



Effect of dietary starch inclusion, feeding level and their combination on nutrient digestibility, bile acid balance, faecal waste production and characteristics of yellowtail kingfish (*Seriola lalandi*)

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ABSTRACT

The effect of dietary starch content, feeding level and their interaction on nutrient digestibility, bile acid balance, faecal waste production and characteristics were investigated in yellowtail kingfish (*Seriola lalandi*). The effect of starch inclusion level was tested in a dose-response trial, using five diets differing in the inclusion level of gelatinized wheat flour (WF) (0, 4, 8, 12 and 16 %) and thus starch level. Each diet treatment was tested in triplicates. A restricted feeding period of 4-wk was followed by a 2-wk period of satiation feeding. Results showed that both starch inclusion level and feeding level (restricted vs. satiation) negatively affected macronutrient digestibility, with fat digestibility being most affected. The latter was associated with a potential depletion of the body bile acid pool as bile acid balances (bile acid intake – faecal bile acid loss) were consistently negative for all diets during both feeding periods. Faecal bile acid loss increased with both increasing feeding level and increasing dietary starch level, which was mainly caused by increased faecal waste production. Furthermore, starch had a negative impact on faecal characteristics. High dietary starch levels resulted in increased faecal waste production, and higher amounts of non-removed faeces by settling due to reduced faecal removal efficiency. The critical dietary starch content was estimated to be 6 %. Above a 6 % dietary starch content, fat digestibility and faecal removal efficiency decreased, while non-removed faeces increased with the dietary starch content. When culturing yellowtail kingfish in RAS, dietary starch levels should be below 6 % from a digestion as well as faecal management point of view.

1. Introduction

Yellowtail kingfish (*Seriola lalandi*) grows fast, is traditionally cultured in cages and currently also in recirculating aquaculture systems (RAS) (e.g., Denmark, Germany, The Netherlands and United States of America) (EUMOFA, 2020). In RAS, waste management is essential for optimal system functioning and to ensure good water quality. The non-digested fraction of feed (faeces) is a principal source of solid waste. To ensure good water quality, faeces must be removed from the water via either sedimentation (settling) or filtration (i.e. drum filter). A major

challenge of farming yellowtail kingfish in RAS is their unstable faeces (Horstmann et al., 2023a, 2023b, 2023c). This instability can enhance the disintegration of faecal matter, resulting in higher level of suspended and dissolved waste within the system (Bureau and Hua, 2010). As a result, RAS may experience high concentrations of total suspended solids (TSS), which can have adverse effects on both water quality and fish health (Badiola et al., 2012).

Compared to a diet that included a mixture of natural food items (krill, squid, smelt and sand eel), feeding yellowtail kingfish only extruded diets resulted in lower digestibility and poorer faecal integrity

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

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(Horstmann et al., 2023b). It was hypothesised that this negative effect of extruded diets was due to the presence of carbohydrates that are poorly digested by yellowtail kingfish (carnivorous species) and potentially affect gut functioning (Horstmann et al., 2023b). In a follow up experiment, it was demonstrated the dietary starch level (i.e., inclusion level of gelatinized wheat flour) reduced the nutrient digestibility (especially fat) and also the faecal integrity (Horstmann et al., 2023c). However, only two starch levels were tested (4 % versus 20 %). Although Booth et al. (2013) did not measure nutrient digestibility, they indicate that dietary carbohydrate levels above 10 % would compromise fish performance, which could be linked to lower fat digestion.

Feeding level can influence nutrient digestibility in fish as was demonstrated in rainbow trout (*Oncorhynchus mykiss*) (Staessen et al., 2020a), Nile tilapia (*Oreochromis niloticus* L.) (El-Sayed, 2002) and African catfish (*Clarias gariepinus*) (Elesho et al., 2021; Henken et al., 1985). The effect of feeding level is not always consistent, but depends on fish species and dietary composition. For example, the effects of feeding level (restricted vs. satiation) on the digestibility of various amino acids (i.e., lysine and methionine) of different feed ingredients was ingredient specific (interaction effect) (Elesho et al., 2021). From a waste management perspective, even a minor 1 % reduction in organic matter digestibility of a common diet (having a digestibility of 90 %) implies a 10 % increase in faecal waste production, which is considerable (from 10 % undigested feed to 9 %). So far the effect of feeding level on digestibility, waste production and faecal characteristics in relation to starch inclusion level has not been studied for yellowtail kingfish. So far most studies on this topic have been done during restrictive feeding (Horstmann et al., 2023c; Horstmann Zuther et al., 2024) while in a commercial setting, yellowtail kingfish are commonly fed (close) to satiation.

Yellowtail kingfish have relatively low fat digestibility when compared to other carnivorous species (Zhang et al., under review). Of all macronutrients, fat digestibility was most influenced by various dietary factors (Horstmann et al., 2023a, 2023b, 2023c). Bile acids aid the breakdown of fat globules into smaller droplets. This emulsification increases the surface area for lipase to hydrolyse fat. Bile acids are synthesised in the liver, stored in the gallbladder and subsequently released into the intestine after a meal. The majority of bile acids are reabsorbed back in the distal intestine and transferred back to the liver through enterohepatic circulation (EHC). Nevertheless, still a small portion of bile acids is lost via the faeces. In rainbow trout, reduced fat digestibility is linked to an increased loss of bile acid with faeces, which was mainly related to more faeces being excreted (Staessen et al., 2022). In yellowtail kingfish, increasing dietary starch levels increases faecal waste production and decreases fat digestibility (Horstmann et al., 2023c). Whether the effects of dietary starch level on fat digestibility and faecal bile acid loss in yellowtail kingfish are related to faecal waste production is still unknown.

Considering the above, the main objective of the study was to explore how yellowtail kingfish respond to a range of dietary starch and to pinpoint the specific dietary starch level at which changes become evident. Therefore, a dose-response of dietary starch on nutrient digestibility, bile acid balance, faecal waste production and characteristics of yellowtail kingfish were investigated. Furthermore it was assessed if these dose-response relationships were affected by feeding level. The effect of dietary starch was tested in a dose-response trial, using five diets different in the inclusion level of gelatinized wheat flour (WF) (0, 4, 8, 12 and 16 %). To determine whether the effect of the dietary starch level was dependent on feeding level, a restrictive feeding period was followed by a satiation feeding period.

