Aquaculture Reports 36 (2024) 102179

Contents lists available at ScienceDirect

Aquaculture Reports

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

Effect of carbohydrate source, feeding level (restricted vs. satiation) and their combination on nutrient digestibility, bile acid balance, faecal waste production and characteristics of yellowtail kingfish (*Seriola lalandi*)

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ARTICLE INFO

Keywords:
Non-starch polysaccharides
fibre
starch
fat digestibility
faecal quality
faeces recovery

ABSTRACT

The effect of carbohydrate source on nutrient digestibility, bile acid balance, faecal waste production and characteristics were investigated in yellowtail kingfish (*Seriola lalandi*). A starch diet and two non-starch polysaccharides (NSP) diets with different NSP sources were used in this study. A diet containing 12 % gelatinized wheat flour as the starch diet, and two diets with distinct non-starch polysaccharides (NSP) sources: soybean hulls (SH) and sugar beet pulp (SBP) at an inclusion level of 10 %, were studied. Each diet was tested in triplicate fish tanks. To determine if feeding level affects diet, restricted and satiation feeding levels were used. After four weeks of restrictive feeding, fish were fed satiation for two weeks. The dietary effect on nutrient digestibility and faecal waste production was dependent on feeding level, whereas the dietary effect on faecal waste characterises was independent of feeding level. SBP diet protein and fat digestibility was highest during restricted and satiation feeding. Satiation feeding reduced nutrient digestibility in all diets, most pronounced in SH and least in SBP. Faecal bile acid loss did not explain fat digestibility differences between the three diets. Both NSP diets increased faecal waste production but decreased bile acid content, resulting in similar bile acid loss to the starch diet. SBP had the highest faecal removal efficiency and the lowest non-removed faeces despite high waste production SH resulted in similar amount of non-removed faeces compared to the starch diet. To conclude, NSP type affected nutrient digestibility, bile acid balance, faecal waste production, and faecal characteristics differently than starch in yellowtail kingfish. Starch resulted in higher FCR and thus lower growth performance in yellowtail kingfish compared to NSP despite higher organic matter digestibility. Adding NSP to their RAS diet may improve faecal integrity without affecting macronutrient digestibility. However, different NSP forms affect nutrient digestion and faeces integrity differently.

1. Introduction

Global aquaculture production has increased from 34 Mt in 1997–120 Mt in 2019 and is expected to continue to grow (Naylor et al., 2021; Verdegem et al., 2023). However, this development faces a challenge: the availability of fishmeal and fish oil has become limited due to the increasing demand for aquafeed and declining stocks of wild fish. This has resulted in a movement toward using more plant-based ingredients in aquafeed (Gatlin et al., 2007), which has led to an increased inclusion of carbohydrates (e.g. starch and non-starch polysaccharides (NSP)). However, carbohydrate-rich ingredients are significantly less digestible by carnivorous fish compared to fishmeal and fish oil. Moreover, they might impair the digestion of other nutrients in the feed (Sinha et al., 2011). A recent study in yellowtail kingfish (*Seriola lalandi*) showed that a dietary starch level above 6 % can have a negative effect on digestibility of dietary protein and dietary fat (Zhang et al., unpublished data).

Additionally, yellowtail kingfish, as a carnivorous species, has

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Abbreviations: NSP, non-starch polysaccharides; PSD, particle size distribution; TSS, total suspended solids; ADC, Apparent digestibility coefficient; EHC, enterohepatic circulation; ANF, anti-nutritional factors.

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https://doi.org/10.1016/j.aqrep.2024.102179
Received 7 December 2023; Received in revised form 29 May 2024; Accepted 30 May 2024

relatively low fat digestion (in a diet with fish oil as the primary fat source; Zhang et al., 2024). Compared to rainbow trout (*Oncorhynchus mykiss*) (Staessen et al., 2020a), this low fat digestibility in yellowtail kingfish was associated with a relatively high loss of bile acids in their faeces. Bile acids are essential for fat digestion, by facilitating fat emulsification and assisting in fat transportation into the gastrointestinal tract through the formation of micelles (Macierzanka et al., 2019). The majority of bile acids are reabsorbed from the gut and recirculated to the liver, while only a small amount is excreted with the faeces. Reduced fat digestibility coincided with increased faecal bile acid losses in yellowtail kingfish when fed high-starch diets (Zhang et al., submitted). A high starch diet increased faecal waste production due to starch's negative effect on overall nutrient digestion. Additionally, increased levels of dietary starch resulted in more unstable faeces (Horstmann et al., 2023c). This puts pressure on waste management in recirculating aquaculture production systems (RAS). To minimize the breakdown of faecal waste into smaller suspended particles, maintain optimal system performance, and provide good water quality for fish health, quick and effective removal of faecal waste is critical in RAS (Amirkolaie, 2011; Brinker et al., 2005; Moran et al., 2009; Schumann et al., 2017).

Although carbohydrates consist of a variety of fractions, only starch (highly digestible) has been studied in yellowtail kingfish (Booth et al., 2013; Horstmann, 2023a; 2023c). There is no information available about the effect of non-digestible carbohydrates, such as NSP on nutrient digestion and faecal characteristics in yellowtail kingfish. In general, NSP are complex groups and can be classified as cellulose, non-cellulosic polymers and pectic polysaccharides . NSP can impair the digestion of other dietary macronutrients and the activity of fish endogenous enzymes (Sinha et al., 2011). Also, the effect of NSP on nutrient digestibility and faecal characteristics may vary depending on the NSP type and fish species (Amirkolaie et al., 2005; Fountoulaki et al., 2022). For example, adding cellulose to diets for seabass (*Dicentrarchus labrax*) did not influence protein digestibility but did enhance faecal firmness (Dias et al., 1998). In contrast, adding different NSP-rich ingredients to a the diet (alginate, guar gum, soyhulls and wheat bran) reduced protein and fat digestibility in rainbow trout (Storebakken, 1985; Staessen et al., 2022; Staessen et al., 2020ab). A mixture of soyhull and wheat bran (rich in NSP) decreased fat digestibility in rainbow trout, which was associated with increased bile acid loss through the faeces due to increased faecal waste production (Staessen et al., 2020a). Hereby, the negative effect of NSP on fat digestibility and faecal waste production was stronger during satiation feeding than during restricted feeding (Staessen et al., 2020a). NSP may also play a crucial role on nutrient digestion and faecal characteristics in carnivorous species (including yellowtail kingfish), but there is not much information on this. Furthermore, yellowtail kingfish are typically fed (close) to satiation in practice, whereas the majority of the current digestive research has been conducted under restricted feeding conditions.

The aim of this study was to determine 1) whether NSP has different effects compared to starch on nutrient digestibility, bile acid balance, faecal waste output, and characteristics in yellowtail kingfish, and 2) whether the response is dependent on the type of NSP. Therefore, an experiment was done using three diets with different carbohydrate sources. A diet containing 12 % gelatinized wheat flour resulted in a starch-rich diet, while two diets including 10 % soyhull (SH) or sugar beet pulp (SBP) were used to increase the NSP content. SH contains relatively high levels of cellulose and hemicellulose, whereas SBP contains fairly high levels of pectins. To determine if feeding level impacts the dietary effect, a restricted feeding period (4-wks) was followed by a satiation feeding period (2-wks).

