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Effect of non-starch polysaccharides and bile acid supplementation on nutrient digestibility, bile acid balance, faecal waste production and characteristics of yellowtail kingfish (*Seriola lalandi*)

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ABSTRACT

Effects of non-starch polysaccharides (NSP) and bile acid supplementation (sodium taurocholate, STC) on nutrient digestibility, bile acid balance, faecal waste production and characteristics were investigated in yellowtail kingfish (*Seriola lalandi*). The experiment was conducted according to a two-by-two factorial design. Four diets were tested, differing in NSP level (CON vs. NSP) and bile acid supplementation (0 % STC vs. 0.4 % STC). The NSP level was altered by the inclusion of either 0 % or 10 % of NSP-rich ingredient mixture, which consisted of wheat bran and soy hull (1:1). Bile acid supplementation was tested by adding either 0 % or 0.4 % STC to the diets. Each dietary treatment was tested in triplicate. Fish were fed to apparent satiation for 32–33 days. Results showed that both the dietary inclusion of NSP and STC increased feed intake of yellowtail kingfish. NSP inclusion had no negative effect on nutrient digestibility. It even improved phosphorus digestibility and tended to increase protein digestibility. STC supplementation improved fat digestibility, independent of NSP level. Dietary STC supplementation increased the size of bile acid body pool, but inhibited bile acid synthesis. Regarding faecal waste, the NSP-supplemented diets had higher faecal waste production but also a higher faecal removal efficiency, resulting in more faecal waste removed faeces than the CON diets. As a result, at both diets (CON and NSP) the amount of non-removed faeces was similar. STC supplementation had no effect on faecal waste production and faecal characteristics. In summary, our results showed that yellowtail kingfish (a carnivorous species) copes well with NSP regarding feed intake and nutrient digestibility. Bile acid supplementation can enter the body bile acid pool and improve fat digestibility of yellowtail kingfish, which can be an effective way to secure proper fat digestion when fed plant-based diets. Regarding faecal waste, a small amount of NSP in the diets results in a large portion in the faeces, which may play an important role in determining faecal characteristics and removal efficiency. Considering its effects, incorporating small amounts of NSP into the feed can be helpful for effective faecal waste management in RAS fish.

1. Introduction

Global aquaculture production has been expanded from 34 million tons in 1997–112 million tons in 2017, and its growth is expected to continue (Naylor et al., 2021). Fishmeal and fish oil remain important ingredients of aquafeed, but their limited availability due to declining wild fish stocks and rising prices makes meeting the increasing demand difficult. Improving the efficiency of the currently used fish resources and increasing the use of plant-based ingredients is of importance for the sustainability of aquaculture (FAO, 2016). However, the shift towards using more plant-based ingredients often coincides with higher dietary inclusion levels of carbohydrates (starch and non-starch polysaccharides, NSP). For carnivorous fish, carbohydrates are far less digestible compared to fishmeal and fish oil and can negatively interfere with other nutrients in the feed, resulting in reduced nutrient digestibility (Sinha et al., 2011). This is especially evident for yellowtail

Abbreviations: STC, sodium taurocholate; NSP, non-starch polysaccharides; PSD, particle size distribution; TSS, total suspended solids; EHC, enterohepatic circulation; RAS, recirculating aquaculture system.

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kingfish (*Seriola lalandi*), where the inclusion of starch has been shown to significantly reduce overall nutrient digestibility, and especially fat digestibility (Horstmann et al., 2023c; Zhang et al., 2025).

A major part of solid waste originates from faeces, which are the undigested part of feed. Faeces require proper management in aquaculture to prevent the release of suspended matter into the environment, which can cause eutrophication. Effective faecal waste management is becoming increasingly important in practical aquaculture, such as in salmon cage culture, where removing solids beneath the cages is being encouraged, and in fish cultured in Recirculating Aquaculture Systems (RAS). Minimizing effluent waste from fish farms (both RAS and open systems such as cages) reduces the impact on the environment, enhances water quality, and reduces waste discharge. One key technical trend in global aquaculture for addressing these challenges is the adoption of RAS. In such closed systems, water is recirculated between fish tanks and water treatment units, the latter consisting of solid waste removal and biofiltration units. To reduce environmental impact, closed containment systems, also known as closed cages, were recently introduced. These systems collect and transport faecal waste to the shore for treatment (Nilsen et al., 2020). A lower nutrient digestibility will result in a higher faecal waste production (Faecal waste production = feed intake × (100 % - nutrient digestibility %). In open systems, such as cages, this results in increased nutrient load, and thus impacts the environment (Elvines et al., 2023). In the case of yellowtail kingfish, a high starch diet resulted in increased waste production, due to starch's negative effect on other macronutrient digestion (Horstmann et al., 2023c). Because NSP are not digested, increased faecal waste production was observed when yellowtail kingfish were fed an NSP-rich diet. NSP, on the other hand, improved faecal characteristics and removal efficiency (Zhang et al., 2024b). In addition, changes in nutrient digestibility can subsequently alter faecal composition (Reid et al., 2009). In turn, changes in faecal composition may affect the overall density of faeces, thereby affecting faecal removal efficiency (Reid et al., 2009) and dispersion in open systems. Solid waste management is an important first step in achieving good water quality in RAS. The culture of yellowtail kingfish in RAS has started relatively recently new, and one major challenge of farming this species in RAS is their poor faecal integrity and thus low faecal removal efficiency (Horstmann et al., 2023a–c). Non-removed faeces can contribute to the accumulation of total suspended solids (TSS) in RAS (Bureau and Hua, 2010). High TSS concentrations can compromise fish growth performance and, in the long run, cause mortality (Badiola et al., 2012). A high starch diet impaired faecal integrity of yellowtail kingfish (Horstmann et al., 2023b), however, starch is essential as a binder for creating stable feed pellets. Based on our previous findings, yellowtail kingfish seems to tolerate NSP better than starch in term of growth performance (Zhang et al., 2024b). Incorporating NSP into their (RAS) diet may be an option, as NSP can improve faecal characteristics without affecting other macronutrient digestion (Zhang et al., 2024b). Therefore, this study aimed to investigate whether including NSP in a high starch diet can improve nutrient digestibility and faecal characteristics of yellowtail kingfish.