2. Materials and methods

2.1. Diets

Five diets were formulated with different gelatinized wheat flour

(WF) inclusion levels (Table 1). WF was used as the starch source to create different levels of dietary starch. A basal mixture was formulated, containing 20 % fish meal and 45 % plant protein ingredients as main protein sources. To obtain diets varying in dietary starch content, the basal diet was diluted by adding different levels of WF. Five inclusion levels of WF were applied: 0, 4, 8, 12 and 16 %. This resulted in an analysed dietary starch contents of 38, 60, 99, 129, and 148 g/kg DM, respectively. To prevent deficiencies and to meet all known nutrient requirements (National Research Council NRC, 2011), diets were

Table 1

Ingredient and analysed nutrient composition of the dietary treatments fed to yellowtail kingfish during restricted feeding (27 days) and satiation feeding (14 days).

	Diet				
	0-WF	4-WF	8-WF	12-WF	16-WF
Ingredients (g/kg)					
Gelatinized wheat flour (WF)	-	40	80	120	160
Basal mixture	1000	960	920	880	840
Ingredients basal 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				
Analysed nutrient content (g/kg DM):					
Dry matter (DM g/kg)	944	927	938	929	950
Crude protein	645	629	604	586	566
Crude fat	177	170	163	155	147
Total carbohydrates ^h	78	102	138	168	201
Starch and sugars	38	60	99	129	148
NSP ⁱ	39	42	39	39	50
Gross energy (kJ/g DM)	23.4	23.1	23.1	22.7	22.5
Crude ash	101	99	95	91	87
Phosphorus	14.6	14.3	14.2	14.0	12.9
Calcium	12.4	11.7	12.0	10.7	10.4
Bile acid (μmol/g DM)	0.14	0.12	0.13	0.12	0.12
Gelatinization degree (%)	37	42	67	76	78

WF - gelatinized wheat flour, the number linked to WF indicates the inclusion level (%);

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.

^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.

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

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

supplemented with taurine, DL-methionine and monocalcium phosphate. All diets contained at least 10 % fish oil to ensure that the requirements for essential fatty acids were met. The ingredient composition and analysed nutrient composition of the diets are given in Table 1.

The diets were produced by cold pelleting by Research Diet Services (Wijk bij Duurstede, The Netherlands) using a Clextal BC45 laboratory scale twin-screw extruder (Clextal, Firminy, France) with a 3 mm die, resulting in 3 mm sinking pellets according to Kals et al. (2019). Thereafter, pellets were dried for 2 h at 45°C followed by 3 h at 70°C. Diets were produced approximately one week prior to the start of the experiment and were stored at 4°C throughout the experiment.

2.2. Experimental procedures

The experiment lasted 41 days, consisting of a 27-d restricted feeding and a 14-d satiation feeding period. 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 prevent feed spillage. For restricted feeding, a feeding level of 23.75 g/kg^{0.8} body weight (BW) day⁻¹ was applied, which is approximately 95 % of the predicted feeding level (Horstmann et al., 2023), whereby the feeding level was increased based on the daily feed intake and calculated growth using an expected FCR of 0.9. During the first five days of satiation feeding, the feeding level was gradually increased until apparent satiation was reached. This allowed the fish to adapt to the increased feeding level. Fish were fed twice daily at 9:00 and 15:00 h. During satiation feeding, feeding was ended when fish stopped eating or when the feeding time exceeded 1 h. Each tank was connected to a swirl separator with a glass bottle for collecting faeces and uneaten feed. Fifteen minutes after feeding, the glass bottles were checked for feed spills to determine feed intake. Mortality was checked at least twice a day before feeding.

2.3. Fish, rearing conditions and housing facilities

The Animal Welfare Body of Wageningen University approved and evaluated this experiment as being a non-invasive animal experiment according to Dutch legislation (Act on Animal Experiments). Fish were kept and handled in agreement with EU-legislation. The experiment was conducted at the Aquaculture Research Facility (ARF) of Wageningen University and Research (Wageningen, The Netherlands). Yellowtail kingfish of mixed sex were obtained from a commercial fish farm (Kingfish Zeeland, Kats, The Netherlands). After arriving at the research facilities, the fish were acclimatized for more than two weeks before the experiment began, under the same conditions (light regime, temperature, etc.) as the experiment. The fish were fed restrictively for 4 weeks, followed by 2 weeks of satiation feeding. At both the start and end of the restricted and satiation feeding period, fish were batch weighted (Mettler-Toledo ICS429) in three rounds (2 rounds of 8 fish and 1 round of 9 fish) to determine the total tank biomass. The day prior to weighing, fish were not fed. Per tank, 25 fish with a mean initial weight of 48 g were stocked (stocking density: 3.2 kg/m³). The number of fish in each tank was reduced to 17 at the start of the satiation feeding period (stocking density: ± 6.1 kg/m³) to ensure that the biofilter carrying capacity of the common RAS was not exceeded and that the oxygen level in the tank remained above the predetermined threshold. Diets were randomly assigned to one of the tanks with 3 replicates per diet. These tanks (n = 15) were connected to a common RAS, which was filled with artificial seawater. This RAS consisted of a sump, settling tank, drum filter (30 µm), protein skimmer, and trickling filter. Water quality parameters were measured daily from the common outflow of the tanks to ensure that the water quality parameters remained within pre-set conditions for yellowtail kingfish (for pre-set conditions of water quality, see Supplementary table 1). The dissolved oxygen concentration in the outlet water was always > 5.5 mg/L/> 94 % saturation (WTW Multi

3630 IDS - FDO 925), and mean water temperature was 23.3 ± 0.1°C (WTW Multi 3630 IDS - FDO 925) during both experimental feeding periods (mean from daily measurements). The pH ranged between 7.3 and 7.6 (WTW Multi 3630 IDS - SenTix 940).

