feed

items as was done in the current study at the *NCD* treatment.

Apart from the fact that natural ingredients contain factors which might have led to faecal pelleting, it could also be possible that the *Marine* and *CD* diets contained factors which induced a poor faeces integrity in yellowtail kingfish. As mentioned earlier, fish at the *NCD* treatment had a lower carbohydrate intake and lower amount of undigested starch along the gastrointestinal tract. As suggested in literature, the high water binding capacity of carbohydrates may lead to osmotic imbalances in the distal intestine (Amirkolaie et al., 2006; Hung et al., 1990; Refstie et al., 1999). In addition, microbial fermentation induced by carbohydrates may result in gas production, which could become trapped in the faecal strands (Amirkolaie et al., 2006; Hung et al., 1990; Kokou and Fountoulaki, 2018; van Barneveld, 1999). On the one hand, the gas could lead to floating faeces if it remains entrapped in the faecal pellet. However, no floating faeces were observed in our experiment (Image 1). On the other hand, the gas could also break up the faecal pellets as they are excreted in the water, making their removal from the system water more difficult. Ultimately, the inclusion of the natural ingredients, resulting in faecal pelleting, improved the faecal removal efficiency of the *NCD* treatment by 29.0% and 23.1% compared to the *Marine* and *CD* treatment, respectively (Fig. 1b). In summary, the combined effects of faecal waste production and faeces removal efficiency resulted in a lower amount of non-removed faeces (g OM/kg OM FI) for fish receiving the *NCD* treatment by 37.7% and 43.7% compared to fish receiving the *Marine* and *CD* treatment, respectively (Fig. 2).

The general assumption prior to the start of the experiment was that increasing faecal particle size would reflect in an increased faeces removal efficiency (Reid et al., 2009; Timmons et al., 2018; Tran-Tu et al., 2018). In our study, the highest faeces removal efficiency (Fig. 1b) and the formation of faecal pellets (Image 1) were observed at the *NCD* treatment, while observing there the smallest faecal particles (Table 5). This is in contrast to the expectation. The absence of a relation between faeces removal efficiency and PSD could be partly explained by the observed segregation of faecal waste at the *NCD* treatment (Image 1). During the experiment, similar amounts of pelleted and natural ingredients (on DM basis, Table 3) were fed at the same feeding moment. However, the respective faeces were excreted at different time windows, which is suggested by the observed segregation of faecal waste occurring from the natural ingredients and the pelleted feed. Although the faecal waste during overnight collection (between 16:30 h and 9:00 h) showed faecal pelleting (originating from the natural ingredients), this was not observed during faecal collection for PSD analysis (obtained between 10:30 h and 13:30 h). Another explanation for the absence of this relationship could be the unequal distribution of the feeding periods during the day, which may have altered the passage rate of digesta resulting in differences in faecal integrity. The absence of a relation between faeces removal efficiency and PSD might be also due to the faecal collection method. Faecal PSD was determined on faeces collected by settling. According to Tran-Tu et al. (2018), this could have resulted in a non-representative sampling of faecal material. Therefore, it might be worth to study faecal particle size of stripped faeces to draw conclusions between faecal characteristics and its removal rate by settling or filtration.

The removal of solids is a key factor in the success of a RAS operation due to their potential impact on animal health, system functioning, operation costs, and environmental eutrophication (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). One of the challenges of farming yellowtail kingfish in RAS is the poor faeces integrity (diarrhoea-like), which makes it difficult to remove the faecal material from the system water. The current study proved that the faeces integrity of yellowtail kingfish fed with natural ingredients is not poor, while pelleted diets resulted in diarrhoea-like faeces (Image 1). Thus, pelleted diets might contain dietary factors, which introduce a poor faeces integrity in yellowtail kingfish or lack dietary factors, which prevent it. Ultimately, it was shown that the inclusion of natural ingredients reduced the amount of non-removed faeces (Fig. 2). In practice, this would result in a reduced build-up of TSS on system level. However, it has to be mentioned that the inclusion of natural ingredients will not be practical under commercial farming conditions (Kolkovski and Sakakura, 2007; Mourente and Tocher, 2009). Nevertheless, the current study clearly shows the potential of dietary intervention to reduce TSS build-up and offers new possibilities for future research. Thus, future research should focus on which dietary factors introduce or hamper the development of diarrhoea-like faeces in yellowtail kingfish. Unravelling mechanisms behind faecal binding would ultimately lead to an increased animal welfare and system performance, while reducing the operating costs and environmental eutrophication of yellowtail kingfish farming.

5. Conclusion

The inclusion of natural feed ingredients into the diet of yellowtail kingfish reduced the faeces quantity and improved integrity (not diarrhoea-like), ultimately resulting in the lowest amount of non-removed faeces among treatments. It was observed that fish receiving pelleted diets had a poor faeces integrity (diarrhoea-like) compared to the *NCD* treatment. Thus, pelleted diets might contain factors, which introduce a poor faeces integrity in yellowtail kingfish or lack dietary factors, which prevent it. One factor that may cause poor faeces integrity may be dietary starch, due to a low amylase activity and relatively short gut length of yellowtail kingfish. Although feeding natural ingredients on farm level is not practical, the potential of dietary intervention on faeces integrity was clearly shown. This offers new possibilities to reduce the TSS load for yellowtail kingfish farming in recirculating aquaculture systems.

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. **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 This work is part of the Healthy Happy Kingfish project applied for by Kingfish Zeeland B.V. under the subsidy scheme Innovation Projects Aquaculture 2019 and, granted by the RVO (Netherlands Enterprise Agency) under the application number 19111000012. This project is partly funded by The European Union with support of the European Maritime and Fisheries Fund (EMFF).

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European Maritime & Fisheries Fund

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.anifeedsci.2023.115625.

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