Yellowtail kingfish has lower fat digestibility than other carnivorous species; improving fat digestibility can lead to lower faecal waste production and altered faecal characteristics and removal efficiency by lowering fat content in the faeces. Reduced fat digestibility in yellowtail kingfish has been suggested to be caused by a high loss of bile acids through faeces in fish fed commercial diets (Zhang et al., 2024a). Bile acids are potent endogenous digestive surfactants that aid in emulsification and absorption of fat. They are synthesised in the liver, stored in the gallbladder and then released into the intestine after a meal. Based on mammalian studies, the majority of bile acids are reabsorbed back in the intestine and are transported back to the liver via enterohepatic circulation (EHC) (Chiang, 2013; Macierzanka et al., 2019). Still, a small amount of bile acids are lost via the faeces, which can be compensated for by synthesis of bile acids from cholesterol in the liver and/or directly supplied by diets containing fishmeal and fish oil (Romano et al., 2020). Plant ingredients, on the other hand, lack bile acids and their precursors, and their inclusion mostly coincided with some amounts of NSP that can further increase faecal bile acid loss (Romano et al., 2020; Sinha et al., 2011). In earlier studies on yellowtail kingfish fed a diet containing 20 % fish meal and 45 % plant protein sources, fish had a negative a bile acid balance, i.e., lost more bile via the faeces then they took in via the diet (Zhang et al., 2024a). Dietary supplementation of bile acids improved fat digestibility in yellowtail kingfish and coincided with a positive bile acid balance (Zhang et al., 2024a). Similarly, in gilthead seabream, supplementing with a bile acid blend improved fat digestibility and growth (Ruiz et al., 2023). Whether in yellowtail kingfish dietary supplementation of bile acid increased the body bile acid pool, like was observed in trout (Staessen et al., 2022) is unclear. Therefore, the effect of bile acid supplementation on bile acid absorption, body bile acid pool size, and bile acid synthesis was quantified in this study. Additionally, it was assessed whether this effect was dependent on dietary NSP level. This study investigated how NSP and bile acid supplementation affect 1) nutrient digestibility and bile acid balance (e.g., faecal bile acid loss, bile acid synthesis and body bile acid pool), and 2) faecal waste production and characteristics in yellowtail kingfish.

2. Materials and methods

2.1. Diets

The experiment was set up according to a two-by-two factorial design. Four diets were tested, differing in NSP level (CON vs. NSP) and bile acid supplementation (0 % STC vs. 0.4 % STC). The NSP level was altered by adding either 0 or 10 % of an NSP-rich ingredient mixture of wheat bran and soy hulls (1:1). Bile acid supplementation was tested at either 0 % or 0.4 % of sodium taurocholate (STC) (Sigma-Aldrich, St. Louis, United States of America). In a previous study using yellowtail kingfish, the applied dose of STC (0.4 %) proved to be effective (Zhang et al., 2025). STC was chosen because it (taurocholate) accounts for the majority of the primary bile acids in fishmeal-rich diets (Staessen et al., 2021, 2022). As a result, reducing the amount of fishmeal used in yellowtail kingfish diets while increasing the amount of plant ingredients will result in a decrease in taurocholate. A basal mixture was formulated with 20 % fish meal and 45 % plant protein ingredients as protein sources, and 20 % gelatinized wheat flour as a binder to create stable feed pellets. The inclusion levels of NSP were altered by diluting the basal mixture with either 0 % or 10 % of the NSP-rich mixture. At the cost of fish meal, 0.4 % STC was added to the diet. Diets were supplemented with taurine, DL-methionine and monocalcium phosphate to prevent deficiencies and meet nutrient requirements. All diets contained at least 10 % fish oil to ensure that essential fatty acids requirements were met. The ingredient composition and analysed nutrient composition of the diets are given in Table 1. The diets were produced by Research Diet Services (Wijk bij Duurstede, The Netherlands) by extrusion using a Clextral BC45 laboratory scale twin-screw extruder (Clextral, Firminy, France) with a 3 mm die, resulting in 4.5 mm sinking pellets. After extrusion, the pellets were dried for 3 h (70 °C) and cooled to room temperature. Diets were produced approximately one week before the experiment began and stored at 4°C for the duration of the experiment.

2.2. Fish, rearing conditions and housing facilities

The Central Committee on Animal Experiments (CCD) approved this study on the advice of The Netherlands' Animal Experiment Committee (DEC) (permit no. AVD10400202216106). It was also approved by Wageningen University's Ethical Committee for Animal Experiments. The experiment was conducted at the Aquaculture Research Facility (ARF) of the Wageningen University and Research (Wageningen, The Netherlands) according to Dutch legislation (Act on Animal Experiments). Yellowtail kingfish (*Seriola lalandi*) of mixed sex were obtained

Table 1
Ingredient composition and analysed nutrient composition of the experimental diet.

Diet	CON		NSP	
	0 %-STC	0.4 %-STC	0 %-STC	0.4 %-STC
Ingredients (g/kg)				
Sodium taurocholate (STC)	-	4	-	4
Wheat bran	-	-	50	50
Soyhulls	-	-	50	50
Fish meal LT[a]	200	196	180	176
Wheat gluten[b]	150	150	135	135
Pea protein concentrate[c]	150	150	135	135
Soya protein concentrate[d]	150	150	135	135
Fish oil[e]	112.8	112.8	101.5	101.5
Gelatinized wheat flour	200	200	180	180
Monocalcium phosphate	10	10	9	9
DL-methionine	4	4	3.6	3.6
Taurine	8	8	7.2	7.2
Premix[f]	15	15	13.5	13.5
Yttrium oxide	0.2	0.2	0.18	0.18
Analysed nutrient content (g/kg DM)				
Dry matter (DM, g/kg)	919	920	944	936
Crude protein	544	540	507	503
Crude fat	164	163	149	154
Total carbohydrates[g]	229	233	280	280
Starch and sugars	203	203	194	191
NSP[h]	26	30	86	88
Gross energy (kJ/g DM)	23.2	23.1	22.8	22.7
Crude ash	63	64	64	63
Phosphorus	9.8	9.9	9.8	9.7
Calcium	8.9	9.1	8.9	8.3
Bile acid (μmol/g DM)	0.16	7.18	0.13	6.98
Gelatinization degree (%)	97.9	98.9	90.1	87.6

CON – control diet; NSP – non-starch polysaccharides supplemented diet; 0 %-STC – no sodium taurocholate supplementation; 0.4 %-STC, 0.4 % sodium taurocholate supplementation

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

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

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

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

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

[f]Premix composition. Vitamins (IU or mg/kg complete diet; 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 basal diet): Fe, (as ferric sulphate), 50 mg; Zn (as zinc sulphate), 80 mg; Co (as cobalt sulphate), 0.2 mg; Cu (as 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.