2.4. Sampling and analysis

During week 4 and 6 of the experiment, faeces collection for digestibility analysis was done overnight for 5 days. Bottles connected beneath the swirl separators, were submerged into ice water to minimize bacterial degradation of faeces with the use of a Styrofoam box. Collected faeces were pooled per tank and stored at -20°C until further analysis. Faeces collection for determination of faecal removal efficiency was done during the weekend of week 4 for restricted feeding and week 6 for satiation feeding. The collection method was the same as for the faeces collected for digestibility, except that faecal material was collected continuously for 48 h (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 the restricted and satiation feeding period (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 taken by pooling 50 g per experimental diet per week (stored at 4°C).

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). Feed and faeces were analysed for dry matter and ash, total nitrogen, gross energy, crude fat, yttrium, phosphorus, and calcium as described by Staessen et al. (2020). The crude protein content was calculated as the total nitrogen content multiplied by 6.25. Gelatinized starch was analysed according to the modified glucoamylase method described by Zhu et al. (2016). With the use of total starch 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 as the protocol described in Horstmann et al., 2023a, with the exception that entire sample collected after morning feeding (3 hour collection) was used. No subsampling was made.

Bile acid content was determined by the enzymatic cycling rate after extraction. Bile acid extraction was performed on feed and faeces according to Porter et al. (2003) and consisted of alkaline hydrolysis in ethylene glycol-KOH and acidification with concentrated HCl. Three rounds of extraction were performed with diethyl ether, and the diethyl ether extract was evaporated at 40°C under a constant 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®, Dresden, Germany).

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). Specific growth rate (SGR; %/d) was calculated as $(\ln W_f - \ln W_i)/t \times 100$ %, where t is the number of days. The daily absolute feed intake (FI_{abs} ; g/d) was calculated as FI_{tot}/t , where FI_{tot} is the total feed intake (g). Daily 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 $e^{(\ln W_f + \ln W_i)/2}$. 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: $ADC (\%) = 100 \times (1 - ((Y_{\text{diet}} / Y_{\text{faeces}}) \times (N_{\text{faeces}} / N_{\text{diet}})))$, where Y is the inert marker content in diet or faeces and N is the nutrient content (or kJ/g for gross energy) in diet or faeces on DM basis. In feed and faeces, organic matter (g/kg DM) content was calculated as $1000 - \text{ash}$ and total carbohydrates content as $1000 - (\text{crude protein} + \text{crude fat} + \text{ash})$.

Faecal waste production, faecal removal efficiency and non-removed faeces per feed were calculated according to Horstmann et al. (2023c). Faecal waste production (g OM/kg DM Feed) was determined on organic matter basis as the amount of non-digested feed per kilogram dry matter feed intake as $(100\% - ADC_{\text{OM}}) \times OM_{\text{diet}}$, where ADC_{OM} is the organic matter digestibility (in %) and OM_{diet} the organic matter (in g/kg DM) of the diet. Faecal removal efficiency (FR, %) was calculated as the percentage of collected faeces by settling throughout 48 h continuous faeces collection in relation to the total amount of faecal waste production. In detail, this was calculated as the amount of yttrium collected by settling (Y_{removed} , g) in relation to the total amount of yttrium given via the feed (Y_{diet} , g) as $Y_{\text{removed}} / Y_{\text{diet}} \times 100\%$. The non-removed faeces per feed intake (g OM/kg DM Feed) was calculated as the difference between the total amount of faecal waste produced and the amount of faeces removed as $((100\% - FR) \times (100\% - ADC_{\text{OM}}) \times OM_{\text{diet}})$, where FR is the faeces removal efficiency during the 48 h continuous faeces collection and ADC_{OM} the organic matter digestibility during week 4 (restricted feeding) and week 6 (satiation feeding).

Faecal PSD was determined as percentage as $P_{\text{fraction}} / P_{\text{total}}$, 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.

Daily bile acid intake ($\mu\text{mol d}^{-1}$) was calculated as daily feed intake \times bile acid content in the feed. Daily faecal bile acid loss ($\mu\text{mol d}^{-1}$) was calculated as the amount of OM excreted via faeces per day (g OM d^{-1}) multiplied by the faecal bile acid content ($\mu\text{mol g}^{-1}$ OM). The amount of faeces produced was calculated as the daily OM intake \times (100% - ADC_{OM}), where ADC_{OM} is the organic matter digestibility during week 4 and 6. Bile acid balance was calculated as bile acid intake - faecal bile acid loss. Daily bile acid intake, faecal bile acid loss, and bile acid balance were expressed per kg BW ($\mu\text{mol kg}^{-1}$ BW d^{-1}), using the mean BW during both experimental periods. Mean BW was calculated as $(W_i + W_f)/2$. Bile acid digestibility (%) was calculated in the same way as the digestibility of the nutrients.

2.6. Statistical analysis

Data were analysed using a mixed model ANOVA for the effect of diet (WF inclusion), feeding level (restricted / satiation) 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 and feeding level, while the interaction with diet was tested against the error within tanks. Because the interaction was significant for several parameters, a one-way ANOVA was performed to examine at the effect of diet on the restricted and satiation feeding periods separately. The residuals were tested for normality (Shapiro-Wilk) and homogeneity (Levene's test). Parameters that did not meet the requirements for normality and/or homogeneity were tested for treatment effect using a non-parametric test (Kruskal-Wallis). There were few parameters that did not meet normality (2 out of 69) and homogeneity (15 out of 69) criteria. Non-parametric testing did not change the statistical effects for those parameters except one (faecal organic matter content at satiation feeding). As a result, it was decided to use ANOVA for all parameters in the main text to keep the statistics consistent and allow for the testing of an interaction effect. The tables indicate which parameters did not meet the normality and/or homogeneity criteria. The supplementary data includes the results of non-parametric testing of all parameters, as well as

the standard error (SE) of the mean of the treatments (Supplementary tables 5 to 10). 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. Additionally, linear plateau analysis were performed for fat ADC, faecal removal efficiency and non-removed faeces in order to estimate if threshold values of starch inclusion levels were present. Linear plateau analysis were done by the NLIN procedure of SAS and in this analysis the analysed dietary starch levels were used. Statistical analyses were performed using the statistical program SAS 9.4, SAS Institute, North Carolina, USA.