2. Materials and methods

2.1. Diets

The effect of carbohydrate source and feeding level on nutrient digestibility, bile acid balance, faecal waste production, and characteristics of yellowtail kingfish was investigated. Three diets were formulated: a starch diet and two NSP diets differing in NSP sources (Table 2). A basal diet was formulated with 45 % plant protein ingredients and 20 % fish meal as protein sources. Gelatinized wheat flour (WF) was added at a 12 % inclusion level of the total diet to create a starch-rich diet. Two NSP sources were investigated: soybean hulls (SH) and sugar beet pulp (SBP), both of which were added at a 10 % level of the total diet. The crude protein, crude fat, and carbohydrate content of the three diets (on dry matter) were comparable. To prevent deficiencies and meet the nutrient requirements, diets were supplemented with taurine, DL-methionine and monocalcium phosphate. All diets contained at least 11 % fish oil to ensure the requirements for essential fatty acids were met. The basal ingredient mixture is shown in Table 1, and the ingredient composition and analysed nutrient composition of diets are given in Table 2. Diets were produced by cold pelleting as described by Horstmann et al. (2023c) in accordance with Kals et al. (2019), resulting in 3 mm sinking pellets.

2.2. Fish, rearing conditions and housing facilities

The experiment was approved by the Animal Welfare Body of Wageningen University, The Netherlands. All procedures applied to the animals were in line with the Dutch legislation (Act on Animal Experiments) and were classified as not being an animal experiment (non-invasive). The experiment was conducted in the research facility of Carus-ARF, Wageningen University. Yellowtail kingfish (*Seriola lalandi*) of mixed sex were obtained from a commercial fish farm (Kingfish Zeeland, Kats, The Netherlands). At both the start and end of the restricted and satiation feeding period, fish were batch weighted (Mettler-Toledo ICS429). The day prior to weighing, fish were not fed (starved). Each tank was stocked with 25 fish, with a mean initial weight

Table 1
Ingredient composition of the basal mixture for the experimental diets.

Basal ingredient mixture (g/kg)	
Fish meal LT[a]	197.05
Wheat gluten[b]	150
Pea protein concentrate[c]	150
Soya protein concentrate[d]	150
Fish oil[e]	130
Monocalcium phosphate	10
DL-methionine	4
Taurine	10
Casein	130
Pellet binders[f]	50
Premix[g]	18.75
Yttrium oxide	0.2

[a] Faroese Fish meal, minimally 71 % CP LT (Köster Marine Proteins GmbH, Hamburg, Germany).

[b] Amygluten (Tereos Starch & Sweeteners, Aalst, Belgium).

[c] Pisane F0 (Cosucra, Warcoing, Belgium).

[d] Soycomil R (ADM Speciality Ingredients B.V., Amsterdam, The Netherlands).

[e] Fish oil (BioCeval GmbH & Co. KG, Cuxhaven, Germany).

[f] Pellet binders – in house composition

[g] Premix composition. Vitamins (IU or mg/kg complete diet): Vitamin B1, 15 mg; Vitamin B2, 15 mg; Vitamin B6, 15 mg; Vitamin B5, 50 mg; Vitamin B3, 150 mg; Biotine, 0.7 mg; Vitamin B12, 0.05 mg; Folic acid, 3 mg; Vitamin C, 500 mg (given as ascorbic acid C phosphate); Vitamin E, 100 IU; Vitamin A, palmitate, 10,000 IU; Vitamin D3–500, 2500 IU; Vitamin K3 (K-menadione sodium bisulphite, 51 %), 15 mg; Inositol, 450 mg; Betaine, 500 mg; Choline (given as choline chloride), 1000 mg. Anti-oxidant BHT (E300–321), 100 mg; Calcium propionate, 1000 mg. Minerals (mg/kg complete diet): Fe, (as ferric sulphate), 50 mg; Zn (as zinc sulphate), 80 mg; Co (as cobalt sulphate), 0.2 mg; Cu (copper sulphate), 8 mg; Se, (as sodium selenite) 0.2 mg; Mn (as manganese sulphate), 30 mg; Mg (as magnesium sulphate), 750 mg; Cr (as chromic chloride), 1 mg; I (as calcium iodate), 2 mg.

Table 2
Ingredient composition and analysed nutrient composition of the experimental diets.

Diet NSP inclusion	Diet WF	Diet SH	Diet SBP
Ingredients (g/kg)			
Gelatinized wheat flour	120	-	-
Soyhulls	-	100	-
Sugar beet pulp	-	-	100
Basal mixture	880	900	900
Analysed nutrient content (g/kg DM):			
Dry matter (DM, g/kg)	929	937	959
Crude protein	586	597	583
Crude fat	155	154	160
Total carbohydrates[a]	168	152	158
Starch and sugars	129	42	38
NSP[b]	39	110	120
Gross energy (kJ/g DM)	22.7	22.9	22.6
Crude ash	91.3	96.6	99.1
Phosphorus	14.0	13.7	13.2
Calcium	10.7	11.8	11.9
Bile acid (umol/g DM)	0.12	0.13	0.13
Starch gelatinization degree (%)	75.8	27.7	27.6
In vitro viscosity (cP[c])	1.82	1.38	1.56

CON – control diet without NSP; SH – soyhulls; SBP - sugar beet pulp;

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

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

[c] cP, centipoise.

of 48 g. The fish were fed restrictively for 4 weeks, followed by 2 weeks of satiation feeding. The number of fish in each tank was reduced to 17 at the start of the satiation feeding period to guarantee that the biofilter carrying capacity of the RAS was not exceeded and that the oxygen level in the tank remained above the predetermined threshold (See supplemental table 1). Diets were randomly assigned to one of the tanks with 3 replicates per diet. Each tank was connected to a swirl separator equipped with a glass bottle for collecting faeces and uneaten feed. These tanks ($n = 9$) were connected to a single RAS that was filled with artificial seawater. The RAS included a sump, settling tank, drum filter, protein skimmer, oxygen cone, UV, and trickling filter. All water quality parameters were measured daily, and stayed withing the pre-set range: 24 ± 0.5°C for water temperature; 8.0 ± 0.05 L/min for water flow; the range of 7.3–8.1 for pH; 34.0 ± 1.0 ppm for salinity. Maximum allowable values for TAN (total ammonium nitrogen), NO_2-N, NO_3-N concentrations were <2 mg/L, <1 mg/L, and <100 mg/L, respectively. The oxygen level in the tanks remained above 5.5 mg/L. The photoperiod was set at 20 L:4D during the entire duration of the experiment. Light went on at 7:30 am and switched off at 3:30 am.