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

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

from a commercial fish farm (Kingfish Zeeland, Kats, The Netherlands). At the beginning of the experiment, fish were batch weighted (Mettler-Toledo ICS429). The day prior to weighing, fish were not fed. Per tank, 24 fish with an average initial weight of 39 g were stocked. Diets were randomly assigned to one of the tanks with 3 replicates per diet. Each tank was connected to a swirl separator with glass bottle for the collecting of faeces and uneaten feed. These tanks (n = 12) were connected to the same RAS (which was filled with artificial seawater). This RAS consisted of a sump, settling tank, drum filter, protein skimmer, and trickling filter. The system's refreshment rate was adjusted to keep the NO_3-N concentration below 100 mg/L. Water quality parameters were measured daily from the common outflow of the tanks to ensure that the pre-set water quality parameters remained within optimum conditions for yellowtail kingfish (see supplementary Table S1). The dissolved oxygen concentration measured in the outlet water was above 5.0 mg/L (WTW Multi 3630 IDS - FDO 925) and water temperature was on average 23.7 ± 0.2°C (WTW Multi 3630 IDS - FDO 925). The pH ranged from 7.0 to 7.6 (WTW Multi 3630 IDS - SenTix 940), and 32.5 ± 2.0 ppm for salinity

2.3. Experimental procedures and sampling

The lasts day of the experiment samples were collected. This sampling was done during 2 consecutive days, at day 32 or 33 of the experiment and per day fish from 6 tanks were sampled. This duration allowed the fish's mean bodyweight increase at least fourfold. At the start of the experiment, 20 fish from the base population were sampled for initial body composition and to determine the initial bile acid body pool size. During the experimental period, fish were hand-fed twice daily (9:00 and 15:00 h) to apparent satiation. Satiation was reached when fish stopped eating, or when the feeding time exceeded 1 h. During the first three feeding moments of the experiment, the feeding level was gradually increased (restricted) until satiation was reached. This allowed the fish to adapt to the diet. Fifteen minutes after feeding, the glass bottles attached to the swirl separators were checked for feed pellets to determine feed spillage. Mortality was checked at least twice a day before feeding.

Faeces for digestibility analysis was collected overnight for 5 days in week 4, starting after afternoon feeding. The glass bottles, which were connected beneath the swirl separators connected to each tank, were submerged in water with ice to minimize bacterial degradation of the faeces with the use of a Styrofoam box. The bottles were removed in the morning (before feeding) and stored on ice until collection. Most of the water was decanted from the bottles, and the faeces were collected in an aluminium tray. Collected faeces were pooled per tank and stored at −20 °C until further analysis. Faeces collection for determination of faeces removal efficiency was done during the weekend of week 4. The collection method was the same as for the faeces collection for digestibility, expect that faecal material was collected continuously for 2 days (including day collection; but excluding feeding moments). During week 4, faeces were collected once after morning feeding for roughly 2 hours to determine faecal particle size distribution (PSD) via sieving. After collection, faecal samples for PSD were stored on ice until further analysis. Feed samples were taken by pooling 100 g per experimental diet per week (stored at 4 °C).

Exactly 5 h after feeding (satiation) started, fish were sampled after being killed by an overdose of benzocaine (1.5 ml/L). First all fish were weighed and counted to determine final body weight. Per tank: 3 fish were directly stored at −20 °C for the determination of the final bile acid body pool size; 7 fish were dissected to remove chyme from the gastrointestinal tract, on the remaining of these fish (n = 7) the proximal composition of the fish was determined.

2.4. Analysis

Faeces collected for digestibility and faeces removal efficiency were dried at 70°C. Thereafter, faeces were pooled per tank for each feeding period and ground (mixer mill, IKA A11 basic). Frozen fish samples were cut into small pieces using a band saw and homogenized by grinding two times in a meat mincer with a 4.5 mm die (TW-R 70, Feuma Gastromaschinen GmbH, Germany). Ground fish samples were subsequently freeze-dried. Dry matter of fish was calculated on fresh basis. Feed, faeces and fish were analysed for dry matter and ash, total nitrogen, gross energy, crude fat, yttrium, phosphorus and calcium as described by Staessen et al. (2020a). For dry matter determination, faeces and feed were analysed gravimetrically by drying for 4 hours at 103°C until constant weight (ISO 6496, 1999). Ash was determined gravimetrically by combustion for 4 hours at 550°C in a muffle furnace (ISO 5984, 2002) until constant weight. The ash fraction was dissolved in concentrated

sulphuric acid by autoclaving (121°C, 20 min) to determine yttrium by ICP-OES (NEN 6966, 2005 and NPR 6425, 1995). Total nitrogen was determined according to Kjeldahl's method (ISO 5983–2, 2009). The crude protein content was calculated as the total nitrogen content multiplied by 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). Gross energy was measured using bomb calorimetry (C7000, IKA werke, IKA analysentechnik, Staufen, Germany). Total starch was analysed enzymatically (without sugars) using amyloglucosidase after washing with 40 % ethanol. Gelatinized starch was analysed according to the modified glucoamylase method described by Zhu et al. (2016). With the use of total starch (without sugar) and gelatinized starch, the gelation degree of starch in the experimental diets were calculated (Nutrilab, Giessen, The Netherlands). For digestibility calculations, starch content (including sugars) of the diets and faeces was analysed as described above for total starch analysis, leaving out the ethanol washing step.

The analysis of faecal PSD served as an indicator for assessing faecal characteristics. Faecal PSD was analysed according to the protocol described in Horstmann et al. (2023a–c), with the exception that the entire sample collected after morning feeding (3-hour collection) was used (No subsampling).

Bile acid content was determined by enzymatic cycling rate after extraction. Bile acid extraction was performed on feed, faeces, liver and gallbladder according to Porter et al. (2003) and consisted of alkaline hydrolysis in ethylene glycol-KOH and acidification by concentrated HCL. Followed by three rounds of extraction with diethyl ether, the diethyl ether extracts were evaporated at 40°C under a continuous stream of air. Dried samples were dissolved in 3 ml of methanol and stored at 4 °C until further analysis. Determination of total bile acid concentration in the methanolic extracts was performed using a commercial kit (Diazyme®, Germany). The Diazyme Total Bile Acids Assay Kit is designed for in vitro quantitative measurement of total bile acids in human serum, but it is also effective in lower vertebrates such as fish.

2.5. Calculations and data analysis

Absolute growth (g) was calculated as the difference between the average individual initial (W_i) and final (W_f) body weight (g). Growth (g/d) was calculated as absolute growth/t, where t is the number of days. Specific growth rate (SGR; %/d) was calculated as $(\ln W_f - \ln W_i)/t \times 100$ %. The absolute feed intake (FI_{abs}; g/d) was calculated as FI_{tot}/t, where FI_{tot} is the total feed intake (g). Feed intake per metabolic body weight (FI_{mbw}; g/kg$^{0.8}$/d) was calculated as FI/MBW, where MBW is the metabolic body weight (kg$^{0.8}$) which was calculated as $(W_G/1000)^{0.8}$. The geometric mean BW (W_G; g) was calculated as $e^{((\ln Wf+\ln Wi)/2)}$. The W_G was used to calculate the mean bodyweight (expected weight halfway through the experiment), accounting for the fish's expected exponential growth as they were fed until apparent satiation. Growth on metabolic body weight (g/kg$^{0.8}$/d) was calculated as growth/MBW. Feed conversion ratio (FCR) was calculated on dry matter (DM) basis (g/g) as $(FI \times Diet_{DM}/1000)/(W_f - W_i)$, where $Diet_{DM}$ is the dry matter content of the diet (g/kg).

Apparent digestibility coefficient (ADC, %) of organic matter, crude protein, crude fat, carbohydrate, starch and gross energy were calculated according to Cheng and Hardy (2002) using yttrium as inert marker: $ADC\ (\%) = 100 \times (1 - ((Y_{diet} / Y_{faeces}) \times (N_{faeces} / N_{diet})))$, 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. In feed and faeces, organic matter (g/kg DM) content was calculated as 1000 – ash and total carbohydrates content as 1000 – (crude protein + crude fat + ash).