3. Results

3.1. Fish performance

Fish performance results are shown in Table 2. Although a statistical interaction effect of diet and feeding level on FCR were found, this finding will not be discussed in depth as they were not the focus of this study (due to different dietary crude protein contents). Initial body weight at day 0 (start restricted feeding period) was equal for all diets ($p > 0.05$). No fish died during the entire experiment. An interaction effect was present for both the absolute daily feed intake (FI_{abs} , $p < 0.001$) and the daily feed intake expressed per unit of metabolic body weight (FI_{MBW} , $p < 0.001$). FI_{MBW} was equal during the restricted feeding period (first four weeks), being 16.5 g/d/kg^{0.8}. During the satiation feeding period, increasing WF levels resulted in an increased daily feed intake. FI_{MBW} increased from 22.3 g/d/kg^{0.8} in fish fed the 0 % WF diet to 27.4 g/d/kg^{0.8} for fish fed the 16 % WF diet ($p < 0.001$).

3.2. Digestibility

Apparent digestibility coefficients (ADC, %) are given in Table 3. An interaction effect between diet and feeding level was observed for all nutrient ADCs ($p < 0.01$), with the exception for fat digestibility. The effect of WF inclusion showed similar impacts/trends during both periods. The ADC of all nutrients (except for starch and sugar during satiation feeding) decreased with increasing WF inclusion during both the restricted feeding and satiation feeding period. During the satiation feeding period, ADC for all nutrients were lower compared to restricted feeding ($p < 0.01$). Averaged over diets, the decrease in ADC between periods was 4.3 % points for organic matter, 2.8 % points for crude protein, and 7.6 % points for crude fat. Moreover, the inclusion levels of WF had the greatest influence on fat ADC ($p < 0.001$); averaged over periods, fat ADC was 6.4 % points lower at the 16 % WF diet than at the 0 % WF diet. In Fig. 1, the relation between fat ADC and analysed dietary starch content (g/kg DM) is shown. Linear plateau analysis showed that until a dietary starch level of 55 and 60 g/kg DM (inflection points) during restricted and satiation feeding respectively, ADC of fat was unaffected by dietary starch content. Above these inflection points fat ADC decreased as dietary starch content increased, with a stronger decrease during the restricted feeding period. Above the inflection point, fat ADC decreased with 7.4 % and 5.4 % points when increasing the dietary starch content with 100 g/kg DM during restricted and satiation feeding respectively (Supplementary table 2; Fig. 1).

3.3. Faecal characteristics and removal efficiency

The total amount of faecal waste produced was affected by an interaction between diet and feeding level ($p < 0.01$, Fig. 2). At all diets, more faecal waste (g OM/kg DM Feed) was produced during satiation feeding compared to restricted feeding. During both periods, increasing the inclusion level of WF in the diet led to an increase in faecal waste (Fig. 2). The increase in total amount of faecal waste was larger during restricted feeding than during satiation feeding; with an increase of 48 % and 8 % between the 0 % WF and 16 % WF diets during the restricted and satiation feeding period, respectively.

Table 2

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

	Feeding period	Diet					SEM	P-value		
		0-WF	4-WF	8-WF	12-WF	16-WF		Diet	F	Diet x F
Initial body weight (g)	R	48	48	49	47	48	1.1	-		
	S	136 ^a	136 ^{ab}	135 ^{ab}	130 ^{bc}	127 ^c	1.3	*** ^a		
	R+S							-	-	-
Final body weight (g)	R	138 ^a	137 ^a	135 ^{ab}	129 ^{bc}	127 ^c	1.6	**		
	S	239 ^{ab}	242 ^a	237 ^{ab}	227 ^{bc}	221 ^c	2.8	**		
	R+S							***	***	ns
FI _{abs} (g/fish/d)	R	2.30	2.34	2.36	2.34	2.30	0.014	-		
	S	6.01 ^a	6.38 ^{ab}	6.69 ^{bc}	6.93 ^c	6.97 ^c	0.108	*** ^a		
	R+S							-	-	-
FI _{MBW} (g/d/kg ^{0.8})	R	16.2	16.2	16.5	16.8	16.9	0.18	ns ^a		
	S	22.3 ^a	23.2 ^{ab}	24.9 ^b	26.4 ^{bc}	27.4 ^c	0.35	***		
	R+S							***	***	***
Growth rate (g/d)	R	3.34 ^a	3.30 ^{ab}	3.21 ^b	3.03 ^c	2.94 ^c	0.024	***		
	S	7.34 ^{ab}	7.58 ^a	7.27 ^{ab}	6.96 ^{ab}	6.82 ^b	1.138	*		
	R+S							**	***	ns
SGR (%/d)	R	3.94 ^a	3.90 ^a	3.79 ^{ab}	3.73 ^{ab}	3.64 ^b	0.046	**		
	S	4.01	4.12	4.01	4.00	4.03	0.050	ns		
	R+S							*	***	ns
FCR	R	0.65 ^a	0.66 ^a	0.69 ^b	0.72 ^c	0.74 ^d	0.0047	***		
	S	0.77 ^a	0.78 ^a	0.86 ^b	0.92 ^c	0.96 ^{cd}	0.008	***		
	R+S							***	***	***

WF - gelatinized wheat flour, the number linked to WF indicates the inclusion level (%); F – feeding level; R – restricted feeding; S – satiation feeding; R+S – restricted feeding and satiation feeding used in statistical analyses; FI_{abs} – daily feed intake absolute; FI_{MBW} – daily feed intake per metabolic body weight; SGR – specific growth rate; FCR – feed conversion ratio (on DM basis). Values are means (n = 3) and the pooled 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 > 0.05; * - p < 0.05; ** - p < 0.01; *** - p < 0.001.

^a The parameter did not meet the criteria for normality and/or homogeneity, but the statistical effect (larger or smaller than p = 0.05) did not change with a non-parametric test.