2.3. *Experimental procedures and sampling*

The experiment lasted 41 days, consisting of a 27-day restricted feeding followed by a 14-day satiation feeding period. During restricted feeding, an estimated feeding level of 20.0 $g/kg^{0.8}$ BW day^{-1} was applied, whereby the feeding level was increased based on the feed intake and expected growth using an expected FCR of 0.9. The daily feed ration was divided equally between morning (9:00 h) and afternoon (15:00 h) feeding, and the fish finished their meals within 30 minutes. During restricted feeding, the feeding level was gradually increased during the first four feeding moments of the experiment until the intended feeding level was reached. This allowed the fish to adapt to the diet and prevented feed spillage. During the satiation feeding period, the feeding level was gradually increased until apparent satiation in the first three days (five days of the SBP diet). This allowed the fish to adapt to the increased feeding level. During satiation feeding, fish were fed from a container containing a predetermined amount of feed until they stopped eating, resulting in significant feed spillage with a maximum of 1 h per feeding moment. Fish were hand fed twice a day at 9:00 and 15:00 h. Fifteen minutes after feeding, the glass bottles attached to the swirl separators were checked for feed spills, and the leftover feed in the cups was weighted to calculate the feed intake per feeding moment per tank. Mortality was checked at least twice a day before feeding.

During week 4 and week 6 of the experiment, faeces collection for digestibility analysis was done overnight (16:00 h – 8:30 h) for 5 days. Bottles connected beneath the swirl separators, were kept submerged in ice water (in a Styrofoam box) to minimize bacterial degradation of collected faeces. Collected faeces were pooled per tank and week (4 and 6) and stored at −20 °C until further analysis. Faeces collection for determination of faecal removal efficiency was done during the weekend of week 4 (restricted feeding) and 6 (satiation feeding). The collection method was the same as for the faeces collection for digestibility, except that faecal material was collected continuously for two days (including day collection; but excluding feeding moments). Faeces collection for determination of faecal particle size distribution (PSD) by sieving was done once during the last week of both restricted and satiation feeding periods (3 h collection during the day after morning feeding in week 4 and week 6). After collection, faecal samples for PSD were stored on ice until further analysis. Feed samples were collected weekly by pooling 50 g of each experimental diet (stored at 4 °C).

2.4. *Analysis*

The analyses of feed and faeces for proximate analyses (for digestibility and faecal removal efficiency) was performed identical as described in Staessen et al. (2020a). Faecal PSD was analysed as described in Horstmann et al., (2023a), (2023b), (2023c), with the exception that the entire sample collected after morning feeding (3 hour collection) was used (no subsampling). The analyses of bile acid content in feed and faeces were identical as described in Zhang et al., (under revision), and based on Porter et al. (2003).

2.5. *Calculations and data analysis*

Absolute growth ($Growth_{abs}$, g/d) was calculated as the difference between the average individual initial (W_i) and final (W_f) body weight (g) divided by the number of days during the experimental period (t). Mean body weight (BW) was calculated as $(W_i + W_f)/2$. Specific growth rate (SGR; %/d) was calculated as $(\ln W_f - \ln W_i)/t \times 100$ %. Absolute feed intake (FI_{abs}; g DM/d) was calculated as FI_{tot}/t, where FI_{tot} is the total feed intake (g DM). Feed intake per metabolic body weight (FI_{mbw}; g $DM/kg^{0.8}/d$) 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 $e^{((\ln Wf+\ln Wi)/2)}$. Feed conversion ratio (FCR) was calculated as $(FI_{tot} \times Diet_{DM}/1000)/(W_f - W_i)$, where $Diet_{DM}$ is the dry matter content of the diet (g/kg). Survival (%) was calculated as $(1 - ((N_i - N_f)/N_i)) \times 100$, where N_i is the number of fish at the beginning and N_f the final number of at the end of the experiment.

Apparent digestibility coefficient (ADC, %) of organic matter, crude protein, crude fat, carbohydrate, starch, energy and bile acids were calculated according to Cheng and Hardy (2002) using yttrium as inert marker: $ADC (\%) = 100 \times (1 - ((Y_{diet}/Y_{faeces}) \times (N_{faeces}/N_{diet})))$, where Y is the inert marker percentage in diet or faeces and N is the nutrient percentage (or kJ/g for gross energy) in diet or faeces.

Faecal waste production (g OM/kg DM feed), faecal removal efficiency (%), removed and non-removed faeces (g OM/kg DM feed) per unit of feed were calculated during week 4 and week 6 according to (Horstmann et al., 2023c). Faecal PSD was determined by sieving as $P_{fraction}/P_{total}$, where $P_{fraction}$ is the collected organic matter within a respective fraction (< 40 μm, 40 – 100 μm, 100 – 250 μm, 250 – 850 μm or > 850 μm) and P_{total} is the total collected organic matter of all fractions.

Bile acid intake (μmol d^{-1}) was calculated as feed intake × bile acid content in the feed. Faecal bile acid loss (μmol d^{-1}) was calculated as the amount of OM excreted via faeces per day (g OM d^{-1}) × faecal bile acid content (μmol g^{-1} OM). The amount of faeces produced was calculated as the daily OM intake × (100 % - ADC$_{OM}$), where ADC$_{OM}$ is the organic matter digestibility during week 4 or week 6, respectively. Bile acid balance was calculated as bile acid intake – faecal bile acid loss. Bile acid intake, faecal bile acid loss, and bile acid balance were expressed per kg body weight (BW) (μmol kg^{-1} BW d^{-1}), using the mean body weight during the experiment (BW).

2.6. Statistical analysis

Tank was used as the experimental unit ($n = 9$) in the statistical analysis for the effect of dietary treatments. Data were analysed using a mixed model ANOVA for the effect of diet, feeding level and their interaction effect. In this model, a random tank effect was taken into account. The effect of diet was tested against the variation between tanks. The effect of feeding level, and its interaction with diet was tested against the variation within tanks. Since the interaction was significant for several parameters, a one-way ANOVA was performed to examine the effect of diet (carbohydrate source) individually for the restricted and satiation feeding period. In the case of a significant diet effect ($p<0.05$), a Tukey HSD test (honest significant difference; 95 % significance level) was performed to compare treatment means. Statistical analyses were performed using the statistical program SAS 9.4, SAS Institute, North Carolina, USA.

3. Results

3.1. Fish performance

The fish performance results are presented in Table 3. Survival was high and unaffected by diet ($p>0.05$). FCR was affected by the interaction effect of diet and feeding level ($p<0.05$). The dietary effect on FCR was more pronounced during the satiation feeding period compared to the restricted feeding period. During restricted feeding, there was a tendency of dietary treatment on the FCR with numerically lower (and thus better) values for both the SH and SBP diet ($p=0.069$) than the starch diet. No difference in FCR was observed between the SH and SBP diets during restricted feeding ($p>0.05$). During satiation feeding, the starch diet had the highest FCR (0.93), and the SBP diet had the lowest FCR (0.82, $p<0.01$). The increase in feed intake relative to metabolic body weight was highest for fish fed the starch diet and lowest for fish fed the SBP diet (FI$_{MBW}$, interaction: $p<0.01$). The higher FCR ($p<0.01$) and feed intake ($p<0.001$) of the starch diet resulted in growth similar to the SH and SBP diets ($p>0.05$).

