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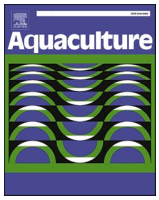

Carbohydrates in dietary ingredients for European seabass: Impact on nutrient digestibility and waste production when reared in recirculating aquaculture systems

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ABSTRACT

The growing demand for aquafeeds along with the limited availability of fish meal has necessitated the use of various alternative ingredients in feed formulations. Depending on the ingredient, such use may also entail higher and diverse carbohydrate inclusion in the diets with implications for fish digestion and environmental output in terms of waste production. The present study examined the impact of different dietary ingredients on nutrient digestibility and waste production focusing on faecal removal efficiency when used in recirculating aquaculture systems (RAS). For this purpose, a trial was performed with juvenile European seabass (*Dicentrarchus labrax*), testing seven ingredients with different carbohydrate levels and types (starch and non-starch polysaccharides, NSP): wheat dried distillers grain with solubles, DDGS; hydrolyzed feather meal I, HFM I; hydrolyzed feather meal II, HFM II; insect meal (black soldier fly larvae, *Hermetia illucens*), IM; single-cell protein, SCP; shrimp-shell meal, SSM; seaweed protein, SWP. The test ingredients were included in a basal (control, CTR) diet at a level of 15 %, and fish were fed restrictively for a period of 4 weeks. Apparent nutrient digestibility was measured, with a focus on carbohydrates, and faecal quantity was determined based on organic matter (OM) digestibility values. Waste production was evaluated based on the amount of removed faeces by settling, and faecal particle size distribution. The results showed that starch digestibility decreased with increasing dietary starch levels, whereas NSP digestibility varied depending on the ingredient source. Moreover, faecal waste production had a stronger correlation with dietary NSP compared to starch. High inclusion levels of both nutrients in the diet were correlated with reduced faecal removal efficiency by settling. Overall, the DDGS and SWP diets scored worse for all indicators used here to assess waste production, whilst SSM excelled at producing highly settleable faeces compared to the rest of the diets. Summarizing, the current findings suggest that the type of dietary ingredients, reflecting also the type and level of dietary carbohydrates, determines faecal quantity and quality, and therefore careful selection of ingredients for RAS aquafeeds should be considered in this context.

1. Introduction

European seabass (*Dicentrarchus labrax*) is a highly-valued marine species which is mainly produced through aquaculture (Vandeputte et al., 2019). As a typical carnivore, seabass has a high protein requirement that has been conventionally met though fish meal inclusion in aquafeeds (Kousoulaki et al., 2015; NRC, 2011). However, due to its high price, scarcity and environmental footprint, there is a perpetual interest in fish meal substitution with alternative ingredients (Oliva-Teles et al., 2015). Although, such replacement is environmentally more sustainable, it induces changes in the chemical composition of the diet with consequences on nutrient digestibility (Antony Jesu Prabhu et al., 2019; Fanizza et al., 2023; Fountoulaki et al., 2022; Prakash et al., 2023).

Besides the often lower protein content and unbalanced amino acid profile, alternative ingredients may additionally contain high levels of carbohydrates (Oliva-Teles et al., 2015). Among them, dietary starch can act as energy source without compromising growth when included

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in limited amounts (Enes et al., 2011; Peres and Oliva-Teles, 2002), but it can impair performance at higher inclusion levels (Moreira et al., 2008). Contrarily, non-starch polysaccharides (NSP), commonly known as fibers, pose always a challenge in aquafeed formulation since they are poorly digestible and interfere with the absorption of other nutrients (Sinha et al., 2011). Although fibers have previously been reported to compose less than 3.2 % of the seabass diets (Kousoulaki et al., 2015), it is foreseen that this percentage may increase when gradually more feedstuffs originating from plants, by-products and low-trophic species are used (Colombo et al., 2023).

Dietary carbohydrates do not only influence fish performance but also waste production. By reducing diet digestibility, fibers increase faecal waste production and alter faecal chemical composition (reviewed in Kokou and Fountoulaki, 2018). A high fraction of NSP in the faeces has direct impact on faecal pellet consistency and may decrease the ratio of settleable to suspended solids (Welker et al., 2021), with the latter being difficult and costly to remove (Timmons et al., 2007). Nonetheless, the effect of fibers on faecal characteristics may vary depending on the NSP type (Amirkolaie et al., 2005). Similarly, starch has also been found to exert diverse effects on faecal integrity which are likely fish species-dependent (Amirkolaie et al., 2006; Horstmann et al., 2023b).

Overall, fish meal substitutes have been reported to reduce faecal stability and increase suspended solids (Brinker and Friedrich, 2012; Davidson et al., 2013; Horstmann et al., 2023a; Schumann et al., 2022). In closed farming systems, like recirculating aquaculture systems (RAS), fine particles will progressively accumulate and eventually undermine fish welfare (Becke et al., 2017, 2018; Bruton, 1985), as well as system functioning (Meriac et al., 2014; Michaud et al., 2006). Given that European seabass is reared in RAS during the early juvenile stages (Vandeputte et al., 2019), knowledge about waste production with novel feed formulations is essential.

As such, the present study aimed to explore the impact of various ingredients on nutrient digestibility and faecal waste production for European seabass. The selected ingredients (wheat dried distillers grain with solubles, hydrolyzed feather meals, insect meal, single-cell protein, shrimp-shell meal, seaweed protein) contained various levels of starch and non-starch polysaccharides, with the latter consisting of different monomer types. This set of ingredients enabled us to additionally assess the impact of the carbohydrate quantity and quality on the aforementioned aspects.

2. Material and methods

2.1. Experimental diets and design

The present experiment was carried out in accordance with the Dutch and European law on the use of experimental animals. The Animal Welfare Body of Wageningen University and Research (The Netherlands) classified it as a non-invasive trial. European seabass juveniles were housed and handled in agreement with the EU legislation in order to assess nutrient digestibility and faecal quality under different dietary treatments. In this regard, eight experimental diets were formulated among which, one served as a control (CTR; basal diet); this reference diet represented a commercial feed formulation with low fish meal levels (Table 1). The remaining diets consisted of the basal mixture diluted at 15 % with a selection of test ingredients (Table 2). The rather low rate of ingredient inclusion was because of the nutritional profile of some of the ingredients used. The test ingredients (wheat dried distillers grain with solubles, DDGS; hydrolyzed feather meal I, HFM I; hydrolyzed feather meal II, HFM II; insect meal (black soldier fly larvae, *Hermetia illucens*), IM; single-cell protein (mixed bacterial culture), SCP; shrimp-shell meal, SSM; seaweed protein, SWP) were chosen based on their carbohydrate profile which has different starch/NSP levels and distinctive fiber types (Supplementary Table S2). As such, dilution of the control diet with the test ingredients resulted in a range in amount and types of carbohydrates. Diets were extruded to produce 3 mm sinking pellets (Research Diet Services, Wijk bij Duurstede, The Netherlands) and stored at 4 °C after being sieved for fines.

Table 1
Basal diet composition

Ingredient	g/kg
Gelatinized maize starch	164.7
Wheat	163.5
Fish meal LT	141.2
Corn gluten	88.3
Wheat gluten	88.3
Pea protein	88.3
Soy protein concentrate	88.3
Fish oil	117.7
Monocalcium phosphate	23.6
Taurine	5.9
DL-methionine	4.7
L-lysine	4.7
L-threonine	1.7
L-tryptophane	0.95
Yttrium oxide	0.2
Premix[a]	10

[a] Premix composition. Vitamins (IU or mg/kg complete diet): Vitamin B1–15 mg; Vitamin B2–15 mg; Vitamin B6–20 mg; Vitamin B5–50 mg; Vitamin B3–150 mg; Biotine – 1.5 mg; B-12–0.1 mg; Folic acid – 5 mg; Vitamin C – 1000 mg (given as ascorbic acid C, phosphate); Vitamin E – 500 IU; A-vitamin A palmitate – 20,000 IU; D-Rovimix D3–500–2500 IU; K_3 K-menadione sodium bisulphite (51 %) – 25 mg; Inositol – 500 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); Iron (as ferric sulphate) – 50 mg; Zinc (as zinc sulphate) – 60 mg; Cobalt (as cobalt sulphate) – 0.6 mg; Copper (as copper sulphate) – 10 mg; Selenium (as sodium selenite) – 0.2 mg; Manganese (as manganese sulphate) – 20 mg; Magnesium (as magnesium sulphate) – 500 mg; Chrome (as chromic chloride) – 1 mg; Iodate (as calcium iodate) – 2 mg.