Nitrogen (N) and energy balance parameters were calculated per diet and expressed as; mg kg$^{-0.8}$ BW d^{-1} and kJ kg$^{-0.8}$ BW d^{-1}, respectively (Supplementary Table 1). The calculation of nitrogen and energy balance parameters was detailed descripted by Saravanan et al. (2012).

Faecal waste production (g OM/kg DM feed), faecal removal efficiency (%), removed and non-removed faeces (g OM/kg DM feed) were calculated during week 4 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}) multiplied by the 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. Bile acid balance was calculated as bile acid intake – faecal bile acid loss. Bile acid intake (BAI), faecal bile acid loss (FBAL), and bile acid balance were expressed per kg body weight (BW) (μmol kg^{-1} BW d^{-1}), using the mean body weight during the experimental period (BW). Mean body weight (BW) was calculated as $(W_i + W_f)/2$. Bile acid digestibility (%) was calculated in the same way as the digestibility of the nutrients.

The total body bile acid pool (μmol/fish) was expressed on individual fish basis. The total body bile acid pool size of individual bile acids expressed per unit of fish weight (μmol kg^{-1} BW). Gain of individual bile acids in the body bile acid pool (BAPG; μmol kg^{-1} BW d^{-1}) was calculated as $(BBAP_f - BBAP_i)/t/(BW/1000)$, where $BBAP_f$ and $BBAP_i$ are respectively the final and initial pool of the individual body bile acid (μmol/fish). Synthesis of individual bile acids (μmol kg^{-1} BW d^{-1}) was calculated as BAPG + FBAL – BAI.

2.6. Statistical analysis

Tanks were used as the experimental unit (n = 12) in the statistical analysis. A two-way ANOVA was used to investigate the effect of NSP level, STC and their interaction. In the case of an interaction effect ($p < 0.05$), a Tukey HSD test (honest significant difference; 95 % significance) was performed to compare treatment means. Statistical analyses were performed by using the statistical program SAS 9.4, SAS Institute, North Carolina, USA.

3. Results

3.1. Fish performance

Fish performance is given in Table 2. Survival was high (>99 %) and unaffected by dietary treatment ($p > 0.05$). The initial body weight was comparable between treatments ($p > 0.05$). Feed intake was increased by dietary NSP addition ($p < 0.05$) and by STC supplementation ($p < 0.05$). NSP supplementation negatively affected FCR ($p < 0.001$); 10 % NSP inclusion increased FCR by 7.6 %. Despite this, dietary NSP inclusion had no effect on growth ($p > 0.05$) or SGR ($p > 0.05$). STC supplementation had no effect on growth, expressed in g/d ($p > 0.05$), but tended to increase SGR ($p < 0.1$) and increased growth expressed on metabolic body weight ($p < 0.05$; Table 2).

3.2. Digestibility

Apparent digestibility coefficients (ADC, %) of nutrients are given in Table 3. The 10 % NSP inclusion reduced the organic matter ADC on average by 6.4 percentage points ($p < 0.001$). Total carbohydrate and energy ADC in fish fed the high NSP diets were lower than fish fed the low NSP diets ($P < 0.01$). However, fat ADC was unaffected by dietary NSP supplementation ($p > 0.05$). Moreover, the NSP supplemented diets had a tendency for higher ADC values for crude protein and starch plus sugars ($p < 0.1$). Bile acid supplementation had a large impact on fat ADC, it increased the fat ADC on average by 6.8percentage points ($p < 0.001$). This increase in fat ADC by bile acid supplementation was independent of dietary NSP supplementation (no interaction effect;

Table 2
Fish performance of yellowtail kingfish fed the experimental diets to apparent satiation for 32–33 days.

Diet	CON		NSP		SEM	P-value		
	0 %-STC	0.4 %-STC	0 %-STC	0.4 %-STC		NSP	STC	NSP x STC
Survival (%)	99	100	100	100	0.7	ns	ns	ns
Initial body weight (g)	39	39	39	39	1.2	-	-	-
Final body weight (g)	177	190	184	183	5.2	ns	ns	ns
FI_{abs} (g/d)	4.57	4.94	4.93	5.08	0.105	*	*	ns
FI_{MBW} (g/d/kg$^{0.8}$)	28.1	29.1	29.7	30.7	0.16	***	***	ns
Growth (g/d)	4.26	4.62	4.43	4.45	0.109	ns	ns	ns
$Growth_{MBW}$ (g/d/kg$^{0.8}$)	26.2	27.2	26.7	26.9	0.15	ns	*	ns
SGR (%/d)	4.67	4.85	4.75	4.78	0.050	ns	#	ns
FCR	0.99	0.98	1.05	1.07	0.010	***	ns	ns

CON – control diet; NSP – non-starch polysaccharides supplemented diet; 0 %-STC – no sodium taurocholate supplementation; 0.4 %-STC, 0.4 % sodium taurocholate supplementation; FI_{abs} – feed intake absolute; SGR – specific growth rate; DGC – daily growth coefficient; FCR – feed conversion ratio (on DM basis). Values are means (n = 3) and the standard error of the means (SEM); ns - not significant $p > 0.1$; # - tendency $p < 0.1$; *- $p < 0.05$; *** - $p < 0.001$.

Table 3
Apparent digestibility coefficient (ADC, %) of yellowtail kingfish fed the experimental diets to apparent satiation for 32–33 days.

Diet	CON		NSP		SEM	P-value		
	0 %-STC	0.4 %-STC	0 %-STC	0.4 %-STC		NSP	STC	NSP x STC
Organic matter	70.5	72.8	65.2	65.4	0.86	***	ns	ns
Crude protein	83.8	84.2	86.6	85.3	0.93	#	ns	ns
Crude fat	65.3	72.7	66.9	73.0	0.99	ns	***	ns
Total carbohydrates	43.0	46.5	25.5	25.4	1.01	***	ns	ns
Starch and sugars	58.5	59.8	64.9	60.9	1.73	#	ns	ns
Energy	72.1	75.7	69.8	70.7	0.94	**	*	ns
Phosphorus	34.1	35.0	44.2	45.8	1.24	***	ns	ns
Bile acids	-1315^{b}	57^{a}	-1486^{c}	55^{a}	19.5	**	***	**

CON – control diet; NSP – non-starch polysaccharides supplemented diet; 0 %-STC – no sodium taurocholate supplementation; 0.4 %-STC, 0.4 % sodium taurocholate supplementation; Values are means (n = 3) and the standard error of the means (SEM); ns - not significant $p > 0.1$; # - tendency $p < 0.1$;*- $p < 0.05$; **- $p < 0.01$; ***- $p < 0.001$.

$p > 0.05$; Table 3).