3.2. Digestibility, faecal waste production and removal efficiency

The results of apparent digestibility coefficients (ADC, %) and faecal waste production are given in Table 4. The ADC of all nutrients was influenced by the interaction of diet and feeding level (interaction: $p<0.05$). The effect of diet was depended on the feeding level. During the restricted feeding period, both NSP diets reduced OM ADC compared to the starch diet ($p<0.001$), with no difference between the two NSP diets ($p>0.05$). While during the satiation feeding period, fish fed the SBP diet had a 3.5 % higher OM ADC than those fed the SH diet ($p<0.001$). In contrast to OM ADC, protein and fat ADC were highest for the SBP diet during both restricted and satiation feeding ($p<0.01$). The SH diet showed higher protein and fat ADC than the starch diet during the restricted feeding period ($p<0.05$), but had similar protein and fat ADC compared to the starch diet during satiation feeding ($p>0.05$). Additionally, a high feeding level had a negative impact on OM ADC, with fat ADC being the most affected ($p<0.001$). The decrease in fat ADC averaged 8.2 % over all diets. The interaction effect of diet and feeding level in fat ADC indicated that the effect was the most pronounced for the SH diet. The decrease in fat ADC was 12.0 % points in the SH diet, whereas it was 6.4 % points for the starch diet and 6.1 % points for the SBP diet (interaction: $p<0.05$).

For all faecal waste parameters, an interaction effect between diet and feeding level was only present for the total amount of faecal waste production ($p<0.01$). For all diets, more faecal waste (g OM/kg DM

Table 3
Fish performance of yellowtail kingfish fed the experimental diets during restricted feeding (27 days) and satiation feeding (14 days).

Diet	Feeding period	Diet			*SEM*	p-value		
Carbohydrate sources		**WF**	**SH**	**SBP**		**Diet**	**F**	**Diet x F**
Survival (%)	**R**	100	98.7	100		ns		
	S	100	100	100	-	-		
	R+S							
Initial body weight (g)	**R**	47	48	47	0.8	-		
	S	130	132	131	1.0	ns		
	R+S					ns	***	ns
Final body weight (g)	**R**	129	131	131	1.1	ns		
	S	227	231	228	2.2	ns		
	R+S					ns	***	ns
FI$_{abs}$ (g DM/fish/d)	**R**	2.17	2.18	2.18	0.000	-		
	S	6.44^{c}	6.05^{b}	5.70^{a}	0.090	***		
	R+S							
FI$_{MBW}$ (g DM/d/kg$^{0.8}$)	**R**	15.5	15.3	15.3	0.14	ns		
	S	25.7^{b}	24.2^{ab}	22.6^{a}	0.40	**		
	R+S					**	**	**
Growth$_{abs}$ (g/d)	**R**	3.03	3.10	3.10	0.016	*		
	S	6.96	7.08	6.93	0.103	ns		
	R+S					ns	***	ns
SGR (%/d)	**R**	3.73	3.76	3.77	0.034	ns		
	S	4.00	4.01	3.95	0.040	ns		
	R+S					ns	***	ns
FCR	**R**	0.72	0.71	0.70	0.004	#		
	S	0.93^{b}	0.87^{ab}	0.82^{a}	0.014	**		
	R+S					**	***	*

WF - gelatinized wheat flour, the starch diet; SH – soyhull; SBP – sugar beet pulp; F – feeding level; R – restricted feeding; S – satiation feeding; FI$_{abs}$ – feed intake absolute; FI – feed intake on metabolic body weight; Growth$_{abs}$ – growth absolute; SGR – specific growth rate; FCR – feed conversion ratio (on DM basis). Values are means (n=3) and the standard error of the means (SEM); in the case of a significant treatment effect, means within the same row not sharing a common letter are different ($p<0.05$); ns - not significant $p>0.1$; # - tendency $p<0.1$. * - $p<0.05$; ** - $p<0.01$. *** - $p<0.001$.

Table 4

Apparent digestibility coefficient (ADC, %) and faecal waste production of yellowtail kingfish fed the experimental diets during restricted feeding (27 days) and satiation feeding (14 days).

Diet	Feeding period	Diet			*SEM*	p-value		
Carbohydrate sources		**WF**	**SH**	**SBP**		**Diet**	**F**	**Diet x F**
Organic matter	**R**	88.4^{a}	82.0^{b}	82.6^{b}	0.22	***		
	S	85.4^{a}	76.0^{c}	79.5^{b}	0.46	***		
	R+S					***	***	**
Crude protein	**R**	96.5^{a}	96.8^{b}	97.2^{c}	0.06	***		
	S	94.2^{a}	94.6^{a}	95.7^{b}	0.17	**		
	R+S					***	***	*
Crude fat	**R**	90.2^{a}	93.2^{b}	94.5^{b}	0.45	**		
	S	83.8^{ab}	81.2^{a}	88.4^{b}	1.05	**		
	R+S					**	***	*
Total carbohydrates	**R**	58.7^{a}	12.5^{b}	16.8^{b}	1.25	***		
	S	56.0^{c}	-2.4^{a}	10.4^{b}	1.64	***		
	R+S					***	***	***
Starch and sugars	**R**	81.0^{a}	83.5^{ab}	85.2^{b}	0.81	*		
	S	81.8	76.3	75.1	2.32	ns		
	R+S					ns	**	*
NSP	**R**	-14.1^{a}	-13.9^{a}	-5.1^{b}	1.76	*		
	S	-28.5^{a}	-31.6^{a}	-10.4^{b}	1.69	***		
	R+S					**	***	***
Energy	**R**	90.6	88.2	88.4	0.68	#		
	S	86.9^{a}	80.2^{c}	84.2^{b}	0.49	***		
	R+S					***	***	*
Phosphorus	**R**	65.4^{a}	69.0^{b}	68.7^{b}	0.41	**		
	S	63.7^{b}	59.5^{a}	60.6^{a}	0.62	**		
	R+S					Ns	***	***
Faecal waste:								
Total amount of faeces (g OM/kg DM Feed)	**R**	116^{a}	180^{b}	174^{b}	2.2	***		
	S	146^{a}	240^{c}	205^{b}	4.6	***		
	R+S					***	***	**
Removed faeces (g OM/kg DM Feed)	**R**	30^{a}	81^{b}	103^{c}	4.9	***		
	S	44^{a}	118^{b}	125^{b}	5.4	***		
	R+S					***	**	ns
Non-removed faeces (g OM/kg DM Feed)	**R**	85^{ab}	100^{b}	70^{a}	4.9	*		
	S	106^{b}	122^{b}	72^{a}	5.3	**		
	R+S					***	*	ns
Faecal removal efficiency (%)	**R**	26^{a}	45^{b}	60^{c}	2.8	***		
	S	29^{a}	49^{b}	65^{c}	2.5	***		
	R+S					***	ns	ns

WF - gelatinized wheat flour, the starch diet; SH – soyhull; SBP – sugar beet pulp; F – feeding level; R – restricted feeding; S – satiation feeding; NSP – non-starch polysaccharides; Values are means (n=3) and the standard error of the means (SEM); in the case of a significant treatment effect, means within the same row not sharing a common letter are different ($p<0.05$); ns - not significant $p>0.1$; # - tendency $p<0.1$. * - $p<0.05$; ** - $p<0.01$. *** - $p<0.001$.

feed) was produced during satiation feeding compared to restricted feeding ($p<0.001$) in line with the lower OM ADC. During both restricted and satiation feeding periods, fish fed the two NSP diets produced a greater amount of faecal waste compared to fish fed the starch diet ($p<0.001$). Furthermore, satiation feeding of the SH diet led to more faecal waste compared to the SBP diet ($p<0.001$), while no difference was found during the restricted feeding period ($p>0.05$). Increasing feeding level did not affect the dietary effect on removed and non-removed faeces. In other words, the differences in removed and non-removed faeces among the three diets during restricted feeding were similar to those during satiation feeding. Faecal removal efficiency was influenced by the diet ($p<0.001$), but not by the feeding level ($p>0.05$). Averaged over feeding levels, faecal removal efficiency of fish fed the SH diet and the SBP diet was 47 % and 63 %, which was 71 % and 127 % higher compared to the faecal removal efficiency of fish fed the starch diet (faeces removal efficiency 28 %). Despite the higher faecal waste production compared to the starch diet, the higher faecal removal efficiency at the SH diet resulted in a similar amount of non-removed faecal waste for both restricted and satiation feeding periods ($p>0.05$;). During restricted feeding, the SBP diet had numerically the lowest amount of non-removed faeces (not statistically different from the starch diet, $p<0.1$). During satiation feeding, the difference was significant, with the SBP diet having 31 % lower non-removed faeces compared to the starch diet and 41 % lower non-removed faeces compared to the SH diet ($p<0.01$).