Dietary treatments were tested in triplicate ($8 \times 3 = 24$ experimental units/tanks) in identical rearing tanks ($90 \times 60 \times 45$ cm; 200 L), which served as experimental units. Due to lack of experimental units, only half of the units/tanks could be concurrently tested. Thus, the experimental period (8 weeks) was divided into two equal sequential phases (4 weeks each), with 12 experimental units/tanks every time. To correct for any phase effect, replicates of the same dietary treatment were included in both phases. Fish originating from Phase I were pooled and randomly redistributed at the same stocking density of 30 individuals per tank in Phase II. Fish were batch-weighed both at the start and end of each phase, with total initial biomass being the same for all units belonging to the same phase (Phase I: 1.83 ± 0.03 kg, Phase II: 2.66 ± 0.05 kg).

2.2. Experimental conditions and sampling

All tanks were connected to a common recirculating aquaculture system (RAS) with each tank being equipped with an individual swirl separator (column height 44 cm; diameter 24.5 cm; Aqua Optima AS, Pulford, United Kingdom). The RAS consisted, in order, of a trickling filter, a sump, a drum filter (Hydrotech 500®, Hydrotech Engineering, Italy), and an oxygenator controlled by a mass flow controller 5850S; Brooks Instruments) and a microprocessor (Brooks Read Out and Control Electronics Model 0154; Brooks Instruments). Water quality parameters were monitored on alternate days to meet the optimal rearing conditions of the species (Blancheton, 2000; Lemarié et al., 2004; Torno et al., 2018). More specifically, temperature was maintained at 21.9 ± 0.1 °C, salinity at 33.8 ± 0.6 ppt, and pH at 7.4 ± 0.3 with the addition of sodium bicarbonate when needed. Mean tank oxygen levels were 6.5 ± 0.2 mg/L, which were ensured by a tank water inflow rate of 7.0 ±

Table 2
Analyzed proximate nutrient composition of the test diets.

	CTR	DDGS	HFM I	HFM II	IM	SCP	SSM	SWP
Inclusion levels as is basis (%)								
Test ingredient	–	15	15	15	15	15	15	15
Basal mixture	100	85	85	85	85	85	85	85
Nutrient composition (g/kg DM)								
Dry matter *(g/kg)*	957	945	955	946	953	949	953	951
Organic matter	929	932	938	938	929	927	885	900
Crude protein (CP)	446	418	515	513	450	480	435	394
Sum amino acids (SAA) [1]	415	393	488	486	423	443	402	368
Crude fat	174	160	151	156	176	160	152	112
Carbohydrates [2]	340	379	299	296	331	324	331	421
Starch	264	233	222	222	228	226	218	233
Non-starch polysaccharides (NSP) [3]	48.2	71.0	41.1	43.7	48.9	46.9	39.8	76.7
Energy *(kJ/g DM)*	22.5	22.4	22.8	22.8	22.6	22.4	21.3	21.0
DP:DE [4]	20.3	19.8	22.8	22.6	20.5	21.8	20.5	19.5

Ctr, Control; DDGS, Dried distillers' grains with solubles (wheat); SSM, Shrimp shell meal; HFM I, Hydrolyzed feather meal (air-dried); HFM II, Hydrolyzed feather meal (disk-dried); IM, Insect meal (black soldier fly larvae, *Hermetia illucens*); SCP, Single-cell protein; SWP, Seaweed protein.
[1] Based on all studied AAs.
[2] Calculated as: OM – SAA – Crude fat.
[3] Calculated as the sum of all analyzed NSP constituents.
[4] Calculated as: Digestible SAA / Digestible Energy.

0.1 L/min. Maximum concentrations for total ammonium nitrogen (TAN; Merck Aquamerck Colorimetric Ammonium test), NO_2-N (Merck Aquamerck Colorimetric Nitrite test) and NO_3-N (Merck Mquant Nitrate test strips) were 0, 0.3 and 113 mg/L, respectively. Photoperiod was set at a 12-h light and 12-h darkness scheme for the entire duration of the trial.

After being feed-deprived for one day before each phase, fish were fed restrictively and during light hours on a dry matter basis. Feeding level was 1.7 % of body weight, with the daily feed amount being calculated according to the mean body weight of all fish at the beginning of each phase and the expected growth rate based on a FCR of 1. Feeding level was set to be lower only for the first 2 days (0.9 % and 1.3 % of body weight, respectively) of each phase to facilitate fish acclimation to the new feeding regime. The daily amount of feed was divided into two equal portions which were hand-fed at fixed time points (08:00 and 16:00). Feed spill was collected in detachable glass bottles mounted to the swirl separators and was removed shortly after the completion of feeding to allow for accurate estimation of feed intake and prevent faecal contamination with feed residues.

Faecal collection was performed in a similar manner, with collection bottles being submerged on ice to minimize microbial degradation of the material (Supplementary Fig. S1). For digestibility analysis, faeces were collected overnight for the first five days of weeks 4 and 8, respectively. For the determination of faecal removal efficiency via settling, collection was extended for another two days during which samples were obtained continuously over a 48-h period (excluding feeding moments). In both cases, faeces were pooled per tank after decanting and stored at −20 °C until analysis. Lastly, particle size distribution (PSD) was measured in faecal samples collected twice in the second half of each phase, 6 h (h) after the first daily feeding.

2.3. Analytical methods

Faeces collected for digestibility and faecal removal efficiency were dried at 70 °C until constant weight. Feed pellets and dried faecal samples were ground with a mixer mill (IKA A11 basic) before chemical analysis. For DM determination, all materials were analyzed gravimetrically by drying at 103 °C for 4 h (ISO 6496, 1999). Ash was determined by combustion in a muffle furnace at 550 °C for 4 h (ISO 5984, 2002). The ash fraction was further dissolved in concentrated sulphuric acid by autoclaving (121 °C, 20 min) to determine yttrium by ICP-AES (NEN 15510, 2007). Total nitrogen was measured using the Kjeldahl method (ISO 5983-2, 2009) and crude protein (CP) was calculated with a 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). Starch, including the free sugar fraction, was analyzed enzymatically using amyloglucosidase without a prior ethanol extraction (Goelema et al., 1998). Gross energy was measured using bomb calorimetry (C7000, IKA werke, IKA analysentechnik, Staufen, Germany). Amino acids (excluding tryptophan and cysteine) were determined by an ultraperformance liquid chromatography (UPLC, Waters Acquity, UPLC systems, Milford, MA, United States). This analysis was based on the method accredited by the Nordic Committee of Food Analysis (NMKL) and the analytical protocol is described in Belghit et al. (2019). NSP was measured according to (Englyst et al., 1994) for all constituents except for uronic acid, which was determined in the final hydrolysate according to (Blumenkrantz and Asboe-Hansen, 1973). Faecal PSD, an additional indicator for faecal quality, was measured via filtration and laser detection methods. For filtration, total samples (without decanting) were gently stirred with a magnet so a homogenous subsample (50 mL) could be withdrawn. The subsample was sieved with an 850 μm screen, making up two particle size groups (1.5–850 μm and > 850 μm). Both fractions were collected on pre-weighed filters (1.5 μm glass-fiber filter, grade 696, VWR, Radnor, USA) which were individually stored at −20 °C until further analysis. Eventually, filters were dried at 103 °C (DM, ISO 6496, 1999) and incinerated at 550 °C (ash, ISO 5985, 2002). For further resolution, PSD was additionally determined using a non-invasive laser particle analyzer (240 s time interval and 90 % confidence interval; DIPA 2000, Donner Technologies, Or Akiva, Isreal). The particle size analyzer was connected to a liquid flow controller (LFC) equipped with a mechanical stirrer (LFC-101; 150 mL/min flow speed; 20 % stirrer speed, around 55 rpm). Before introduction of the material into the LFC, faeces were sieved using an 850 μm screen and only the filtrate (< 850 μm) was further analyzed.

2.4. Data treatment and statistical analysis

Feed conversion ratio (FCR) and specific growth rate (SGR) were calculated as: FCR (g DM/g) = feed intake / body weight gain, and SGR (g/day) = $100 \times (\ln W_i - \ln W_0) / t$, where Wi is the final body weight, W0 is the initial body weight, and t is the phase duration. Considering proximate compositions, the sum of amino acids (SAA) was calculated after adding up all essential and non-essential amino acids analyzed in the present study (Supplementary Table S3 & S4). Non-protein nitrogen

(NPN) was then estimated as: $N_{CP} - N_{SAA}$, with N_{CP} corresponding to the Kjeldahl N and N_{SAA} was calculated based on individual AA coefficients provided by (Sosulski and Imafidon, 1990). Organic matter (OM) and carbohydrates were calculated as: DM – ash and DM – SAA – crude fat – ash, respectively. The latter formula was chosen instead of the conventional one which uses CP instead of SAA as an estimate of protein (NRC, 2011). This was because most of the ingredients used in the present study contain a substantial amount of NPN (Biswas et al., 2021; Glencross et al., 2020; Kono et al., 1987a; Peng et al., 2015) which results in an overestimation of CP. NSP as presented here equate the sum of the analyzed arabinosyl, fucosyl, galactosyl, glucosyl, mannosyl, rhamnosyl, xylosyl, and uronyl monomers.