3.3. Body composition

Table 4 shows initial and final fish body composition. Fish fed the NSP diets had lower fat content than those fed the CON diets ($p < 0.01$). Similarly, STC supplementation resulted in a higher fat content of fish fed the 0.4 %-STC diets compared to fish fed the 0 %-STC diet. In fish fed the STC diets, fat content increased and concurrent energy content (fat: $p < 0.01$; energy: $p < 0.05$). The NSP inclusion and STC did not affect body protein and ash content ($p > 0.05$).

In Supplementary Table S2 the complete nitrogen and energy balances are given. Retained energy as protein was similar for all diets ($p > 0.05$; Fig. 1). In contrast, retained energy as fat was affected both by dietary NSP supplementation ($p < 0.05$) and STC supplementation ($p < 0.01$; Fig. 1). Dietary NSP supplementation reduced the energy retention as fat, whereas STC supplementation increased fat retention. The effects of NSP and STC on fat retention were additive (no interaction effect; $p > 0.10$).

3.4. Bile acid balance

The results on bile acid balance are given in Table 5. Fish fed the STC supplemented diets had a higher bile acid intake compared to fish fed the non-supplementation diets ($p < 0.001$). Fish were fed to satiation. As feed intake increased with NSP supplementation ($p < 0.001$) and also with STC supplementation ($p < 0.05$), the "NSP 0.4 %-STC" diet had the highest bile acid intake (299.8 μmol/kg/d; $p < 0.01$). Faecal bile acid content decreased with increasing dietary NSP supplementation. Since faeces production increased with dietary NSP supplementation, there was a tendency to affect faecal bile acid loss ($p < 0.1$) when diets were supplemented with NSP. This was especially the case for the diets supplemented with STC (interaction, $p < 0.05$; Table 5). However, NSP supplementation had no effect on bile acid balance ($p > 0.05$). Both the CON and NSP Diets without STC supplementation (0 %-STC) resulted in a negative bile acid balance. At these treatments, the loss of bile acid via

Table 4
Initial and final body composition (on fresh weigh basis, g/kg) of yellowtail kingfish fed the experimental diets to apparent satiation for 32–33 days.

Diet		CON		NSP		SEM	P-value		
		0 %-STC	0.4 %-STC	0 %-STC	0.4 %-STC		NSP	STC	NSP x STC
Final body composition	**Initial body composition**								
Dry matter	245	286	292	276	285	3.4	*	#	ns
protein	176	194	192	192	193	1.2	ns	ns	ns
Fat	43	68	77	58	69	2.4	**	**	ns
Energy	5.7	7.0	7.3	6.6	7.0	0.12	*	*	ns
Ash	30	28	27	28	29	0.5	ns	ns	ns
Phosphorus	5.1	4.9	4.9	4.9	5.0	0.09	ns	ns	ns
Calcium	6.1	5.7	5.7	5.6	6.0	0.20	ns	ns	ns

CON – control diet; NSP – non-starch polysaccharides supplemented diet; 0 %-STC – no sodium taurocholate supplementation; 0.4 %-STC, 0.4 % STC supplementation; Values are means (n = 3) and the standard error of the means (SEM); ns - not significant $p > 0.1$; # - tendency $p < 0.1$; * - $p < 0.05$; **$p < 0.01$.

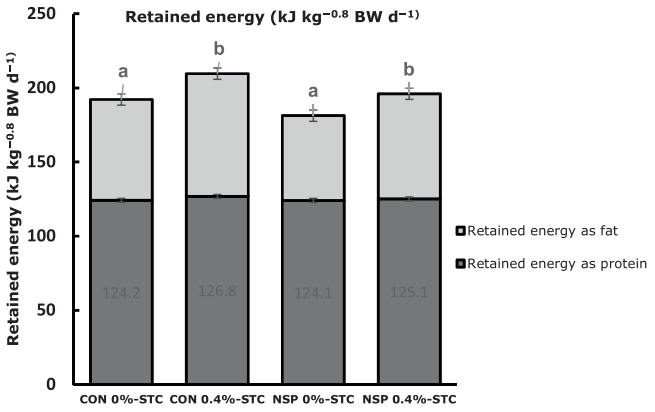

Fig. 1. Total retained energy, retained energy as fat, and retained energy as protein (kJ $kg^{-0.8}$ BW d^{-1}) of yellowtail kingfish fed the experimental diets to apparent satiation for 32–33 days. Total retained energy is represented by the entire bar, retained energy as fat by light grey part of the bar and retained energy as protein by dark grey. CON – control diet; NSP – non-starch polysaccharides supplemented diet; 0 %-STC – no sodium taurocholate supplementation; 0.4 %-STC, 0.4 % sodium taurocholate supplementation; Values are means (n = 3) and the standard error of the means (SEM); Retained energy as fat was affected by NSP level ($p < 0.05$) and STC ($p < 0.01$), but not by their interaction ($p > 0.5$); Retained energy as protein was neither affected by NSP, STC nor their interaction ($p > 0.05$).

the faeces was more than ten-fold higher than the bile acid intake via the diets, which was also reflected in the negative bile acid digestibility (average −1400 % for the non-supplemented diets; Table 3). Dietary STC supplementation did increase bile acid intake, faecal bile acid content and faecal bile acid losses ($p < 0.001$; Table 5). Dietary STC supplementation increased faecal bile acid content on average from 7.3 μmol/g at the 0 %-STC diets to 11.0 μmol/g at the 0.4 %-STC diets. STC supplementation had a greater effect on bile acid loss in the NSP diets than in the CON diets (interaction: $p < 0.05$). STC supplementation resulted in a positive bile acid balance in both CON and NSP diets ($p < 0.001$; Table 5).

The size of the bile acid pool at the end of the experiment was not influenced by dietary NSP supplementation ($p > 0.05$; Table 5) and consequently, the body bile acid pool gain was unaffected by NSP addition ($p > 0.05$). This, combined with a similar bile acid balance in the CON and NSP diets, bile acid synthesis was not influenced by NSP supplementation. In contrast to NSP addition, STC supplementation altered the size of the bile acid pool at the end of the experiment ($p < 0.001$). Averaged over the CON and NSP diets, the final body bile acid pool of fish fed the STC diets was 115 μmol/fish, which was approximately twice the size of fish fed the non-supplemented diets (52.7 μmol /fish). However, at the STC supplemented diets, the increase in body bile acid pool size was smaller than the bile acid balance, consequently the calculated bile acid synthesis was negative in fish fed the STC diets, averaged −140.5 μmol kg^{-1} BW d^{-1}, being different from fish fed diets without STC supplementation ($p < 0.001$). The bile acid synthesis was 92 μmol kg^{-1} BW d^{-1} in fish fed the diets without bile acid supplementation. The effect of STC supplementation on bile acid balance, body pool size and synthesis was independent of the dietary NSP supplementation (no interaction effect; $p > 0.05$; Table 5).