Table 3

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

	Feeding period	Diet					SEM	P-value		
		0-WF	4-WF	8-WF	12-WF	16-WF		Diet	F	Diet x F
Digestibility:										
Organic matter	R	91.6 ^a	91.7 ^a	89.8 ^b	88.6 ^c	87.8 ^c	0.28	***		
	S	85.2 ^{ab}	86.5 ^a	86.1 ^{ab}	85.6 ^{ab}	84.6 ^b	0.35	*		
	R+S							***	***	***
Crude protein	R	97.0 ^a	97.0 ^a	96.7 ^{ab}	96.5 ^{bc}	96.2 ^c	0.11	***		
	S	93.3 ^c	94.2 ^{ab}	94.4 ^a	94.3 ^a	93.7 ^{bc}	0.13	***		
	R+S							**	***	***
Crude fat	R	96.4 ^a	96.6 ^a	92.4 ^b	90.3 ^b	89.7 ^b	0.62	*** ^a		
	S	87.4 ^a	88.6 ^a	85.0 ^b	84.1 ^b	82.4 ^c	0.34	***		
	R+S							***	***	ns
Total carbohydrates	R	35.5 ^a	50.9 ^b	56.1 ^{bc}	59.4 ^c	63.1 ^c	1.36	***		
	S	12.5 ^d	35.8 ^c	51.2 ^b	56.7 ^{ab}	60.7 ^a	1.84	***		
	R+S							***	***	***
Starch and sugars	R	84.9	85.6	84.8	81.3	80.1	1.27	*		
	S	72.6 ^b	79.2 ^{ab}	83.6 ^a	82.1 ^{ab}	80.3 ^{ab}	1.96	*		
	R+S							ns	**	**
Energy	R	93.7 ^a	94.3 ^a	91.5 ^b	90.8 ^b	90.4 ^b	0.44	*** ^a		
	S	87.2 ^b	88.7 ^a	87.8 ^{ab}	87.1 ^{bc}	86.2 ^c	0.28	**		
	R+S							***	***	***
Phosphorus	R	66.1 ^{ab}	69.0 ^{bc}	69.7 ^c	65.9 ^a	66.0 ^a	0.57	***		
	S	51.0 ^a	55.9 ^b	63.6 ^{cd}	64.3 ^d	61.7 ^c	0.54	*** ^a		
	R+S							***	***	***

WF - gelatinized wheat flour, the number linked to WF indicates the inclusion level (%); F – feeding level; R – restricted feeding; S – satiation feeding; R+S – restricted feeding and satiation feeding used in statistical analyses; 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 > 0.05; * - p < 0.05; ** - p < 0.01; *** - p < 0.001.

^a The parameter did not meet the criteria for normality and/or homogeneity, but the statistical effect (larger or smaller than p = 0.05) did not change with a non-parametric test.

In contrast to faecal waste production, no interaction effect between diet and feeding period was present for faecal removal efficiency (p > 0.5; [Supplementary table 2](#)). The main effect of feeding level on faecal removal efficiency tended to significance (p = 0.053), with a slightly lower removal efficiency during restricted feeding than during satiation feeding. Faecal removal efficiency decreased with increasing WF inclusion in the diet (p < 0.001). In [Fig. 3](#) the effect of dietary starch

content on the faecal removal efficiency averaged over both feeding periods is given (no significant effect of feeding level). Linear plateau analysis did not give consistent results for faecal removal efficiency. The estimated inflection point was dependent on the starting values used in the NLIN procedure; either starting with a plateau that ended at a dietary starch content of 43 g/kg DM or ending with a plateau at dietary starch levels above 140 g/kg DM ([Supplementary figure 1](#)). Linear

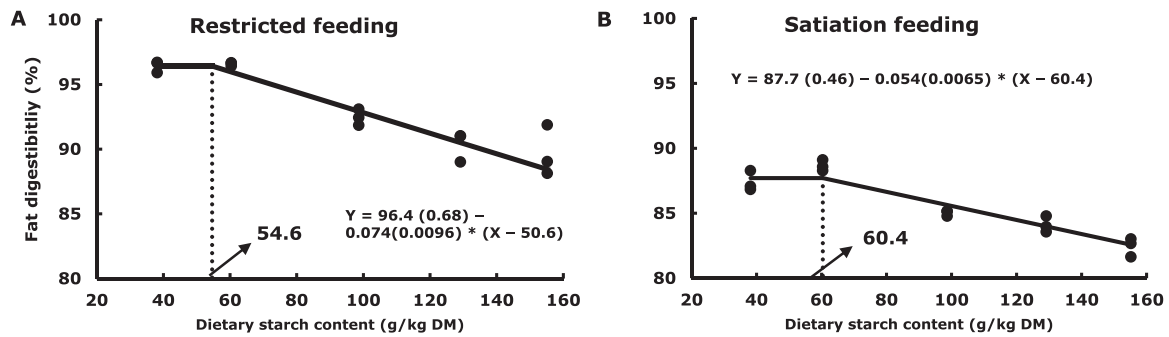


Fig. 1. The relationship between dietary starch content (expressed in g/kg DM) and fat digestibility (%) during restricted (panel A) and satiation feeding (panel B) in yellowtail kingfish. A linear plateau model was used for estimating the breakpoint (see dotted line). DM – dry matter. All estimated equations and R^2 of the models are provided in [Supplementary table 3](#).