Apparent digestibility coefficient (ADC) of all nutrients (and gross energy) in diets was calculated using yttrium as inert marker: $ADC\ (\%) = 100 \times (1 - ((Y_{diet} / Y_{faeces}) \times (N_{faeces} / N_{diet})))$, where Y stands for yttrium and N denotes for any nutrient (or gross energy) in the diet or faeces. Nutrient ADCs of ingredients were calculated as: $ADC_{ti}\ (\%) = ADC_{td} + (ADC_{td} - ADC_{rd}) \times (0.85 \times D_{ctr} \times DM_{ctr})/(0.15 \times D_{ing} \times DM_{ing}))$, where ADC_{ti} is the ADC of the test ingredient, ADC_{td} (%) is the ADC of the test diet, ADC_{rd} is the ADC of the control (basal) diet, D_{ctr} is the nutrient content (g/kg DM) (or kJ/g gross energy) of the control diet, DM_{ctr} is the DM content of the control diet (pre-mix), D_{ing} is the nutrient content (g/kg DM) (or kJ/g gross energy) of the ingredient, and DM_{ing} is the DM content of the ingredient. The inclusion of the basal diet and the ingredient content in the test diet are represented as 85 % and 15 % in the above formula.

Faecal waste production was determined, on organic matter basis, as the amount of non-digested feed per kg feed intake DM using the calculated ADC_{OM}: $(100\ \% - ADC_{OM}) \times 1000$. Here, ADC OM was used instead of the previously suggested ADC DM (Bureau and Hua, 2010; Schumann and Brinker, 2020) since the latter would have been underestimated due to the presence of salt in the collected faeces. Faecal removal efficiency (FR, %) was calculated as the percentage of collected faeces by settling throughout the 48-h continuous faeces collection in relation to the total amount of faecal waste production over the same period. Accordingly, the non-removed faeces per feed intake DM was calculated as the difference between the total amount of faecal waste produced and the amount of faeces removed: $((100\ \% - FR) \times (100\ \% - ADC_{OM})) \times 1000$.

PSD data as obtained by filtration were expressed on weight basis, whereas the PSD data obtained by the particle size analyzer were expressed on volumetric basis in size classes of 1 μm (upper size class 850 μm). Data was then converted into cumulative volume percentages making up four fractions: 10–100 μm, 100–200 μm, 200–400 μm, and 400–850 μm.

All data were analyzed with R software (R Core Team 2022; version 4.2.1) and are reported as least square means of three replicates ($n = 3$). Pairwise correlations between variables were analyzed with either linear regression or Spearman's rank correlation. To investigate the effect of diet on the different parameters, two-way analysis of variance (ANOVA) was performed using general linear models that included diet and phase as explanatory variables (main effects only). Normality was assessed using a Shapiro-Wilk test and data transformation was carried out when needed to improve it. When assumptions were not met (3 cases; Supplementary Table S4), normality was assumed due to the absence of an equivalent non-parametric analysis. A Tukey honest significant difference (HSD) test was performed as a post-hoc analysis when applicable. Differences in faecal composition among the dietary treatments were tested with both univariate (ANOVA) and multivariate (permutational multivariate ANOVA; PERMANOVA) statistics, with the former accounting for each macronutrient separately and the latter for all macronutrients together. After PERMANOVA, post-hoc analysis was performed using the adonis2 R package. Confidence level for all analyses was set to 95 %.

3. Results

3.1. Digestibility

Overall, digestibility of macronutrients (Table 3) and minerals (Supplementary Table S4) differed among diets and was significantly affected by the test ingredient included. This was the result of different nutrient digestibilities for each of the ingredients (Supplementary Table S3). Organic matter (OM) ADC was highest for both feather meal diets (HFM I, HFM II) and lowest for the DDGS and SWP diets. Similar results were obtained for carbohydrate and energy ADCs, with DDGS having the lowest digestibility. The differences observed for carbohydrate ADC was due to differences in starch and non-starch polysaccharides (NSP) ADCs. More specifically, starch was better digested for feather meal diets, whilst it was least digestible for both DDGS and CTR diets. However, it should be noted that starch ADC was also influenced by phase. ADC of crude protein (CP) was similar for all diets. However when expressed as sum amino acids (SAA), protein was more digestible for IM diet and less for HFM II, SCP and SWP diets. This outcome was induced by accumulated differences in individual AAs ADC (Supplementary Table S4). Fat ADC levels were highest for IM and SSM, whereas SWP demonstrated the lowest fat digestibility. Overall, similar ADC trends as the above were also observed for the test ingredients given in the Supplementary material (Supplementary Table S3). However, these are not thoroughly discussed as diet digestibility effects on waste production was the main focus of this study. Similarly, selective growth parameters (FCR, SGR) are provided for context (Supplementary Fig. S2) but are not directly addressed in the discussion.

In Fig. 1, correlations are given between the dietary content of different carbohydrate-related nutrients and their ADC. OM ADC was positively correlated to carbohydrate digestibility; this relationship was mainly due to the positive correlation observed between OM ADC and starch ADC, since OM ADC was not correlated to NSP ADC. Despite that, OM ADC correlated with the dietary levels of both starch and NSP, with the latter relationship being stronger. On the contrary, carbohydrate digestibility was found to be negatively correlated only with the dietary starch content. Starch digestibility was negatively correlated with dietary starch content, while NSP digestibility did not seem to relate with the dietary NSP levels.

3.2. Faecal waste production

Faecal removal efficiency varied among the test diets (ANOVA: $p < 0.01$; Fig. 2A), with the proportion of faeces being removed to be the highest for SSM diet (66.5 %) and the lowest for the DDGS (45.6 %) and SWP (42.9 %) diets. This outcome did not seem to relate to production of larger faecal particles in the SSM group (Supplementary Table S5). Regarding the accumulation of faecal OM in the system (i.e., the amount of non-removed faeces; Fig. 2B), feather meal diets (HFM I, HFM II) were found to induce a lower accumulation compared to the rest of the diets (ANOVA: $p < 0.01$) due to a lower amount of faeces produced (ANOVA: $p < 0.001$).

Faecal removal efficiency was negatively correlated with dietary carbohydrate levels ($p < 0.05$, $r_s = -0.420$). This correlation was due to both dietary NSP ($p < 0.001$, $r_s = -0.656$) and starch levels ($p < 0.01$, $r_s = -0.573$), since both were negatively correlated with faecal removal efficiency. Moreover, faecal removal efficiency was linearly negatively related to the dietary NSP content; increasing NSP levels corresponded to a decline in faecal settleability (Fig. 3A). Contrarily, faecal removal efficiency was curvilinearly related to the dietary starch content ($p < 0.001$, $r_s = 0.530$; Fig. 3B); at low dietary starch levels (<~235 g/kg), faecal removal efficiency declined with dietary starch level, whereas above a level of ~235 g/kg, this negative effect of starch on faecal removal efficiency seemed to attenuate.

Faecal composition was affected by diet (PERMANOVA: $p < 0.001$) and significant differences among treatments were detected for all faecal

Table 3
Apparent digestibility coefficient (ADC, %) of nutrients in the test diets fed to European seabass over a 4-week experimental period.