3.3. *Faecal characteristics*

Image 1 shows the faeces collected overnight for every experimental diet during the restricted feeding period. There quantity of collected faeces varies considerably (visually) depending on the carbohydrate source. Notably, it can be observed that the visual appearance of the faeces from the two NSP diets, in particular the SBP diet, seemed to consist of more faecal pellets, whereas the faeces at the starch diet had a poor faecal integrity.

The data on faecal composition and particle size distribution (PSD) are shown in Table 5. Faecal composition was expressed on OM basis to alleviate the effect of salt coming from the water during faecal collection. An interaction effect between diet and feeding level was observed for crude fat and NSP content in the faeces ($p<0.01$). The largest portion of faecal waste consisted of carbohydrates, with the major fraction of carbohydrate being NSP for all three diets. During both feeding periods, the carbohydrate and NSP content in the two NSP diets were higher ($p<0.001$) compared to that in the starch diet. Furthermore, when fish were fed to satiation, differences in faecal composition were observed between the two NSP sources; faeces from fish fed the SBP diet had a higher carbohydrate and NSP content, along with a lower fat content compared to faeces from fish fed the SH diet ($p<0.001$). Additionally, satiation feeding resulted in higher protein and fat content in the faeces at the expense of carbohydrate (starch and NSP) compared to the restricted feeding ($p<0.001$).

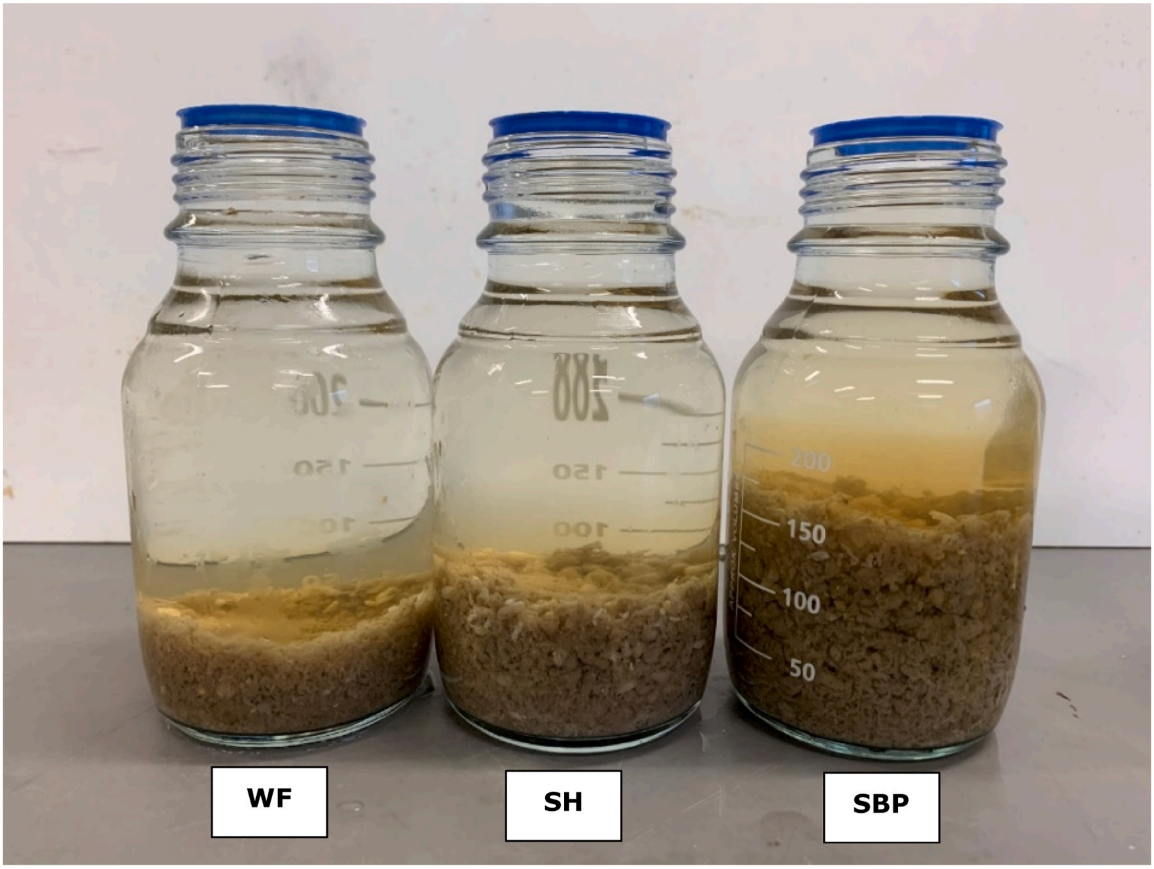

Image 1. Overnight collected faeces of yellowtail kingfish fed the starch diet, the SH diet, and the SBP diet (left to right) to restricted for 27 days. WF - gelatinized wheat flour, the starch diet; SH – soyhull; SBP – sugar beet pulp.

Faecal particle size distribution (PSD) is shown in Table 5. There was no interaction effect between diet and feeding level on PSD ($p>0.1$), with only a tendency for interaction for the fraction 250–850 μm ($p<0.1$). A main effect of diet and feeding level were found in the large PSD fractions (above 100 μm, $p<0.05$). Specifically, larger particles (>850 μm) were observed during satiation feeding period compared to restricted feeding ($p<0.001$). During the restricted feeding, the NSP sources had an effect on the larger PSD fractions of 250 μm-850 μm ($p<0.05$) and >850 μm ($p<0.05$). Fish fed the SH diet had a larger amount of 250 μm-850 μm particles and a smaller amount of >850 μm particles compared to those fed the starch diet and the SBP diet. However, no effect of diet on PSD was found during satiation feeding period ($p>0.05$).