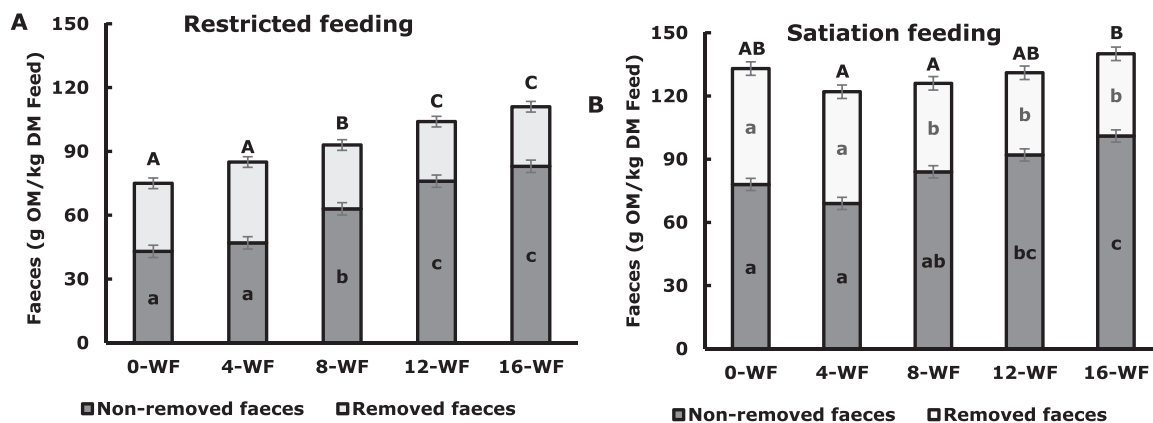


Fig. 2. Total, removed and non-removed faeces in of yellowtail kingfish fed the diets during restricted feeding (27 days, panel A) and satiation feeding (14 days, panel B). Total amount of faeces produced (entire bar), removed (light grey) and non-removed (dark grey) faeces per kg DM feed intake (g DM) during restricted feeding (panel A) and satiation feeding (panel B) in yellowtail kingfish. WF-gelatinized wheat flour; DM – dry matter. Values are means (n = 3) and standard error of the mean (SEM). Different superscripts in white, grey and above the bars (capitals) indicate dietary differences for removed faeces ($p > 0.05$ during restricted feeding; $p < 0.001$ during satiation feeding), and non-removed faeces ($p < 0.001$ for restricted and satiation feeding) and total faeces produced ($p < 0.001$ during restricted feeding; $p < 0.05$ during satiation feeding).

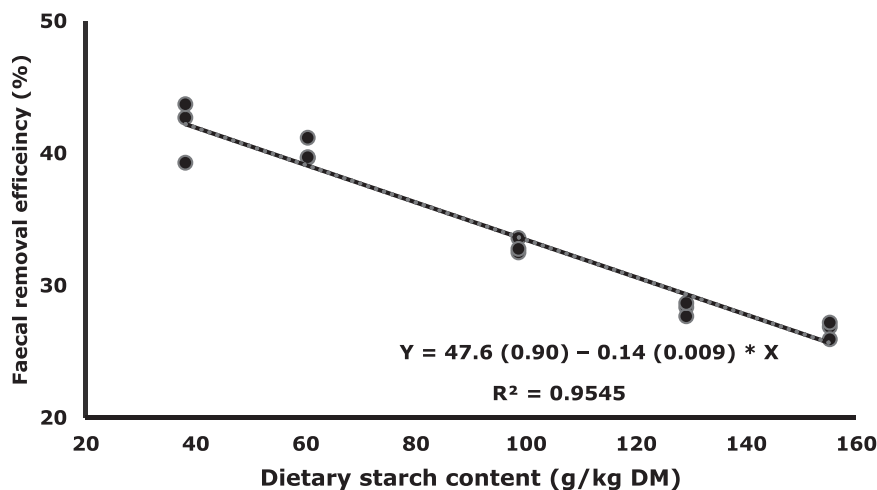


Fig. 3. The linear relationship between dietary starch content (X variable in the equation, in g/kg DM) and faecal removal efficiency (estimated value (Y), in %) averaged over the restricted and satiation feeding periods in yellowtail kingfish. Each dot represents a single data point. DM – dry matter. All estimated equations and R^2 of the models are provided in [Supplementary table 3](#).

regression on data averaged over both feeding periods (Fig. 3) showed that faecal removal efficiency decreased by 14 % point for every 100 g/kg DM dietary starch increase.

The amount of faecal waste produced, separated into removed and

non-removed faeces, is presented in Fig. 2. The interaction between diet and feeding level on non-removed faeces ($p < 0.01$) and removed faeces ($p < 0.01$; Fig. 2) was attributed to the interaction effect on the total amount of faecal waste produced, as faecal removal efficiency was

unaffected by this interaction. The amount of non-removed faecal waste increased with increasing dietary WF inclusion and expressed in absolute values the effect of dietary WF level was larger during restricted than during satiation feeding. In Fig. 4, the relation between the non-removed faecal waste (in g OM/kg DM feed) and dietary starch content (g/kg DM) is shown. Linear plateau analysis demonstrated that below a dietary starch level of 50 and 67 g/kg DM the non-removed faecal was constant during the restricted and satiation feeding period, respectively. Above these inflection points the amount of non-removed faecal waste increased by 38 and 31 g OM/kg DM feed when the dietary starch content increased with 100 g/kg DM during the restricted and satiation feeding periods, respectively (Supplementary table 3; Fig. 4).

Data on faecal composition are shown in Table 4. An interaction effect between starch inclusion level and feeding level was only found on faecal fat content ($p < 0.05$). There was a lower fat content in faeces at the low WF diets (0 % and 4 % WF) compared to the high WF diets (8 %, 12 % and 16 % WF), and this difference became larger when fish were fed to satiation. Starch inclusion levels had an effect on overall faecal composition ($p < 0.05$) during both restricted and satiation feeding periods. Carbohydrates were the largest fraction in faeces. Averaged over diets, the carbohydrate fraction in the faeces was 66 %, 54 % for the restricted and satiation feeding period, respectively. The faecal carbohydrate content was increased by increasing WF inclusion levels during satiation feeding ($p < 0.05$), but not during restricted feeding ($p > 0.05$). Crude protein was decreased with increasing WF inclusion levels for both restricted and satiation feeding periods ($p < 0.001$). Satiation feeding resulted in higher protein and fat content in the faeces compared to restricted feeding for all diets ($p < 0.001$).