	CTR	DDGS	HFM I	HFM II	IM	SCP	SSM	SWP	SEM	p-value
Organic matter	82.4^{bc}	77.6^{a}	85.1^{c}	85.0^{c}	82.2^{bc}	80.5^{ab}	82.6^{bc}	78.4^{a}	0.6	***
Crude protein (CP)	93.4	92.3	92.7	92.8	93.3	92.0	91.5	92.3	0.2	ns
Sum amino acids (SAA) [1]	94.9^{ab}	94.2^{ab}	94.1^{ab}	93.9^{a}	95.6^{b}	94.0^{a}	95.0^{ab}	94.2^{a}	0.2	**
Crude fat	93.6^{ab}	92.6^{ab}	93.2^{ab}	93.0^{ab}	94.2^{b}	92.0^{ab}	94.1^{b}	91.5^{a}	0.2	*
Carbohydrates [2]	56.2^{ab}	54.0^{a}	66.1^{c}	66.3^{c}	59.3^{b}	56.6^{ab}	60.0^{b}	61.1^{b}	0.9	***
Starch	82.4^{a}	81.3^{a}	92.2^{c}	91.4^{c}	87.2^{b}	86.8^{b}	90.8^{bc}	88.6^{bc}	0.8	***
Non-starch polysaccharides (NSP) [3]	1.2^{a}	7.6a^{b}	9.2^{abc}	14.4^{bc}	17.0^{bc}	16.5^{bc}	9.4^{abc}	21.5^{c}	1.5	**
Arabinosyl	2.5	11.5	8.1	14.2	17.6	15.6	3.3	7.5	1.5	*
Fucosyl	12.9^{ab}	5.4^{a}	14.6^{ab}	10.5^{ab}	16.9^{ab}	19.4^{ab}	30.2^{b}	4.6^{a}	2.3	*
Galactosyl	32.1	31.7	28.5	29.1	37.9	38.1	29.3	35.8	1.5	ns
Glucosyl	(−21.6)	(−15.4)	(−20.6)	(−7.4)	(−8.5)	(−9.0)	(−16.5)	(−7.0)	1.8	na
Mannosyl	46.6^{a}	46.5^{a}	56.1^{bcd}	58.6^{cd}	60.7^{d}	58.5^{cd}	52.8^{abc}	49.2^{ab}	1.2	***
Rhamnosyl	19.9^{ab}	17.6^{a}	35.5^{abc}	31.0^{abc}	32.1^{abc}	31.5^{abc}	42.7^{bc}	47.9^{c}	2.6	**
Xylosyl	(−10.1)	4.5	4.2	8.8	10.1	9.6	(−4.2)	5.8	1.7	ns
Uronyl	(−18.3)^{a}	(−4.3)^{ab}	(−3.8)^{ab}	3.3^{ab}	3.6^{ab}	10.5^{ab}	14.4^{bc}	41.2^{c}	3.9	***
Energy	86.3^{bcd}	83.5^{a}	88.5^{e}	88.6^{e}	87.2^{cde}	85.4^{bc}	87.6^{de}	84.5^{ab}	0.4	***

Ctr, Control; DDGS, Dried distillers' grains with solubles (wheat); SSM, Shrimp shell meal; HFM I, Hydrolyzed feather meal (air-dried); HFM II, Hydrolyzed feather meal (disk-dried); IM, Insect meal (black soldier fly larvae, *Hermetia illucens*); SCP, Single-cell protein; SWP, Seaweed protein.
Values are least square means (n = 3) with a standard error of the mean (SEM) and lowercase letters indicate significant differences among diets. P-value: >0.05, ns; *; <0.05, **; <0.01, ***; <0.001.
[1] Based on the all studied AAs.
[2] Calculated as: OM – SAA – Crude fat.
[3] Calculated as the sum of all analyzed NSP constituents.

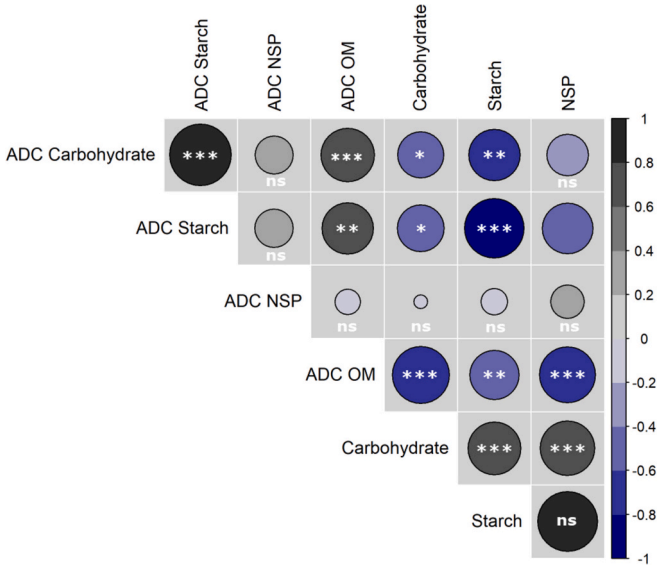

Fig. 1. Spearman correlations between carbohydrate-related nutrients (g/kg DM) in the test diets fed to European seabass and their respective apparent digestibility coefficients (ADCs, %). Circle size reflects the magnitude of correlation, with larger circles representing stronger correlations, while circle color indicates the sign of correlation (positive or negative) according to the provided color scale. Stars denote significant differences. NSP: Non-starch polysaccharides, OM: organic matter. p-value: >0.05, ns; *; <0.05, **; <0.01, ***; <0.001.

nutrients except for OM (Table 4). In particular, faeces originating from the feather meal diets contained the highest amount of SAA and fat, and the lowest amount of carbohydrates. Faecal starch content was high for the DDGS and CTR diets, whilst NSP content was highest in the faeces from the SWP and SSM diets. Faecal waste differed mainly in their protein and carbohydrate content, with the relative ratios between the two (SAA: carbohydrates) ranging from 0.13 to 0.3.

4. Discussion

The present study aimed to explore the impact of dietary ingredients on nutrient digestibility focusing on the carbohydrate fraction, faecal waste production and removal. Specifically, the focus was on understanding the effects of different levels and types of carbohydrates, starch and NSP, originating from those distinctive ingredient sources. To address the above objectives, faecal quantity was estimated based on OM digestibility values and faecal quality was evaluated by examining faecal properties such as settling and PSD. Although, the study did not investigate the effect of diet on fish growth — due to the diets being nutritionally unbalanced and the influence of age from testing in two phases — it is noteworthy that the observed FCR and SGR (Supplementary Fig. S2) align with values reported for commercial diets (Kousoulaki et al., 2015), while no welfare issues were noted.

4.1. Digestibility

In this study, a number of ingredients were used as protein and/or protein replacement sources in European seabass diets. This inclusion resulted in changes in dietary composition with significant consequences for nutrient digestibility. Alternative ingredients, like the ones presently used, have been formerly reported to reduce feed efficiency in marine warmwater species including European seabass (Batista et al., 2020; Biswas et al., 2020; Davies et al., 2009; Diógenes et al., 2018; Mastoraki et al., 2022; Xu et al., 2021; Yi et al., 2015). This is typically because new formulations lead to the introduction of more dietary carbohydrates which make up a large fraction of the dietary OM. Indeed, we observed that diet digestibility on OM basis was the lowest for DDGS and SWP (Table 3), both of which contained the highest carbohydrate levels.

A clear, positive relationship between OM digestibility and carbohydrate digestibility was observed (Fig. 1); however, this correlation is not definitive. For instance, despite the low OM ADC found in SWP diet, this was not reflected in its carbohydrate ADC (Table 3). This is because carbohydrate digestibility was found to be affected by the starch fraction of the dietary carbohydrate (Fig. 1) and not the NSP fraction; SWP had a relatively low starch-to-NSP ratio. In general, starch is highly digestible by European seabass, since this species has been reported to possess amylolytic enzymes (Enes et al., 2011). However, starch digestibility in marine fish has been shown to decrease with increasing starch dietary levels (Dias et al., 1998; Enes et al., 2006; Glencross et al., 2012; Horstmann et al., 2023b; Moreira et al., 2008), as observed also in this study (Fig. 1). On the contrary, NSP digestibility was found to be