3.4. *Bile acid balance*

The bile acid balance results are given in Table 6. An interaction effect between carbohydrate source and feeding level was found on bile acid intake and faecal waste production ($p<0.01$). There was a tendency for an interaction effect on faecal bile acid losses and bile acid balance ($p<0.1$), but not on faecal bile acid content ($p>0.1$). Feed bile acid content was the same across the diets (0.13 μmol/g DM, see Table 2), resulting in similar bile acid intake during restricted feeding. Due to different feed intake under satiation, bile acid intake was lower in fish fed the SBP diet compared to fish fed the starch diet, while bile acid intake in fish fed the SH diet was not different from the starch diet. During restricted feeding, the averaged faecal bile acid content for the two NSP diets was 38 % lower (mean 3.65 μmol g^{-1} OM) compared to the starch diet (5.9 μmol g^{-1} OM, $p<0.001$), with no difference found in faecal bile acid content between the two NSP diets ($p>0.05$). However, this dietary effect was not present during satiation feeding (tendency; $p<0.1$). Satiation feeding resulted in a 21 % increase in faecal bile acid content as compared to restricted feeding, when averaged across the diets. On the other hand, both NSP diets led to a higher amount of faecal waste production (g OM kg^{-1} BW d^{-1}) compared to the starch diet for both feeding periods ($p<0.001$). Overall, all diets resulted in similar negative bile acid balance for both feeding periods (only a tendency for a diet effect when feeding was restricted). Faecal bile acid loss was approximately five times higher than bile intake for restricted feeding and seven times higher for satiation feeding.

4. Discussion

In the current study, the effect of carbohydrate source, feeding level and their combination on nutrient digestibility, bile acid balance, faecal waste production and faecal characteristics of yellowtail kingfish were investigated. A starch diet and two NSP diets with varying NSP sources were used. Although the NSP composition of SH and SBP was not analysed in this study, literature has shown that SH contains mostly insoluble NSP (Liu and Li,2017), whereas SBP contains relatively more soluble NSP (Liu et al., 2011). Soluble NSP form a network with water, increasing digesta viscosity, whereas insoluble NSP have a lower water-holding capacity and have a lesser impact on digesta viscosity (Sinha et al., 2011). *In vitro* dietary viscosity was 1.82 for the starch diet, 1.38 for the SH diet, and 1.56 for the SBP diet. The carbohydrate content of the three diets (on dry matter) was comparable, but their composition differed (starch vs. NSP). Averaged over feeding level periods, carbohydrate digestibility of yellowtail kingfish fed the starch diet was 57 %, which was substantially higher than that of fish fed the two NSP diets (averaged 9 %). Yellowtail kingfish appears to digest a certain amount of starch but has no capacity to digest NSP, most likely due to a lack of specific enzymes. This is partially supported by the low α-amylase

Table 5

Faecal composition and particle size distribution (PSD, %) of yellowtail kingfish fed the experimental diets during restricted feeding for 27 days and satiation feeding for 14 days.

Diet	Feeding period	Diet			*SEM*	p-value		
Carbohydrate sources		**WF**	**SH**	**SBP**		**Diet**	**F**	**Diet x F**
Organic matter (DM)	**R**	469^{a}	575^{c}	499^{b}	3.1	***		
	S	499^{a}	589^{c}	523^{b}	4.9	***		
	R+S					***	***	ns
Crude protein (g/kg OM)	**R**	197^{c}	119^{b}	104^{a}	1.7	***		
	S	256^{b}	149^{a}	134^{a}	3.6	***		
	R+S					***	**	ns
Crude fat (g/kg OM)	**R**	145^{b}	64^{a}	56^{a}	6.9	***		
	S	189^{c}	133^{b}	101^{a}	7.1	***		
	R+S					***	***	**
Total carbohydrates (g/kg OM)	**R**	658^{a}	817^{b}	840^{b}	6.1	***		
	S	555^{a}	718^{b}	765^{c}	5.9	***		
	R+S					***	***	ns
Starch and sugars (g/kg OM)	**R**	233^{a}	42^{b}	36^{b}	7.7	***		
	S	176^{a}	45^{b}	51^{b}	8.3	***		
	R+S					***	***	#
NSP (g/kg OM)	**R**	425^{a}	774^{b}	803^{c}	4.0	***		
	S	379^{a}	673^{b}	714^{c}	7.2	***		
	R+S					***	**	***
Particle size distribution (%)								
< 20 μm	**R**	2.7	1.9	1.1	0.64	ns		
	S	2.0	1.6	1.6	0.59	ns		
	R+S					ns	ns	ns
20 – 40 μm	**R**	1.3	0.9	0.6	0.48	ns		
	S	0.7	0.6	0.3	0.27	ns		
	R+S					ns	ns	ns
40 – 100 μm	**R**	3.6	4.1	2.1	1.29	ns		
	S	1.9	1.2	1.0	0.46	ns		
	R+S					ns	#	ns
100 – 250 μm	**R**	6.1	6.2	4.1	1.10	ns		
	S	3.4	2.8	1.7	0.91	ns		
	R+S					ns	**	ns
250 – 850 μm	**R**	21.0^{a}	46.1^{b}	16.4^{a}	3.83	**		
	S	13.2	22.5	8.8	5.29	ns		
	R+S					*	**	#
> 850 μm	**R**	65.2^{b}	42.8^{a}	73.8^{b}	5.04	*		
	S	78.8	71.2	86.6	6.70	ns		
	R+S					*	**	ns

WF - gelatinized wheat flour, the starch diet; SH – soyhull; SBP – sugar beet pulp; F – feeding level; R – restricted feeding; S – satiation feeding; NSP – non-starch polysaccharides Values are means (n=3) and the standard error of the means (SEM); in the case of a significant treatment effect, means within the same row not sharing a common letter are different ($p<0.05$); ns - not significant $p>0.1$; # - tendency $p<0.1$. * - $p<0.05$; ** - $p<0.01$. *** - $p<0.001$.

Table 6

Bile acid intake, faecal bile acid loss and bile acid balance of yellowtail kingfish fed the experimental diets during restricted feeding for 27 days and satiation feeding for 14 days.

Diet	Feeding period	Diet			*SEM*	p-value		
Carbohydrate sources		**WF**	**SH**	**SBP**		**Diet**	**F**	**Diet x F**
Bile acid intake (μmol kg^{-1} BW d^{-1})	**R**	2.9	2.8	2.9	0.03	ns		
	S	4.2^{b}	3.9^{ab}	3.8^{a}	0.07	*		
	R+S							
Faecal bile acid content (μmol g^{-1} OM)	**R**	5.9^{b}	3.4^{a}	3.9^{a}	0.15	***		
	S	6.2	4.5	5.3	0.41	#		
	R+S					***	**	ns
Faecal waste production (g OM kg^{-1} BW d^{-1})	**R**	2.6^{a}	4.0^{b}	3.8^{b}	0.05	***		
	S	4.8^{a}	7.6^{c}	5.9^{b}	0.15	***		
	R+S					***	***	**
Faecal bile acid losses (μmol kg^{-1} BW d^{-1})	**R**	15.3	13.4	15.0	0.51	#		
	S	30.0	32.5	30.7	2.05	ns		
	R+S					ns	***	#
Bile acid balance (μmol kg^{-1} BW d^{-1})	**R**	-12.5	-10.6	-12.1	0.52	#		
	S	-25.6	-28.6	-27.0	2.07	ns		
	R+S					ns	***	#

WF - gelatinized wheat flour, the starch diet; SH – soyhull; SBP – sugar beet pulp; F – feeding level; R – restricted feeding; S – satiation feeding; Values are means (n=3) and the standard error of the means (SEM); in the case of a significant treatment effect, means within the same row not sharing a common letter are different ($p<0.05$); ns - not significant $p>0.1$; # - tendency $p<0.1$. * - $p<0.05$; ** - $p<0.01$. *** - $p<0.001$.

activity found in the gastrointestinal tract of yellowtail (*Seriola quinqueradiata*)(Shimento, 1977), whereas no endogenous enzymes for the breakdown of NSP was found in any carnivorous fish species (Kuz'mina, 1996).