3.5. Faecal characteristics

The total amount of faecal waste produced by fish fed the NSP supplemented diets (325 g OM/kg Feed) was 22 % higher than fish fed the CON diets (266 g OM/kg Feed; $p < 0.001$; Table 6). Fish fed the NSP diets, on the other hand, had a faecal removal efficiency of 39 % compared to 28 % for fish fed the CON diets ($p < 0.001$). Consequently, all the diets had comparable amounts of non-removed faeces ($p > 0.05$). STC supplementation had no effect on faecal waste production, faecal removal efficiency, or non-recovered faeces ($p > 0.05$).

Data on the composition and PSD of the collected faecal waste by settling are shown in Table 6. Overall, the proximate composition of the faeces was affected by dietary NSP supplementation ($p < 0.001$). Dietary supplementation of NSP increased the total carbohydrate content of faeces but it reduced the content of crude protein, crude fat, starch and sugars and energy in the faeces (Table 6). Averaged over the CON and NSP diets, dietary supplementation of STC only increased the fat content and thereby also the energy content of faecal waste ($p < 0.001$). Fish fed STC supplemented diets had a lower faecal fat content (Table 6). Dietary NSP addition affected all fractions of the PSD ($p < 0.05$; Table 6), but only a tendency for the fraction 250–850 μm ($p < 0.10$). NSP supplemented diets had a higher percentage of large particles (>850 μm, $p < 0.05$) and a lower percentages of small particles (<250 μm, $p < 0.05$). Regarding dietary STC supplementation, only the fraction 40–100 μm differed ($p < 0.05$) between the 0 %-STC and 0.4 %-STC diets, indicating that STC acid supplementation had little effect on PSD.

4. Discussion

In this study, averaged over all diets the digestibilities of all nutrients (on average: 68.5 % OM, 85 % crude protein, and 69.5 % fat) were lower than previously published values for yellowtail kingfish within the same facilities (OM: 72–92.9 %; crude protein: 90.2–96.5 %; fat: 72.9–93.4 %) (Horstmann et al., 2023a–c). Yellowtail kingfish were fed to apparent satiation in the current study and realized a feed intake of

Table 5
Bile acid balance of yellowtail kingfish fed the experimental diets to apparent satiation for 32–33 days.

Diet	CON		NSP		SEM	P-value		
	0 %-STC	0.4 %-STC	0 %-STC	0.4 %-STC		NSP	STC	NSP x STC
Feed bile acid content (μmol^{-1}g OM)	0.17^{b}	7.70^{d}	0.14^{a}	7.50^{c}	0.00	-	-	-
Feed intake (g kg^{-1} BW d^{-1})	36.5	37.2	39.1	40.2	0.34	***	*	ns
Bile acid intake (μmol kg^{-1} BW d^{-1})	6.3^{a}	284.9^{b}	5.4^{a}	299.8^{c}	1.57	**	***	**
Faecal bile acid content (μmol g^{-1} OM)	8.3	12.2	6.3	9.8	0.33	***	***	ns
Faeces production (g OM kg^{-1} BW d^{-1})	10.7	10.1	13.6	13.9	0.33	***	ns	ns
Faecal bile acid losses (μmol kg^{-1} BW d^{-1})	89^{a}	123^{b}	86^{a}	136^{c}	2.70	#	***	*
Bile acid balance (μmol kg^{-1} BW d^{-1})	−83	162	−81	164	2.1	ns	***	ns
Initial body bile acid pool (μmol/fish)	19.0	19.0	19.0	19.0	0.58	ns	ns	ns
Initial body bile acid pool size (μmol kg^{-1} BW)	487	-	-	-	-	-	-	-
Final body bile acid pool (μmol/fish)	50.0^{a}	117.7^{b}	55.4^{a}	113.2^{b}	10.49	ns	***	ns
Final body bile acid pool size (μmol kg^{-1} BW)	283	619	303	619	56.5	ns	***	ns
Gain of bile acid pool (μmol kg^{-1} BW d^{-1})	9.7	23.5	10.5	22.4	1.88	ns	***	ns
Bile acid synthesis (μmol kg^{-1} BW d^{-1})	92	−139	91	−142	3.0	ns	***	ns

CON – control diet; NSP – non-starch polysaccharides supplemented diet; 0 %-STC – no sodium taurocholate supplementation; 0.4 %-STC, 0.4 % STC supplementation; Values are means (n = 3) and the standard error of the means (SEM); ns - not significant $p > 0.1$; # - tendency $p < 0.1$; *- $p < 0.05$; **- $p < 0.01$; *** - $p < 0.001$.

Table 6
Faecal composition (on organic matter [OM] basis) and faecal particle size distribution (PSD, %) of yellowtail kingfish fed the experimental diets to apparent satiation for 32–33 days.

Diet	CON		NSP		SEM	P-value		
	0 %-CON	0.4 %-STC	0 %-CON	0.4 %-STC		NSP	STC	NSP x STC
Faecal waste (g OM/kg DM Feed)	276	255	326	324	8.0	***	ns	ns
Faecal removal efficiency (%)	28	27	40	38	0.9	***	ns	ns
Non-recovered faeces (g OM/kg DM Feed)	212	198	211	214	5.9	ns	ns	ns
Removed faeces (g OM/kg DM Feed)	78	70	129	124	3.80	***	ns	ns
Organic matter (g/kg DM)	547	545	619	612	14.2	**	ns	ns
Crude protein (g/kg OM faeces)	319	335	209	228	10.0	***	ns	ns
Crude fat (g/kg OM faeces)	207	174	151	128	3.2	***	***	ns
Total carbohydrates (g/kg OM faeces)	474	490	640	643	9.4	***	ns	ns
Starch and sugars (g/kg OM faeces)	307	320	208	231	11.8	***	ns	ns
Energy (g/kg OM faeces)	23.4[c]	22.1[b]	21.1[a]	20.5[a]	0.18	***	***	*
Particle size distribution (PSD, %)								
< 40 μm	4.4	4.9	3.0	2.9	0.67	*	ns	ns
40–100 μm	2.3	3.5	1.4	1.8	0.31	**	*	ns
100–250 μm	6.7	6.9	3.4	3.6	0.92	**	ns	ns
250–850 μm	25.9	21.8	15.7	14.0	4.12	#	ns	ns
> 850 μm	60.8	62.8	76.5	77.7	5.63	*	ns	ns

CON – control diet; NSP – non-starch polysaccharides supplemented diet; 0 %-STC – no sodium taurocholate supplementation; 0.4 %-STC, 0.4 % STC supplementation; OM – organic matter; DM – dry matter; Values are means (n = 3) and the standard error of the means (SEM); ns - not significant $p > 0.1$; # - tendency $p < 0.1$; *- $p < 0.05$; **- $p < 0.01$;*** - $p < 0.001$.