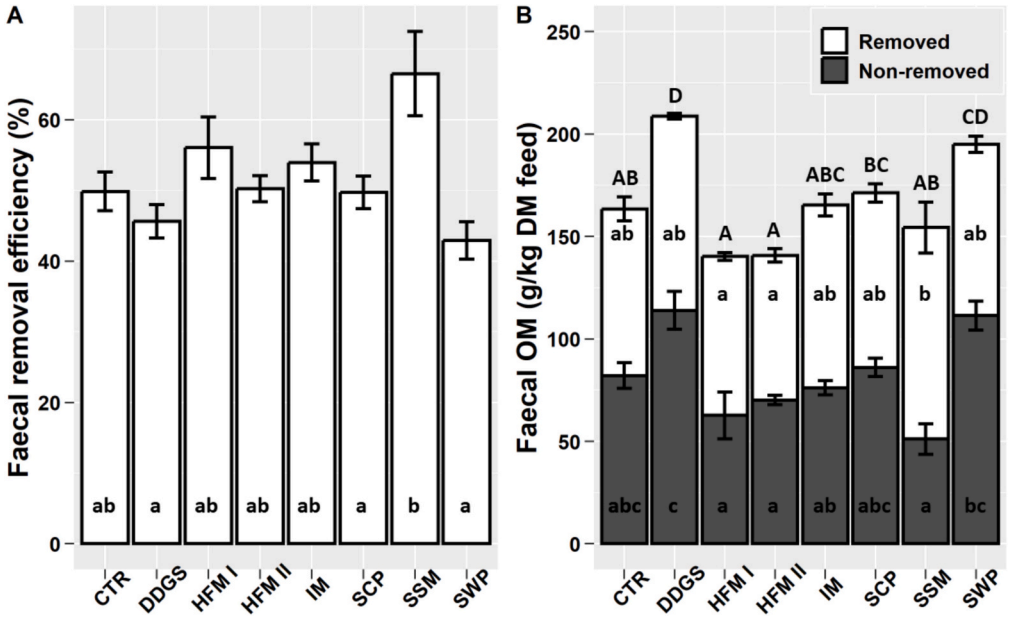

Fig. 2. Faecal waste production by European seabass fed with the test diets. Values are least square means ($n = 3$) with a standard error of the mean (SEM); A) The proportion of faeces that was removed by settling with lowercase letters indicating significant differences among diets. B) The amount of faeces produced (total column height) split into the fraction that was removed by settling (grey bar) and the one that accumulated in the system (white bar). Uppercase letters indicate for significant differences among diets in terms of total faecal organic matter produced, while lowercase letters in each of the stacked bars indicate significant differences among diets in terms of removed and non-removed faecal organic matter, respectively. Ctr, Control; DDGS, Dried distillers' grains with solubles (wheat); SSM, Shrimp shell meal; HFM I, Hydrolyzed feather meal (air-dried); HFM II, Hydrolyzed feather meal (disk-dried); IM, Insect meal (black soldier fly larvae, *Hermetia illucens*); SCP, Single-cell protein; SWP, Seaweed protein.

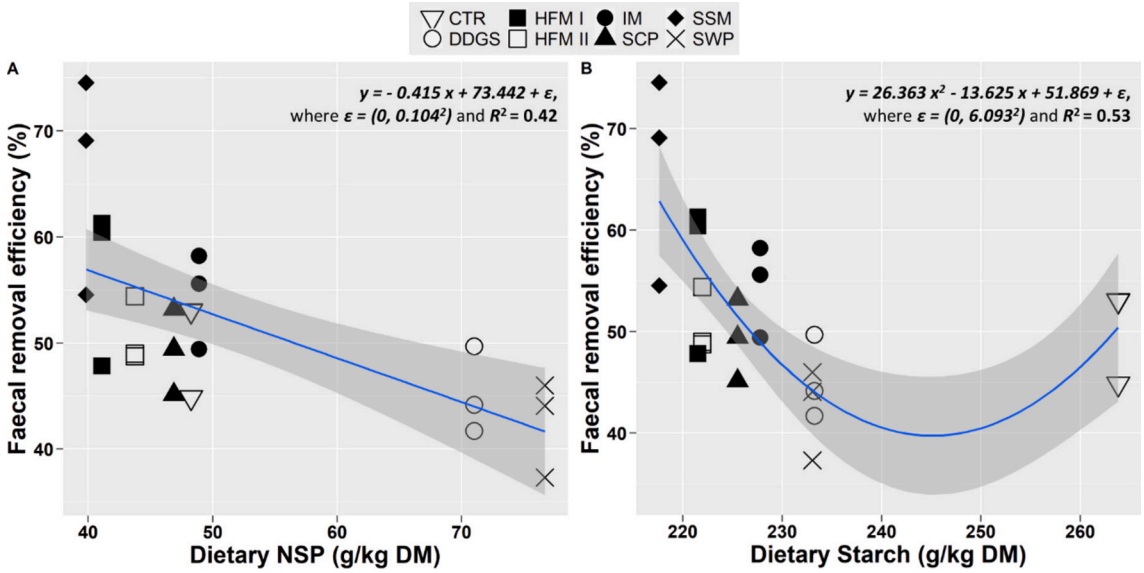

Fig. 3. Linear regressions describing the effect of A) dietary NSP and B) dietary starch on faecal removal efficiency. Marked areas denote confidence intervals at p = 0.05. Ctr, Control; DDGS, Dried distillers' grains with solubles (wheat); SSM, Shrimp shell meal; HFM I, Hydrolyzed feather meal (air-dried); HFM II, Hydrolyzed feather meal (disk-dried); IM, Insect meal (black soldier fly larvae, *Hermetia illucens*); SCP, Single-cell protein; SWP, Seaweed protein.

independent of the dietary NSP (Fig. 1) indicating that the type rather than the amount of NSP determines its digestibility, at least under the tested dietary NSP levels in this study.

In principle, fish lack enzymes for hydrolyzation of NSPs, and that is why these nutrients are considered indigestible (Choct, 1997; Sinha et al., 2011). This is however a simplistic premise since chitinolytic activity in the fish stomach suggests that chitin may be endogenously catabolized despite being an NSP (Gasco et al., 2016; Kono et al., 1987a, 1987b; Reyes et al., 2020). This is also corroborated in the present study since carbohydrate digestibility for IM and SSM diets was quite high compared to the rest of the test diets (Table 3) despite being both rich in chitin (Díaz-Rojas et al., 2006; Eggink and Dalsgaard, 2023). Furthermore, particular NSP may undergo microbial fermentation in the gut of fish like European seabass (Fountoulaki et al., 2022; Leenhouwers et al., 2008). Fermentability will increase the NSP ADC but fermentation capacity is low for ingredients, such as DDGS. DDGS is already an end product of fermentation and its NSP fraction comprises primarily of lignin (reviewed in Böttger and Südekum, 2018), arabinoxylan and

Table 4
Analyzed proximate nutrient composition (g/kg OM) of the faeces produced by European seabass after being fed with the test diets over a 4-week experimental period.

	CTR	DDGS	HFM I	HFM II	IM	SCP	SSM	SWP	SEM	p-value
Organic matter (g/kg)	419	465	394	420	438	408	411	408	6	ns
Crude protein (CP)	162^{a}	154^{a}	267^{e}	263^{de}	186^{ab}	212^{bc}	230^{cd}	156^{a}	9	***
Sum amino acids (SAA) [1]	116^{a}	109^{a}	202^{c}	211^{c}	113^{a}	149^{b}	124^{a}	111^{a}	8	***
Crude fat	61^{abc}	57^{ab}	$73b^{c}$	77^{c}	62^{abc}	70^{bc}	57^{ab}	49^{a}	2	***
Carbohydrates [2]	823^{c}	834^{c}	725^{a}	712^{a}	825^{c}	781^{b}	820^{bc}	840^{c}	10	***
Starch	255^{d}	210^{c}	124^{a}	135^{ab}	178^{bc}	166^{ab}	124^{a}	136^{ab}	10	***
Non-starch polysaccharides (NSP) [3]	264^{ab}	314^{c}	254^{a}	259^{a}	247^{a}	236^{a}	227^{a}	309^{bc}	7	***

Ctr, Control; DDGS, Dried distillers' grains with solubles (wheat); SSM, Shrimp shell meal; HFM I, Hydrolyzed feather meal (air-dried); HFM II, Hydrolyzed feather meal (disk-dried); IM, Insect meal (black soldier fly larvae, *Hermetia illucens*); SCP, Single-cell protein; SWP, Seaweed protein.
Values are least square means ($n = 3$) with a standard error of the mean (SEM). Lowercase letters indicate significant differences among diets. p-value: >0.05, ns; *; <0.05, **; <0.01, ***; <0.001.
[1] Based on the all studied AAs.
[2] Calculated as: OM – SAA – Crude fat.
[3] Calculated as the sum of all analyzed NSP constituents.

cellulose (Pedersen et al., 2014). Since all these residual NSP are poorly fermentable, it was expected that NSP digestibility would be lower for this ingredient (Table 3).

The fact that different NSP types have different apparent digestibility potentials is additionally demonstrated here by differences in the digestibility of the various NSP monomers. Although arabinosyl and xylosyl are universally poorly digestible, uronyl can sometimes be well-digestible. This was the case for the SWP diet where 21 % of the NSP consisted of highly digestible uronyl units (Table 3; Supplementary Table S1). As such, despite former beliefs that European seabass cannot digest seaweed fibers (Wassef et al., 2013), the current findings suggest that it is capable of degrading ulvan, which is mostly composed of uronic acid (Siddhanta et al., 2001).