Previous research showed that starch has a strong negative effect on nutrient digestibility in yellowtail kingfish (Horstmann, et al., 2023c). It was hypothesized that undigested starch has a similar negative impact on nutrient digestibility as NSP. (Fountoulaki et al., 2005; Hemre et al., 1995). Surprisingly, the results of this study revealed that NSP had different effects on nutrient digestibility than starch in yellowtail kingfish. Starch, while better digested than the two NSP sources, had a greater negative impact on the protein and fat digestibility. Notably, during restricted feeding, the starch diet had the lowest protein and fat digestibility of the three diets. The differences in nutrient digestibility between the three diets may be related to the differences in water solubility, which may result in differences in chyme characteristics such as chyme viscosity. Increased dietary viscosity corresponded with increased digesta viscosity in Nile tilapia (*Oreochromis niloticus L.*) and African catfish (*Clarias gariepinus*) (Amirkolaie et al., 2006; Leenhouwers et al., 2006; 2007ab). In general, increasing digesta viscosity can slow down intestinal transit time by suppressing intestinal contractions (Cherbut et al., 1990), resulting in reduced mixing of dietary components including endogenous digestive enzymes (Johnston et al., 2003). In this study, the reduced protein and fat digestibility observed for the starch diet could be due to high dietary viscosity, and thus digesta viscosity. Furthermore, the reduced protein and fat digestibility in the starch diet may explain the numerically lower growth (g/d) of fish fed the starch diet compared to fish fed the NSP diets. Notably, during satiation, even though fish fed the starch diet had higher carbohydrate digestibility and comparable protein and fat digestibility than fish fed the SH diet, they still had a numerically higher FCR (tendency; 0.93 vs. 0.87). Accordingly, it seems that the digested carbohydrates or starch did not contribute to the growth of yellowtail kingfish.

Although both NSP rich ingredients were not digested (negative NSP digestibility), their effect on other macronutrient digestibility differed between the two NSP diets. The dietary effect also depended on the feeding level. Satiation feeding lowered all nutrient digestibilities in the current study, which is consistent with prior studies in yellowtail kingfish (Horstmann et al., 2023a), Atlantic Salmon (*Salmo salar*) (Rørvik et al., 2010), gilthead sea bream (*Sparus aurata*) (Fernández et al., 1998), African catfish (Elesho et al., 2021; Henken et al., 1985), Nile tilapia (Haidar et al., 2016; Schrama et al., 2012) and rainbow trout (Staessen et al., 2020b). Satiation feeding led to a reduction in nutrient digestibility in all diets, with the decrease being most pronounced in the SH (more insoluble NSP) diet and least pronounced in the SBP (more soluble NSP) diet. In contrast, in pigs soluble NSP was found to lower the digestibility of other macronutrient more than insoluble (Dégen et al., 2007). Similar findings were observed in Atlantic Salmon (Refstie et al., 1999), rainbow trout (Storebakken, 1985), African catfish (Leenhouwers et al., 2006), and Nile tilapia (Amirkolaie et al., 2006). One possible explanation is that the higher dietary solubility causes an increase in intestinal transit time (Burrows et al., 1982), which may benefit digestion by giving endogenous digestive enzymes more time to act and nutrients to be absorbed. Another study with African catfish found that a diet containing a mixture of maize and rye led to intermediate digesta viscosity, resulting in the highest macronutrient digestibility (Leenhouwers, et al., 2007). Perhaps there is a viscosity optimum for nutrient digestion. More research is needed to determine how the solubility of starch and NSP sources affects nutrient digestion kinetics in yellowtail kingfish and other fish species.

Among digestibility of nutrients analysed, fat digestibility was the most affected by the carbohydrate source, this suggests that factors other than physical characteristics (e.g., viscosity) should be considered, such as bile acids. All diets resulted in a negative bile acid balance where the total amount of faecal bile acid loss exceeds the amount of dietary bile acid intake. This would result in a decrease of the total body bile acid pool if endogenous synthesis is not sufficient. Furthermore, the faecal bile acid losses were comparable between the starch diet and the two NSP diets during restrictive and satiation feeding. This indicates that in yellowtail kingfish, NSP do not increase faecal bile acid loss compared to starch. Additionally, there was no difference in FABL between SH and SBP. In contrast, Staessen et al., (2020b) found that the inclusion of NSP increased bile acid loss in the faeces in rainbow trout. Despite similar faecal bile acid losses, the fat digestibility of the three diets differed. This contradicts the studies on rainbow trout (Staessen, 2021; Staessen et al., 2020a; Staessen, et al., 2020b) and our previous research in yellowtail kingfish (Zhang et al., submitted) that found an inverse relationship between fat digestibility and faecal bile acid loss. One possible explanation for this discrepancy could be the presence of saponins in soy hulls, which are known to degrade digestive enzymes (del Hierro et al., 2018; Sinha et al., 2011) and cause enteritis in Atlantic Salmon at high levels (Krogdahl et al., 2015). The relatively large amount of saponins from diets during satiation feeding could influence enzyme activity and gut integrity, evemtuallly affecting fat digestion and absorption, whereas the amount could be too low to cause such adverse effects under restricted feeding, or it could be a lagged response. An early study found a lower total bile acid level and lipase activities in anterior intestinal digesta of yellowtail (*Seriola quinqueradiata*) fed a soybean meal diet than fish fed a fishmeal diet, indicating that soybean meal inhibited bile acid secretion into the intestine,