29.4 g/kg$^{0.8}$ BW/d averaged over all dietary treatments. The current feed intake was much higher (> 40 % higher) than in previous studies (Dam et al., 2019; Horstmann et al., 2023a–c; Liu et al., 2019). The feed intake in the current study even exceeded the expected satiation level of 25.0 g/kg$^{0.8}$ BW/d. Feed intake increased when diets were supplemented (i.e., diluted) with NSP, which aligns with earlier findings that fish compensate for a dietary dilution of energy by increasing feed intake (Jobling, 1983; Sinha et al., 2011). The low nutrient digestibilities in the current study may relate to the high feed intake. It has been suggested that high feeding levels may cause an overload of the fish's digestive capacity (Jobling, 1986; Schrama et al., 2012). Increasing feed intake decreases the digestive transit time, resulting in a shorter time for digestion (Bromley, 1994; Dias et al., 1998). The negative effect of feeding level on nutrient digestibility was also found in other aquaculture species such as African catfish (*Clarias gariepinus*) (Elesho et al., 2021; Henken et al., 1985), Nile tilapia (*Oreochromis niloticus*) (Schrama et al., 2012) and rainbow trout (*Oncorhynchus mykiss*) (Staessen et al., 2020b). In the current study, dietary STC supplementation increased feed intake of yellowtail kingfish, which is similar to findings in turbot (*Scophthalmus maximus*) and European seabass (*Dicentrararchus labrax*) (Gu et al., 2017; Sallam et al., 2017). But in rainbow trout dietary STC addition reduced feed intake (Staessen et al., 2022). According to literature, bile acids have high olfactory potential and can act as taste stimuli in some fish (Buchinger et al., 2014; Kasumyan and Vinogradskaya, 2019; Rolen and Caprio, 2008). Most likely such an effect of STC on feed palatability is fish species dependent (e.g., herbivorous vs carnivorous fish). In summary, the current study shows that yellowtail kingfish can adjust feed intake based on dietary composition, but higher feed intake might negatively impact nutrient digestibility.

Reduced fat digestibility has been observed in yellowtail kingfish fed high wheat flour diets (Horstmann et al., 2023c), which may be related to the presence of starch and/or small amounts of NSP originating from plant ingredients. Dietary starch levels negatively affect protein and fat digestibility already at levels > 6 % (Horstmann et al., 2023c; Zhang et al., 2024b). NSPs are well-known for their antinutritional properties, including induction of changes in digesta viscosity, enzyme activities and gut physiology in fish (Sinha et al., 2011). The NSP applied in the current study was a mixture of wheat bran and soy hulls. Both these ingredients primarily contain insoluble NSP (Englyst, 1989; Knudsen, 2015). The same NSP mixture reduced protein and fat digestibility in rainbow trout (Staessen, 2020a; 2020b; 2021). Interestingly in the current study with yellowtail kingfish, this applied NSP mixture had no effect on fat digestibility and it even tended to improve protein digestibility. This is in line with findings in Nile tilapia, where insoluble NSPs improved the nutrient digestibility (Amirkolaie et al., 2005; Jiang et al., 2022). Yellowtail kingfish and Nile tilapia are warm-water species. Therefore, fermentation in the intestine may have been related to this positive effect of insoluble NSP, however, other mechanisms might also be involved. The current results show that insoluble NSPs have no negative effects on the digestibility of other macronutrient in yellowtail kingfish.

When fishmeal and fish oil have been reduced in the diet, shortage of bile acids was suggested to cause low fat digestibility in yellowtail kingfish (Zhang et al., 2024a, 2025). In the present study, fat digestibility at the 0 %-STC diets was 66 %, which is 20.1 % points lower than in the study of Zhang et al. (2024a). In line with the differences in fat digestibility, faecal bile acid losses were almost seven times higher in the current study compared to Zhang et al. (2024a) when fed 0 %-STC diets (87.5 versus 12.6 μmol kg^{-1} BW d^{-1}). In the study of Zhang et al. (2024a) fish were restrictively fed about 70 % the amount of the feed intake on metabolic body weight in the current study. The differences in fat digestibility and faecal bile acid losses between studies might be due to differences in feed intake (as discussed above), batch differences in feed ingredients and/or different fish batches (genetics). Interestingly, dietary NSP inclusion did not increase faecal bile acid loss, despite the fact that it increased the amount of faecal waste, because dietary NSP inclusion reduced the faecal bile acid content. This current observation confirms our earlier observations on different types of NSP (Zhang et al., 2024b), that insoluble NSP have no impact on the amount of bile acid lost via faeces in yellowtail kingfish. This differs from rainbow trout, where dietary NSP inclusion increases faecal bile acid losses mainly because of increased faeces being excreted (Staessen et al., 2020b; 2022). Thus, it seems that there are fish species related differences in the impact of dietary factors effecting faecal bile acid losses and consequently fat digestion. Yellowtail kingfish seems to tolerate NSP better than rainbow trout.

In the present study, bile acid balances were negative for fish fed diets without STC supplementation. In other words, the daily amount of bile acids lost via faeces exceeded the amount bile acid consumed with the feed. For maintaining a sufficient bile acid body pool size, endogenous synthesis is required (Chiang, 2009). Dietary NSP supplementation had no effect on fish body bile acid pool size. This is consistent with the absence of a NSP supplementation effect on the bile acid balance and bile acid synthesis. In contrast, dietary supplementation of STC led to

positive bile acid balance, increased the fish bile acid body pool size while it reduced the bile acid synthesis. This suggests that STC supplemented to the diets enters the EHC, which is supported by the positive bile acid digestibility value.

The final body bile acid pool size relative to body weight (292 μmol kg^{-1} BW) declined during the experimental period in fish fed the 0 %-STC diets, as the initial body bile acid pool size was 487 μmol kg^{-1} BW. This might be an indication that the growth of the yellowtail kingfish's body bile acid pool did not keep up with the overall body growth. In contrast, the bile acid pool size increased linearly with body weight in rainbow trout (Staessen, 2021). However, it is unclear whether this difference between these species is a characteristic of yellowtail kingfish having a low capacity for enlarging its bile acid pool size through synthesis. Alternatively, diets contained only 20 % fish meal and 45 % plant protein ingredients, may have a too low bile acid/cholesterol content (i.e. limitation) to achieve a sufficient growth of the bile acid body pool as bile acid synthesis might be limited.

In the current study, dietary STC supplementation increased fat digestibility by 6.8 percentage points, which is much larger than the 2.2 % in an earlier study with using a similar diet but fed restrictively (Zhang et al., 2024a). The effect of STC supplementation was unaffected by diet (no interaction), which is consistent with a study by Staessen et al. (2022) on rainbow trout, in which the effect of STC supplementation was comparable between diets with varying dietary NSP levels. In contrast, a study by Staessen et al. (2020) (rainbow trout) found that an NSP-rich diet resulted in higher improvement than a low-NSP diet. Likewise, another study using rainbow trout found that the extent of improvement in fat digestibility with bile acid supplementation was dependent on the lipid source used (Amirkolaei et al., 2024). Both in the current study and Staessen et al. (2022), whereby the effect of STC supplementation was independent of diet, the fat digestibility was comparable between the low and high NSP diet without STC supplementation and the low and high NSP diet with supplementation (no diet effect), whereas in Staessen et al. (2020), the fat digestibility was considerably lower for the high NSP diet without STC supplementation (diet effect) and showed a larger improvement with STC supplementation. This suggests that the extent of improvement with STC supplementation can be affected by the diet, as fat digestibility with STC supplementation was found to be comparable irrespective of the diet (dietary NSP levels) within trials (current; Staessen et al., 2020, 2022). As a result, the current study does not show an interaction effect between diet and STC supplementation because the diet (NSP level) had no effect on fat digestibility. Despite a large improvement in the current study, fat digestibility still remained very low. Likewise, in gilthead seabream, supplementing a blend of bile acids (including STC) to a commercial-like diet improved fat digestibility by nearly 5 percentage points, while protein digestibility remained unaffected (Ruiz et al., 2023).