Faecal particle size distribution (PSD) was unaffected by diet and the interaction between diet and feeding level ($p > 0.05$, Supplementary table 4). PSD was only affected by feeding level ($p < 0.001$). Therefore, Fig. 5 presents PSD of faeces averaged over diets for restricted and satiation feeding period, respectively. More large particles ($>850 \mu\text{m}$) were observed during satiation feeding compared to restricted feeding ($p < 0.001$). Fig. 6 shows a picture of faeces collected overnight for all experimental diets during the satiation feeding period. It can be observed that the visual appearance of the faeces integrity seemed similar across the dietary treatments, but there was an observable decrease in the amount of collected faeces with increased WF inclusion.

3.4. Bile acid balance

Bile acid related parameters are given in Table 5. Bile acid content was similar for all the diets, thus bile acid intake followed the same trend as feed intake. Due to the small differences in feed intake also small difference between bile acid intake were present between diets ($p < 0.001$; Table 5). The higher feed intake during the satiation period resulted also in a higher bile acid intake ($p < 0.001$). During restricted

feeding, fish fed the 0 % WF diet had the highest faecal bile acid content, while those fed the 16 % WF diet had the lowest faecal bile acid content ($p < 0.05$). However, difference in faecal bile acid content between diets were not observed during satiation feeding ($p > 0.05$). Due to the small difference in faecal bile acid content between treatments, the effects in faecal bile acid losses almost fully paralleled the pattern observed in the amount of faecal waste produced. Also faecal bile acid losses were affected by the interaction between WF inclusion and feeding level ($p < 0.01$). During satiation feeding, with increasing dietary WF inclusion the faecal bile acid loss increased, while this effect was less clear during restrictive feeding. During satiation feeding, increasing the dietary WF content from 0 % to 16 % caused the faecal bile acid loss to increase from 24.1 to 38.5 $\mu\text{mol kg}^{-1} \text{BW d}^{-1}$). Averaged over all diets, the faecal bile acid loss in fish fed to satiation was more than double that of fish fed restrictively ($p < 0.001$; Table 5). Because bile acid intake was similar in value among diets, the same trend of faecal bile acid loss was observed in bile acid balance.

4. Discussion

In the current study, the effects of starch inclusion level and feeding level on nutrient digestibility, bile acid balance, faecal waste production and faecal characteristics of yellowtail kingfish were investigated. Gelatinized wheat flour was used as starch source, with five different inclusion levels i.e., 0 %, 4 %, 8 %, 12 %, and 16 %, which resulted in an analysed dietary starch content of 38, 60, 99, 129, and 148 g/kg DM, respectively.

All nutrient digestibilities were lower at the higher feeding level in the current study, which is consistent with a prior study on yellowtail kingfish (Horstmann et al., 2023a). Though, the effect of feeding level on nutrient digestibility was larger in the current study than in Horstmann et al. (2023a). This might be due to the greater contrast in feed intake between restricted and satiation feeding in the current study. Based on metabolic body weight, feed intake during restricted feeding turned out to be around 60 % of that during satiation feeding in the current study, while it was around 80 % in the study by Horstmann et al. (2023a). The negative effect of feeding level on nutrient digestibilities in yellowtail kingfish is consistent with findings in other species such as African catfish (Elesho et al., 2021; Henken et al., 1985), Nile tilapia (Schrama et al., 2012) and rainbow trout (Staessen et al., 2020b). This has been suggested to be due to limitations of the digestive capacity fish being surpassed at higher feeding levels (Jobling, 1986; Schrama et al., 2012) and/or to an increase in digesta passage rate (Bromley, 1994; Dias et al., 1998). Furthermore, the current study showed that the decrease in nutrient digestibility with feeding level depended on the diet composition (i.e., starch content). The decrease in organic matter ADC during satiation feeding was more pronounced for the diets with a low starch content compared to diets with a high starch content. Similar findings were found in rainbow trout, where satiation feeding resulted in a higher

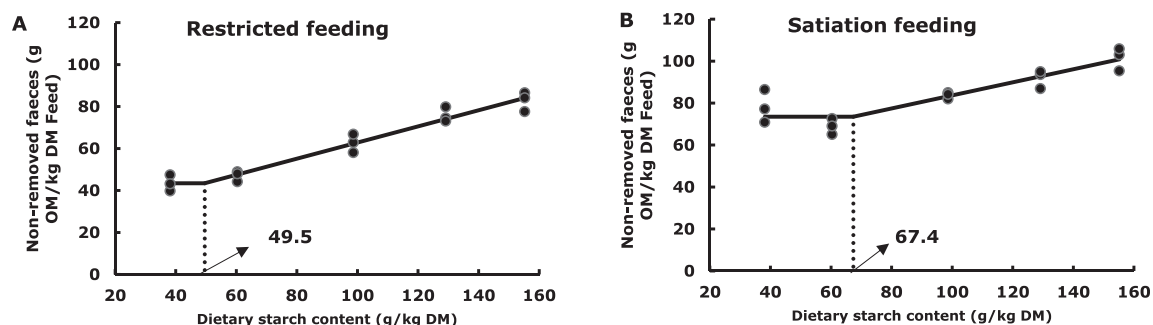


Fig. 4. The relationship between dietary starch content (expressed in g/kg DM) and non-removed faeces (g OM/kg DM) during restricted feeding (panel A) and satiation feeding (panel B) in yellowtail kingfish. DM – dry matter. A linear plateau model was used for estimating the breakpoint/optimal of dietary starch content regarding non-removed faeces. The estimated equations and R^2 for the linear plateau model are provided in Supplementary table 3.

Table 4

Faecal composition of yellowtail kingfish fed the experimental diets during restricted feeding (27 days) and satiation feeding (14 days).