4.2. Faecal waste production

Since carbohydrates were not only a major component of the test diets (~30–40 % DM; Table 2) but also the least digestible among macronutrients (Table 3), it was of no surprise that they exerted a major effect on OM digestibility (Fig. 1) and thus faecal loss. Even though, both the starch and NSP fraction of the dietary carbohydrate were found to determine faecal production, fibers had a stronger effect on the latter (Fig. 1) due to their poorer digestibility potential. This was also reflected on the faecal composition (Table 4), which was highly dominated by undigested carbohydrates (~70–85 % OM) and high NSP/starch ratios (up to 2.7), as previously reported for diets rich in fibers (Antony Jesu Prabhu et al., 2019; Fountoulaki et al., 2022; Prakash et al., 2023). This was however less evident for diets with nitrogen-containing fibers, like keratin, where CP still makes up a large fraction of the faecal composition. As such, faeces from both feather meal diets used in the present study had the highest protein content (Table 4), corroborating former results on European seabass (Fountoulaki et al., 2022).

It is likely that the high protein-to-carbohydrate ratio in HFM faeces accommodated their removal (Fig. 2a), since faeces with low protein (Schumann et al., 2022) and high NSP levels (Prakash et al., 2023) are commonly considered unstable and are therefore composed of small particles. To an extent, particle size may determine faecal settleability and thus waste removal efficiency via swirl separators. The present findings indicate no major differences in the faecal PSD (Supplementary Table S5) and it can therefore be concluded that other faecal properties, like density, may affect faecal removal efficiency via settling in European seabass.

Dietary factors may also influence faecal removal efficiency in a direct manner. In this regard, increased levels of both starch and NSP were found to correlate with reduced faecal settling (Fig. 3). This trend has also been observed in other warm-water, carnivorous species, such as yellowtail kingfish (Horstmann et al., 2023b), where increasing dietary starch from 54 to 144 g/kg DM feed resulted in a 12 % decrease in faecal removal efficiency. Here, a marginal increase of 15 g/kg DM feed in starch levels corresponded to a dramatic 23.6 % reduction in faecal removal efficiency. This may indicate that when starch inclusion exceeds 20 % of DM feed, like in the current study, slight increments in dietary starch may vastly impair faecal settleability. However, the present findings also suggest that the negative impact of starch on faecal quality may plateau at very high inclusion levels (>23.3 % of DM feed; Fig. 3B), though further research is needed to confirm this.

Despite the generally negative effects of high NSP levels, insoluble fibers have been previously reported to positively influence faecal stability in Nile tilapia (Amirkolaie et al., 2005). This indicates that both the quantity and type of dietary NSP may interfere with faecal properties. Here, the SSM diet exhibited the highest faecal removal efficiency (Fig. 2A); this might be related to its lower dietary NSP content but also its content in chitin, an insoluble NSP, the deacetylated form of which (chitosan) has been shown to reduce suspended solids (Abdel-Ghany and Salem, 2020) and accommodate faecal removal (Horstmann et al., 2023b; Prakash et al., 2023). IM, as another chitin-rich diet, exhibited also a competitively high feacal removal efficiency, but lower to SSM. In addition to differences in chitin forms (Henry et al., 2015), the small difference in NSP levels between the IM and SSM diet may have contributed to the observed differences in faecal removal efficiency. Similarly, the presence of other dietary factors, such as keratin, may have assisted in the reduction of the non-removed OM fraction for feather meals (Fig. 2B).

Even though the SSM diet did not produce the lowest amount of faeces among the diets, it led to the lowest amount of non-removed OM (Fig. 2B). Since settleable particles may be easily recovered via various mechanical methods like sedimentation or dual-drain tanks (Timmons et al., 2007), the remaining suspended solids present a challenge for RAS operations (Bureau and Hua, 2010); accumulating fine particles originating from undigested OM have been extensively reported to interfere with both fish (e.g. Becke et al., 2017, 2018) and system performance (Meriac et al., 2014; Rojas-Tirado et al., 2018). It is thus important to realize that RAS water quality may be improved not only by feed formulations that facilitate digestibility, but also faecal characteristics.

5. Conclusion

Dietary carbohydrates appear to influence both the quantity and properties of faecal excretions of European seabass farmed in RAS, with effects proportional to their inclusion level in the diet. However, the impact may vary per carbohydrate type; while high dietary levels of both starch and NSP increase faecal output, starch is comparatively more digestible than NSP. Additionally, although high dietary starch levels (>20 % of DM feed) can severely impair faecal settleability, the effect of dietary NSP on feacal settleability depends not only on their quantity but also on their type, with dietary inclusion of NSPs like chitin

enhancing faecal removal efficiency. These findings highlight the importance of balancing carbohydrate levels and types in aquafeed formulations to optimize both nutrient digestibility and waste management, particularly as new ingredients with diverse carbohydrate profiles are increasingly integrated in RAS feeds.

CRediT authorship contribution statement

Elisavet Syropoulou: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Satya Prakash:** Writing – review & editing, Investigation, Formal analysis. **Daan Smeenge:** Writing – review & editing, Investigation. **Detmer Sipkema:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Johan W. Schrama:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Fotini Kokou:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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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.2025.742182.

Data availability

Data will be made available on request.

References

Abdel-Ghany, H.M., Salem, M.E.S., 2020. Effects of dietary chitosan supplementation on farmed fish; a review. In: Reviews in Aquaculture, 12, Issue 1. Wiley-Blackwell, pp. 438–452. https://doi.org/10.1111/raq.12326.

Amirkolaie, A.K., Leenhouwers, J.I., Verreth, J.A.J., Schrama, J.W., 2005. Type of dietary fibre (soluble versus insoluble) influences digestion, faeces characteristics and faecal waste production in Nile tilapia (Oreochromis niloticus L.). Aquac. Res. 36 (12), 1157–1166. https://doi.org/10.1111/j.1365-2109.2005.01330.x.

Amirkolaie, A.K., Verreth, J.A.J., Schrama, J.W., 2006. Effect of gelatinization degree and inclusion level of dietary starch on the characteristics of digesta and faeces in Nile tilapia (Oreochromis niloticus (L.)). Aquaculture 260 (1–4), 194–205. https://doi.org/10.1016/j.aquaculture.2006.06.039.

Antony Jesu Prabhu, P., Fountoulaki, E., Maas, R., Heinsbroek, L.T.N., Eding, E.H., Kaushik, S.J., Schrama, J.W., 2019. Dietary ingredient composition alters faecal characteristics and waste production in common carp reared in recirculation system. Aquaculture 512. https://doi.org/10.1016/j.aquaculture.2019.734357.

Batista, S., Pintado, M., Marques, A., Abreu, H., Silva, J.L., Jessen, F., Tulli, F., Valente, L. M.P., 2020. Use of technological processing of seaweed and microalgae as strategy to improve their apparent digestibility coefficients in European seabass (Dicentrarchus labrax) juveniles. J. Appl. Phycol. 32, 3429–3446. https://doi.org/10.1007/s10811-020-02185-2/Published.

Becke, C., Steinhagen, D., Schumann, M., Brinker, A., 2017. Physiological consequences for rainbow trout (Oncorhynchus mykiss) of short-term exposure to increased suspended solid load. Aquac. Eng. 78, 63–74. https://doi.org/10.1016/j.aquaeng.2016.11.001.

Becke, C., Schumann, M., Steinhagen, D., Geist, J., Brinker, A., 2018. Physiological consequences of chronic exposure of rainbow trout (Oncorhynchus mykiss) to suspended solid load in recirculating aquaculture systems. Aquaculture 484, 228–241. https://doi.org/10.1016/j.aquaculture.2017.11.030.

Belghit, I., Lock, E.J., Fumière, O., Lecrenier, M.C., Renard, P., Dieu, M., Berntssen, M.H. G., Palmblad, M., Rasinger, J.D., 2019. Species-specific discrimination of insect meals for aquafeeds by direct comparison of tandem mass spectra. Animals 9 (5). https://doi.org/10.3390/ani9050222.

Biswas, A., Takakuwa, F., Yamada, S., Matsuda, A., Saville, R.M., LeBlanc, A., Silverman, J.A., Sato, N., Tanaka, H., 2020. Methanotroph (Methylococcus capsulatus, Bath) bacteria meal as an alternative protein source for Japanese yellowtail, *Seriola quinqueradiata*. Aquaculture 529. https://doi.org/10.1016/j.aquaculture.2020.735700.

Biswas, A., Takakuwa, F., Yamada, S., Furukawa, A., Shiratori, M., Koshiishi, T., Tomokane, H., Tanaka, H., 2021. Partial replacement of fishmeal by a protein-rich product from methanotrophic bacteria in juvenile red sea bream (Pagrus major). Aquac. Nutr. 27 (6), 2726–2738. https://doi.org/10.1111/anu.13398.