Other factors that were not investigated in this study could contribute to low fat digestibility. One noteworthy factor is that all diets in this study contained approximately 19 % starch. Starch has been shown to have a strong negative impact on nutrient digestibility in yellowtail kingfish, especially fat digestibility (Horstmann et al., 2023c). Yellowtail kingfish might experience hyperglycaemia due to a low glucose utilisation (Booth et al., 2013). It would be valuable to explore in-depth the strong negative effect of starch in this species, for instance, hyperglycaemia-related parameters, enzyme activities, health-related parameters.

Including wheat brand and soy hulls, both high in NSP, did not reduce growth performance, despite a decrease of approximately 9 % in organic matter digestibility and a lower protein and fat content in the diet due to NSP dilution (10 %). The absence of a negative effect on growth was primarily due to increased feed intake and a tendency for improved protein digestibility, resulting in comparable N (protein) retention (as shown in Fig. 1). This higher feed intake but comparable growth resulted in an impaired FCR, which increased by about 7 %. Although STC supplementation significantly improved fat digestibility, it did not improve growth statistically (only numerically). STC improved fat digestibility, which led to more energy retained as fat while keeping energy retained as protein comparable. Because fat retention is associated with minor water loss, whereas protein retention, which is the primary driver of growth, is associated with water gain (3–4 g per gram N retained), the effect of increased fat digestibility and retention on growth is not as apparent. As a result of the increased feed intake with STC supplementation and numerically higher growth, the FCR was comparable to the 0 %-STC treatments.

For farming yellowtail kingfish in RAS, faeces production has been one of the major challenges, as they produce poor, unstable faeces that are difficulty to be removed by drum filter or sedimentation (Horstmann et al., 2023a–c). Dietary interventions that improve nutrient digestibility and/or enhance the characteristics of egested faeces have been considered as potential approaches to improve faecal waste management (Tran-Tu et al., 2018). In a previous study, replacing starch by NSPs from soy hulls or sugar beet pulp increased faecal waste production but improved the faecal removal efficiency in comparison to a starch diet (Zhang et al., 2024b). In the current study, the addition of NSP (a mixture of wheat bran and soy hulls) increased faecal waste production due to a lower organic matter digestibility, in line with the previous study on yellowtail kingfish (Zhang et al., 2024b). The increased amount in faecal waste was expected to primarily come from indigestible dietary NSP fraction. As the NSP inclusion had no negative effects on other macronutrient digestibility, it showed only a dilution effect resulting in increased faecal bulk. Furthermore, supplementing STC to the diets was expected to improve nutrient digestibility and thus reduce faecal waste production. In the current study, fat digestibility was improved by bile acid supplementation but not the overall nutrient digestibility. As a result, it did not have an effect on the amount of faeces produced.

Efficient removal of faeces is crucial in RAS to maintain good water quality. Faecal characteristics, such as particle size and density, directly influence faecal removal efficiency (Reid et al., 2007, 2009). The current study found a low faecal removal efficiency of approximately 33 %, which means that only a small portion of faeces was effectively removed from the system by settling. The inclusion of NSP improved faecal removal efficiency. Despite different NSP sources and inclusion level used between studies, this improvement in faecal removal efficiency by NSP is in line with previous findings for yellowtail kingfish (Zhang et al., 2024b) and other fish species (Prabhu et al., 2019; Fountoulaki et al., 2022; Prakash et al., 2023). Moreover, dietary NSP supplementation resulted in a larger portion of large particle size and a smaller portion of small particle size compared to the CON diets. In addition, the composition of faeces was altered by dietary NSP inclusion, which could have influenced the overall density of faeces and, as a result, faecal removal efficiency (Amirkolaie et al., 2005; Prabhu et al., 2019; Fountoulaki et al., 2022; Reid et al., 2007; Sinha et al., 2011). Fish fed the STC supplemented diets had a lower fat content in their faeces compared to fish fed the non-supplemented diets. However, the difference in faecal fat content did not result in changes in faecal removal efficiency nor particle size distribution. This is consistent with the previous findings (Reid et al., 2009; Zhang et al., 2024a). It is unlikely that faecal fat content has a decisive influence on the faecal quality because the proportion of fat in faeces is relatively small compared to carbohydrates (starch and NSP) in yellowtail kingfish (Reid et al., 2009; Zhang et al., 2024a).

Overall, high faecal waste production combined with high faecal removal efficiency in the NSP diets, led to more removed faeces compared to the CON diets. As a result, yellowtail kingfish fed the CON or NSP diets had comparable amounts of non-removed faeces. Despite of high faecal production, high NSP had no negative effect on non-removed faeces, which is line with previous findings in common carp (Prabhu et al., 2019) and European seabass (Fountoulaki et al., 2022). In practice, NSP supplementation will increase the amount of faeces to be removed. Thus, a higher waste loads for drum filers, which can increase

the backwash frequency. This will increase the cost for removal of waste and the water use for backwashing. Since the drumfilter backwash water is originated from the system water, this may increase the systems water refreshment rate. Additionally, the bile acid supplementation had no effect on non-removed faeces.

Our findings showed that yellowtail kingfish are less sensitive to dietary NSP compared to other carnivorous species regarding feed intake and nutrient digestibility; feed intake in yellowtail kingfish increased with dietary NSP inclusion. NSP did not affect fat digestibility, and even improved phosphorus digestibility and tended to improve protein digestibility. Dietary STC supplementation increased feed intake and improved fat digestibility through enlarging the size of the body bile acid pool of yellowtail kingfish, independent of dietary NSP level. Furthermore, 10 % inclusion of NSP in diets influenced faecal waste production, faecal composition and particle size distribution. Regarding faecal waste, a small amount of NSP in the diets results in a relative large portion in the faeces, which may play an important role in determining faecal characteristics and removal efficiency. Considering its effects, incorporating small amounts of NSP into the feed can be helpful for effective faecal waste management in RAS fish. However, dietary supplementation with STC did not alter faecal waste production or faecal removal efficiency.

CRediT authorship contribution statement

Schrama Johan: Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **Staessen Thomas:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Kokou Fotini:** Writing – review & editing, Conceptualization. **Horstmann Peter:** Writing – review & editing, Methodology, Conceptualization. **Hilgers Pim:** Writing – review & editing, Methodology, Investigation. **Maas Roel:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Zhang Yaqing:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

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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.2025.102708.

Data availability

Data will be made available on request.

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