Previous studies attributed increased faecal bile acid loss to the presence of anti-nutritional factors (ANF) and NSP in plant-based ingredients, which can bind bile acids (Li et al., 2017) and/or lead to increased faecal waste production (Staessen, 2021; Staessen et al., 2020a; Staessen, et al., 2020b). The results of this study showed that the NSP diets had a lowered faecal bile acid content compared to the starch diet, but showed to produce more faecal waste than the starch diet. This supports the finding of Staessen et al., (2020ab) that the main factor driving faecal bile acid loss by NSP was faecal waste production. However, differences in faecal bile acid loss did not reflect on fat digestibility, especially for the SBP diet. This could be attributed to the aforementioned impact of soluble NSP on increasing intestinal transit time, which could lead to increased nutrient digestion and absorption. The highest fat digestibility at the SBP diet may be attributed to the pectin in SBP, which are found to have emulsifying properties (Chen et al., 2016; Funami et al., 2007; Williams et al., 2005).This implies that pectin may break down fat globules into smaller droplets, increasing the surface area available for bile acid and lipase activity. As a result, this emulsifying property may improve the efficiency of bile acid in breaking down fat globules into smaller droplets, increasing the surface area available for bile acid and lipase activity to act on. Furthermore, the loss of bile acids can be compensated by de novo synthesis of bile acids from cholesterol in the liver, a process that varies on species and diet. Furthermore, the loss of bile acids can be compensated by *de novo* synthesis of bile acids from cholesterol in the liver, a process that varies on species and diets (Chiang, 2009; Romano et al., 2020). Staessen et al. (2023) showed that the body bile pool size of rainbow trout fed a starch diet was larger than that of fish fed a fat diet, suggesting a stronger bile acid synthesis, possibly driven by a positive feedback due to low levels of bile acids detected in the intestine and liver (Murashita et al., 2018; Staessen et al., 2021). Although bile acid synthesis was not measured in this study, it is possible that yellowtail kingfish fed the SBP diet during the restricted feeding period had a low bile acid content in the intestine (as indicated by a low faecal bile acid content), which in turn led to upregulated bile acid synthesis and an enlarged body bile acid pool. Furthermore, researchers found that both bile acid pool size and synthesis increased in response to pectin, which was linked to the interaction with bile acid transport in the intestine Cai et al., (2020); Fang et al., (2018). As a result, this could explain the highest fat digestibility in both feeding periods and the smallest reduction in fat digestibility during satiation feeding for the SBP diet, despite similar faecal bile acids loss compared to the other diets. In contrast, fish fed the starch diet had higher faecal bile acid content compared to the SBP diet, potentially lacking this positive feedback mechanism on bile acid synthesis. Overall, our results show that the impact of NSP and starch on nutrient digestion and bile acid balance in yellowtail kingfish differs between.

Faecal waste production, which is the non-digestible fraction of diets, follows OM digestibility. This study found that NSP increased faecal waste production in yellowtail kingfish, which aligns with other fish species e.g., European seabass, Nile tilapia, and common carp (Amirkolaie et al., 2005; Prabhu et al., 2019; Fountoulaki et al., 2022). Furthermore, the SH diet with low dietary viscosity (high level of insoluble NSP) produced more faecal waste than the SBP diet with relatively high dietary viscosity (high level of soluble NSP). Similar results were observed in sea bass (Fountoulaki et al., 2022) and common carp (Prabhu et al., 2019), while no difference was found between soluble and insoluble NSP in Nile tilapia on faecal waste production (Amirkolaie et al., 2005). The discrepancies could be related to differences between fish species, as well as the source and level of NSP in diets. During restricted feeding, there were no differences in faecal waste production between yellowtail kingfish fed the SH diet and those fed the SBP diet. However during satiation feeding, fish fed the SH diet produced 15 % more faecal waste than those fed the SBP diet. The differences in faecal waste production between the two NSP diets were attributed to higher feed intake and lower OM digestibility in the SH diet when compared to the SBP diet during satiation feeding.

The amount of faeces produced by fish has to be efficiently removed to ensure good water quality and optimal system performance. In this study, a low removal efficiency of the starch diet using settling was observed, which is in line with previous observations for yellowtail kingfish (Horstmann et al., 2023a; Moran et al., 2009). High starch diets induced poor faecal integrity in yellowtail kingfish (Horstmann et al., 2023a), most likely due to starch's high water absorption capacity (Greer et al., 1959) and faecal matter breakdown by microbiota through fermentation on undigested (Amirkolaie et al., 2006; Hung et al., 1990; Kokou and Fountoulaki, 2018). On the other hand, this study found that NSP improved faecal removal efficiency in yellowtail kingfish, which is consistent with previous researchin seabass (Fountoulaki et al., 2022) and common carp (Prabhu et al., 2019). However, the mechanism underlying this improvement by NSP is not clear. It is possible that fish produce mucus in response to NSP (Sinha et al., 2011), which improves faecal integrity by forming a mucus envelope around the digesta. When comparing the two NSP sources, the SBP diet resulted in 33 % higher faecal removal efficiency than fish fed the SH diet in this study. The highest faecal removal efficiency was also observed in seabass and common carp fed diets containing 30 % ingredients rich in soluble NSP (Prabhu et al., 2019; Fountoulaki et al., 2022). Despite the relative low inclusion level of 10 % NSP in this study, the effect was relatively larger compared to the aforementioned studies. This difference could be attributed to fish species, but it could also be influenced by the amount and type of NSP. It was shown in another study that starch can impair faecal integrity of yellowtail kingfish; the faecal removal efficiency of fish fed the starch diet (12 % WF) was 28 %, whereas fish fed the 0 % WF diet had a removal efficiency of 42 % (Zhang et al., submitted). Nonetheless, including 10 % SBP resulted in a 63 % increase in removal efficiency. During this experiment, the undigested SBP in faeces of fish fed the diet expanded greatly overnight, indicating a high water binding capacity. Their matrix structure and high water binding capacity may also aid in the absorption/trapping of small particles, potentially contributing to the observed improved faecal removal efficiency. Furthermore, the SBP diet's improved faecal removal efficiency was associated with larger particle size during restricted feeding, resulting in a lower amount of non-removed faeces than the starch and SH diets. However, due to the high variability, the effect of diet on PSD disappeared with satiation feeding.

To conclude, the effect of carbohydrate on nutrient digestibility of yellowtail kingfish differed between the different three sources used in this study (starch, soy hulls and sugar beet pulp) and varied with feeding level. The starch diet had a higher OM digestibility than the two NSP diets, but it lowered protein and fat digestibility. The two NSP diets contained different types of NSP, which showed different effects on protein and fat digestibility. Furthermore, feeding level affected these differences. Satiation feeding reduced nutrient digestibility in all diets, with the SH diet showing the biggest decrease and the SBP diet showing the least reduction. However, the variation in fat digestion among the three diets cannot be explained by the bile acid balance or loss. The two NSP diets increased faecal waste production while lowering faecal bile acid content, resulting in a similar amount of bile acid loss as the starch diet. The effect of carbohydrate source on faecal characteristics (faecal removal efficiency and particle size distribution), was independent of feeding level. SBP had the highest faecal removal efficiency, resulting in the least amount of non-removed faeces while producing the most faecal waste. Whereas, SH resulted in similar amount of non-removed faeces compared to starch. In summary, starch did not result in higher growth (numerically lower)in yellowtail kingfish compared to NSP. Incorporating NSP to their RAS diet may be a way to improve faecal quality without affecting the digestibility of other macronutrients.

CRediT authorship contribution statement

Thomas W.O. Staessen: Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Satya Prakash:** Writing – review & editing, Investigation, Formal analysis. **Peter Horstmann:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Roel M. Maas:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Yaqing Zhang:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Johan W. Schrama:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **Fotini Kokou:** Writing – review & editing, Conceptualization. **Jeroen Kals:** Writing – review & editing, Methodology, Conceptualization.

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).

Data availability

Data will be made available on request.

Acknowledgements

We would like to thank the staff of the aquaculture research facility (in particular Sander Visser and Menno ter Veld) and Marit Nederlof for their technical support in conducting the experiment. Furthermore, we would like to acknowledge Tino Leffering, Troy Spekking, Joop Arts, Ronald Booms, and Samara Hutting for their support during the lab analysis. Laura Ossendrijver contributed to the work as part of her MSc thesis. 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). The author Yaqing Zhang received financial support from the China Scholarship Council (Grant N. 201907720014).

European Maritime
& Fisheries Fund

During the preparation of this work the author(s) used ChatGTP in order to perform a grammar check. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2024.102179.

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