Blancheton, J.P., 2000. Developments in recirculation systems for Mediterranean fish species. In: Aquacultural Engineering, 22. www.elsevier.nl/locate/aqua-online.

Blumenkrantz, N., Asboe-Hansen, G., 1973. New method for quantitative determination of uranic acids. In: Analytical biochemistry, 54.

Böttger, C., Südekum, K.H., 2018. Protein value of distillers dried grains with solubles (DDGS) in animal nutrition as affected by the ethanol production process. Anim. Feed Sci. Technol. 244, 11–17.

Brinker, A., Friedrich, C., 2012. Fish meal replacement by plant protein substitution and guar gum addition in trout feed. Part II: effects on faeces stability and rheology. Biorheology 49 (1), 27–48. https://doi.org/10.3233/BIR-2012-0605.

Bruton, M.N., 1985. The effects of suspensoids on fish. Hydrobiologia 125, 221–241.

Bureau, D.P., Hua, K., 2010. Towards effective nutritional management of waste outputs in aquaculture, with particular reference to salmonid aquaculture operations. In: Aquaculture Research, 41, issue 5, pp. 777–792. https://doi.org/10.1111/j.1365-2109.2009.02431.x.

Choct, M., 1997. Feed Non-Starch Polysaccharides: Chemical Structures and Nutritional Significance. Feed Milling International, June, pp. 13–26.

Colombo, S.M., Roy, K., Mraz, J., Wan, A.H.L., Davies, S.J., Tibbetts, S.M., Øverland, M., Francis, D.S., Rocker, M.M., Gasco, L., Spencer, E., Metian, M., Trushenski, J.T., Turchini, G.M., 2023. Towards achieving circularity and sustainability in feeds for farmed blue foods. In: Reviews in Aquaculture, 15, issue 3, pp. 1115–1141. John Wiley and Sons Inc. https://doi.org/10.1111/raq.12766.

Davidson, J., Good, C., Barrows, F.T., Welsh, C., Kenney, P.B., Summerfelt, S.T., 2013. Comparing the effects of feeding a grain- or a fish meal-based diet on water quality, waste production, and rainbow trout Oncorhynchus mykiss performance within low exchange water recirculating aquaculture systems. Aquac. Eng. 52, 45–57. https://doi.org/10.1016/j.aquaeng.2012.08.001.

Davies, S.J., Gouveia, A., Laporte, J., Woodgate, S.L., Nates, S., 2009. Nutrient digestibility profile of premium (category III grade) animal protein by-products for temperate marine fish species (European sea bass, gilthead sea bream and turbot). Aquac. Res. 40 (15), 1759–1769. https://doi.org/10.1111/j.1365-2109.2009.02281.x.

Dias, J., Christine Huelvan, I., Dinis, M.T., 1998. Influence of dietary bulk agents (silica, cellulose and a natural zeolite) on protein digestibility, growth, feed intake and feed transit time in European seabass (Dicentrarchus Zabrax) juveniles. In: Aquat. Living Resour. I I, 2, issue 4.

Díaz-Rojas, E.I., Argüelles-Monal, W.M., Higuera-Ciapara, I., Hernández, J., Lizardi-Mendoza, J., Goycoolea, F.M., 2006. Determination of chitin and protein contents during the isolation of chitin from shrimp waste. Macromol. Biosci. 6 (5), 340–347. https://doi.org/10.1002/mabi.200500233.

Diógenes, A.F., Castro, C., Miranda, A.C., Oliva-Teles, A., Peres, H., 2018. Dietary replacement of fishmeal by corn distillers dried grains with solubles (DDGS) in diets for turbot (Scophthalmus maximus, Linneaus, 1758) juveniles. Aquaculture 492, 113–122. https://doi.org/10.1016/j.aquaculture.2018.04.005.

Eggink, K.M., Dalsgaard, J., 2023. Chitin contents in different black soldier fly (Hermetia illucens) life stages. J. Insects Food Feed 1–10. https://doi.org/10.3920/jiff2022.0142.

Enes, P., Panserat, S., Kaushik, S., Oliva-Teles, A., 2006. Effect of normal and waxy maize starch on growth, food utilization and hepatic glucose metabolism in European sea bass (Dicentrarchus labrax) juveniles. Comp. Biochem. Physiol. -Part A Mol. Integr. Physiol. 143 (1), 89–96. https://doi.org/10.1016/j.cbpa.2005.10.027.

Enes, P., Panserat, S., Kaushik, S., Oliva-Teles, A., 2011. Dietary carbohydrate utilization by European Sea bass (Dicentrarchus labrax L.) and gilthead sea bream (Sparus Aurata L.) Juveniles. Rev. Fish. Sci. 19 (3), 201–215. https://doi.org/10.1080/10641262.2011.579363.

Englyst, H.N., Quigley, M.E., Hudson, G.J., 1994. Determination of dietary fibre as non-starch polysaccharides with gas-liquid chromatographic, high-performance liquid chromatographic or spectrophotometric measurement of constituent sugars. In: Analyst, 11.

Fanizza, C., Trocino, A., Stejskal, V., Prokešová, M.D., Zare, M., Tran, H.Q., Brambilla, F., Xiccato, G., Bordignon, F., 2023. Practical low-fishmeal diets for rainbow trout (Oncorhynchus mykiss) reared in RAS: effects of protein meals on fish growth,

nutrient digestibility, feed physical quality, and faecal particle size. Aquacult. Report. 28. https://doi.org/10.1016/j.aqrep.2022.101435.
Fountoulaki, E., Vasilaki, A., Nikolopoulou, D., Schrama, J., Kaushik, S.J., Antony Jesu Prabhu, P., 2022. Faecal waste production, characteristics and recovery in European seabass (Dicentrarchus labrax) is affected by dietary ingredient composition. Aquaculture 548. https://doi.org/10.1016/j.aquaculture.2021.737582.
Gasco, L., Henry, M., Piccolo, G., Marono, S., Gai, F., Renna, M., Lussiana, C., Antonopoulou, E., Mola, P., Chatzifotis, S., 2016. Tenebrio molitor meal in diets for European sea bass (Dicentrarchus labrax L.) juveniles: growth performance, whole body composition and in vivo apparent digestibility. Anim. Feed Sci. Technol. 220, 34–45. https://doi.org/10.1016/j.anifeedsci.2016.07.003.
Glencross, B., Rutherford, N., Bourne, N., 2012. The influence of various starch and non-starch polysaccharides on the digestibility of diets fed to rainbow trout (Oncorhynchus mykiss). Aquaculture 356–357, 141–146. https://doi.org/10.1016/j.aquaculture.2012.05.023.
Glencross, B.D., Huyben, D., Schrama, J.W., 2020. The application of single-cell ingredients in aquaculture feeds—a review. Fishes 5 (3), 1–39. https://doi.org/10.3390/fishes5030022.
Goelema, J.O., Spreeuwenberg, M.A.M., Hof, G., Van Der Poel, A.F.B., Tamminga, S., 1998. Effect of pressure toasting on the rumen degradability and intestinal digestibility of whole and broken peas, lupins and faba beans and a mixture of these feedstuffs. In: Animal Feed Science and Technology, 76.
Henry, M., Gasco, L., Piccolo, G., Fountoulaki, E., 2015. Review on the use of insects in the diet of farmed fish: Past and future. In: Animal Feed Science and Technology, 203, Issue 1. Elsevier B.V, pp. 1–22. https://doi.org/10.1016/j.anifeedsci.2015.03.001.
Horstmann, P., Maas, R.M., de Boer, X.V., de Jong, T.M.B., Staessen, T.W.O., Kokou, F., Schrama, J.W., 2023a. 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. Aquaculture 562. https://doi.org/10.1016/j.aquaculture.2022.738875.
Horstmann, P., Maas, R.M., de Boer, X.V., Staessen, T.W.O., Kokou, F., Schrama, J.W., 2023b. Faecal waste characteristics of yellowtail kingfish (Seriola lalandi) fed with pelleted and natural feed. Anim. Feed Sci. Technol. 299. https://doi.org/10.1016/j.anifeedsci.2023.115625.
Kokou, F., Fountoulaki, E., 2018. Aquaculture waste production associated with antinutrient presence in common fish feed plant ingredients. In: Aquaculture, vol. 495. Elsevier B.V, pp. 295–310. https://doi.org/10.1016/j.aquaculture.2018.06.003.
Kono, M., Matsui, T., Shimizu, C., 1987a. Effect of chitin, chitosan, and cellulose as diet supplements on the growth of cultured fish. Nippon Suisan Gakkaishi 53 (1), 125–129. https://doi.org/10.2331/suisan.53.125.
Kono, M., Matsui, T., Shimizu, C., 1987b. Effect of chitin, chitosan, and cellulose as diet supplements on the growth of cultured fish. Nippon Suisan Gakkaishi 53 (1), 125–129.
Kousoulaki, K., Sether, B.S., Albrektsen, S., Noble, C., 2015. Review on European sea bass (Dicentrarchus labrax, Linnaeus, 1758) nutrition and feed management: a practical guide for optimizing feed formulation and farming protocols. Aquac. Nutr. 21 (2), 129–151. https://doi.org/10.1111/anu.12233.
Leenhouwers, J.I., Pellikaan, W.F., Huizing, H.F.A., Coolen, R.O.M., Verreth, J.A.J., Schrama, J.W., 2008. Fermentability of carbohydrates in an in vitro batch culture method using inocula from Nile tilapia (Oreochromis niloticus) and European sea bass (Dicentrarchus labrax). Aquac. Nutr. 14 (6), 523–532. https://doi.org/10.1111/j.1365-2095.2007.00558.x.
Lemarié, G., Dosdat, A., Covès, D., Dutto, G., Gasset, E., Person-Le Ruyet, J., 2004. Effect of chronic ammonia exposure on growth of European seabass (Dicentrarchus labrax) juveniles. Aquaculture 229 (1–4), 479–491. https://doi.org/10.1016/S0044-8486(03)00392-2.
Mastoraki, M., Panteli, N., Kotzamanis, Y.P., Gasco, L., Antonopoulou, E., Chatzifotis, S., 2022. Nutrient digestibility of diets containing five different insect meals in gilthead sea bream (Sparus aurata) and European sea bass (Dicentrarchus labrax). Anim. Feed Sci. Technol. 292. https://doi.org/10.1016/j.anifeedsci.2022.115425.
Meriac, A., Eding, E.H., Schrama, J., Kamstra, A., Verreth, J.A.J., 2014. Dietary carbohydrate composition can change waste production and biofilter load in recirculating aquaculture systems. Aquaculture 420–421, 254–261. https://doi.org/10.1016/j.aquaculture.2013.11.018.
Michaud, L., Blancheton, J.P., Bruni, V., Piedrahita, R., 2006. Effect of particulate organic carbon on heterotrophic bacterial populations and nitrification efficiency in biological filters. Aquac. Eng. 34 (3), 224–233. https://doi.org/10.1016/j.aquaeng.2005.07.005.
Moreira, I.S., Peres, H., Couto, A., Enes, P., Oliva-Teles, A., 2008. Temperature and dietary carbohydrate level effects on performance and metabolic utilisation of diets in European sea bass (Dicentrarchus labrax) juveniles. Aquaculture 274 (1), 153–160. https://doi.org/10.1016/j.aquaculture.2007.11.016.
NRC, 2011. Nutrient Requirement of Fish and Shrimp. National Academies Press.
Oliva-Teles, A., Enes, P., Peres, H., 2015. Replacing fishmeal and fish oil in industrial aquafeeds for carnivorous fish. In: Feed and Feeding Practices in Aquaculture. Elsevier, pp. 203–233. https://doi.org/10.1016/B978-0-08-100506-4.00008-8.
Pedersen, M.B., Dalsgaard, S., Knudsen, K.B., Yu, S., Lærke, H.N., 2014. Compositional profile and variation of distillers dried grains with solubles from various origins with focus on non-starch polysaccharides. Anim. Feed Sci. Technol. 197, 130–141.
Peng, Y., Hu, J., Yang, B., Lin, X.P., Zhou, X.F., Yang, X.W., Liu, Y., 2015. Chemical composition of seaweeds. In: Seaweed Sustainability: Food and Non-Food Applications. Elsevier Inc., pp. 79–124. https://doi.org/10.1016/B978-0-12-418697-2.00005-2
Peres, H., Oliva-Teles, A., 2002. Utilization of raw and gelatinized starch by European sea bass (Dicentrarchus labrax) juveniles. Aquaculture 205, 287–299. www.elsevier.com/locate/aqua-online.
Prakash, S., Maas, R.M., Fransen, P.M.M.M., Kokou, F., Schrama, J.W., Philip, A.J.P., 2023. Effect of feed ingredients on nutrient digestibility, waste production and physical characteristics of rainbow trout (Oncorhynchus mykiss) faeces. Aquaculture 574. https://doi.org/10.1016/j.aquaculture.2023.739621.
Reyes, M., Rodríguez, M., Montes, J., Barroso, F.G., Fabrikov, D., Morote, E., Sánchez-Muros, M.J., 2020. Nutritional and growth effect of insect meal inclusion on seabass (Dicentrarchuss labrax) feeds. Fishes 5 (2). https://doi.org/10.3390/fishes5020016.
Rojas-Tirado, P., Pedersen, P.B., Vadstein, O., Pedersen, L.F., 2018. Changes in microbial water quality in RAS following altered feed loading. Aquac. Eng. 81, 80–88. https://doi.org/10.1016/j.aquaeng.2018.03.002.
Schumann, M., Brinker, A., 2020. Understanding and managing suspended solids in intensive salmonid aquaculture: a review. Rev. Aquac. 12 (4), 2109–2139. https://doi.org/10.1111/raq.12425.
Schumann, M., Holm, J., Brinker, A., 2022. Effects of feeding an all-plant diet on rainbow trout performance and solid waste characteristics. Aquac. Nutr. 2022. https://doi.org/10.1155/2022/1694245.
Siddhanta, A.K., Goswami, A.M., Ramavat, B.K., Mody, K.H., Mairh, O.P., 2001. Water soluble polysaccharides of marine algal species of Ulva (Ulvales, Chlorophyta) of Indian waters.
Sinha, A.K., Kumar, V., Makkar, H.P.S., De Boeck, G., Becker, K., 2011. Non-starch polysaccharides and their role in fish nutrition - a review. In: Food Chemistry, 127, issue 4, pp. 1409–1426. https://doi.org/10.1016/j.foodchem.2011.02.042.
Sosulski, F.W., Imafidon, G.I., 1990. Amino acid composition and nitrogen-to-protein conversion factors for animal and plant foods. J. Agric. Food Chem. 38 (6), 1351–1356. https://pubs.acs.org/sharingguidelines.
Timmons, M.B., Michael, B., Ebeling, J.M., Northeastern Regional Aquaculture Center (U.S.), 2007. Recirculating aquaculture. Cayuga Aqua Ventures.
Torno, J., Einwächter, V., Schroeder, J.P., Schulz, C., 2018. Nitrate has a low impact on performance parameters and health status of on-growing European sea bass (Dicentrarchus labrax) reared in RAS. Aquaculture 489, 21–27. https://doi.org/10.1016/j.aquaculture.2018.01.043.
Vandeputte, M., Gagnaire, P.A., Allal, F., 2019. The European sea bass: A key marine fish model in the wild and in aquaculture. In: Animal Genetics, 50, Issue 3. Blackwell Publishing Ltd., pp. 195–206. https://doi.org/10.1111/age.12779
Wassef, E.A., El-Sayed, A.F.M., Sakr, E.M., 2013. Pterocladia (Rhodophyta) and Ulva (Chlorophyta) as feed supplements for European seabass, Dicentrarchus labrax L., fry. J. Appl. Phycol. 25 (5), 1369–1376. https://doi.org/10.1007/s10811-013-9995-5.
Welker, T.L., Liu, K., Overturf, K., Abernathy, J., Barrows, F.T., 2021. Effect of soy protein products and gum inclusion in feed on fecal particle size profile of rainbow trout. Aquacult. J. 1 (1), 14–25. https://doi.org/10.3390/aquacj1010003.
Xu, B., Liu, Y., Chen, K., Wang, L., Sagada, G., Tegomo, A.F., Yang, Y., Sun, Y., Zheng, L., Ullah, S., Shao, Q., 2021. Evaluation of Methanotroph (Methylococcus capsulatus, Bath) Bacteria meal (FeedKind®) as an alternative protein source for juvenile Black Sea bream, Acanthopagrus schlegelii. Front. Mar. Sci. 8. https://doi.org/10.3389/fmars.2021.778301.
Yi, X., Li, J., Xu, W., Zhou, H., Smith, A.A., Zhang, W., Mai, K., 2015. Shrimp shell meal in diets for large yellow croaker Larimichthys croceus: effects on growth, body composition, skin coloration and anti-oxidative capacity. Aquaculture 441, 45–50. https://doi.org/10.1016/j.aquaculture.2015.01.030.