	Feeding period	Diet					SEM	P-value		
		0-WF	4-WF	8-WF	12-WF	16-WF		Diet	F	Diet x F
Organic matter (g/kg DM)	R	425 ^a	435 ^a	462 ^b	469 ^{bc}	475 ^c	2.9	***		
	S	494 ^{ab}	486 ^b	487 ^b	490 ^{ab}	506 ^b	4.5	* ^b		
	R+S							***	***	**
Crude protein (g/kg OM)	R	253 ^c	250 ^c	214 ^b	197 ^a	196 ^a	3.7	***		
	S	323 ^a	302 ^a	271 ^b	256 ^b	256 ^b	6.1	***		
	R+S							***	***	ns
Crude fat (g/kg OM)	R	84 ^a	78 ^a	133 ^b	145 ^b	136 ^b	7.6	***		
	S	168 ^{ab}	158 ^a	195 ^{bc}	189 ^{bc}	183 ^b	5.5	**		
	R+S							***	***	*
Total carbohydrates (g/kg OM)	R	663	672	653	658	668	7.8	ns		
	S	509 ^a	539 ^{ab}	534 ^{ab}	555 ^{ab}	561 ^b	10.3	*		
	R+S							ns	***	ns
Starch and sugars (g/kg OM)	R	56 ^a	90 ^a	139 ^b	206 ^c	251 ^d	9.7	*** ^a		
	S	77 ^a	98 ^a	122 ^a	175 ^b	212 ^b	15.0	***		
	R+S							***	ns	ns
Energy (g/kg OM)	R	19.4	17.5	21.0	20.3	19.4	0.78	ns ^a		
	S	22.4 ^a	21.5 ^b	22.5 ^a	22.5 ^a	22.1 ^{ab}	0.20	* ^a		
	R+S							*	***	ns

WF - gelatinized wheat flour, the number linked to WF indicates the inclusion level (%); F – feeding level; R – restricted feeding; S – satiation feeding; R+S – restricted feeding and satiation feeding used in statistical analyses; DM – dry matter. OM – organic matter. Values are means (n = 3) and the pooled 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 > 0.05; * - p < 0.05; ** - p < 0.01; *** - p < 0.001.

^a The parameter did not meet the criteria for normality and/or homogeneity; using a non-parametric test, the effect becomes non-significant (p = 0.053).

^b The parameter did not meet the criteria for normality and/or homogeneity, but the statistical effect (larger or smaller than p = 0.05) did not change with a non-parametric test.

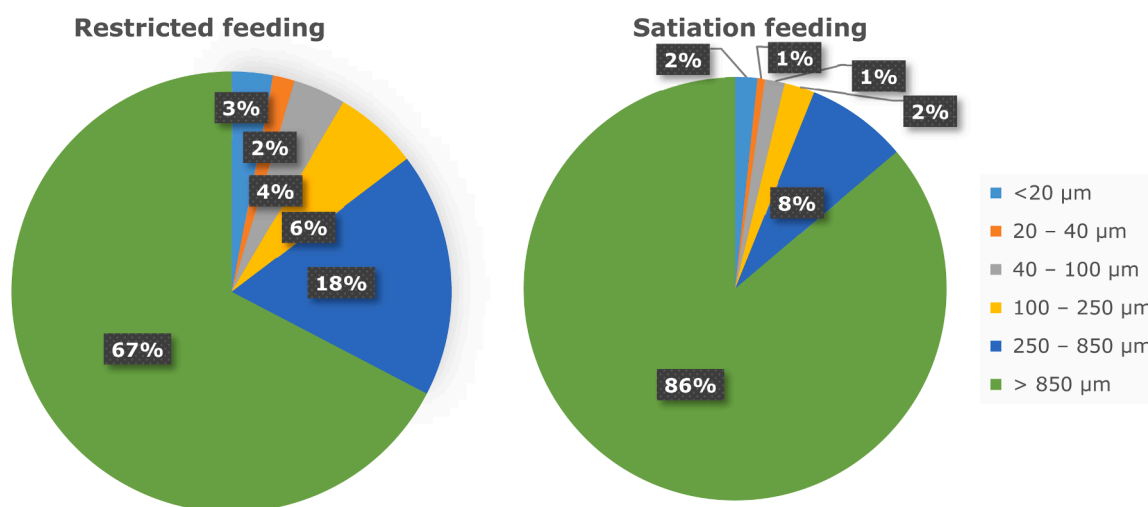


Fig. 5. Particle size distribution of yellowtail kingfish faeces averaged over diets (classes were unaffected by diet) for restricted and satiation feeding periods; [Supplementary table 4](#) provides a complete overview, including statistics.

reduction in digestibility in fishmeal-based diets compared to plant-based diets (Staessen et al., 2020b). This may be explained by a difference in adaptation time to the experimental diets. Early-feeding of a plant-based diet improved future acceptance and utilization of plant based diets in a study with rainbow trout (Geurden et al., 2013). In the current study, the fish were obtained from a commercial company, where fed diets containing at least 70–90 g/kg DM of starch (personal communication, Kingfish Zeeland, The Netherlands). This implies that the fish may have been better adapted to the high starch level diets. However, there is a simpler explanation; the highest ADC of OM was observed during the restrictive feeding period at the 0 % and 4 % WF diets (Table 3); with a high ADC as a reference point, the potential for an adverse impact is higher compared to treatments with a relatively low ADC of OM.

Compared to protein and starch digestibility, fat digestibility was most strongly reduced by dietary starch level in yellowtail kingfish. This

result is consistent with earlier studies conducted in yellowtail kingfish (Horstmann et al., 2023a), Atlantic salmon (*Salmo salar*) (Hemre et al., 1995), rainbow trout (Staessen et al., 2021), and gilthead sea bream (*Sparus aurata*) (Fountoulaki et al., 2005). Likewise, in Booth and Pirozzi (2021), despite lower absolute dietary fat levels (16.5 % vs. 22.2 %), the fat digestibility decreased by ± 7 % when the level of wheat flour was 54.3 g/kg diet higher. The larger impact of starch on fat digestibility cannot simply be explained by the factors such as a shorter digesta transit time, a negative effect of osmolarity, and/or viscosity, as suggested for impacts of NSP (Amirkolaie et al., 2006; Leenhouders et al., 2007; Refstie et al., 1999). Literature on rainbow trout has suggested that this comparable strong/high reduction in fat digestibility can occur due to a depletion of the total body bile acid pool size caused by enhanced faecal bile acid loss (Staessen et al., 2020b). The results from the current study showed that yellowtail kingfish consistently lost bile acids in their faeces even at the diet with the lowest starch, and that this

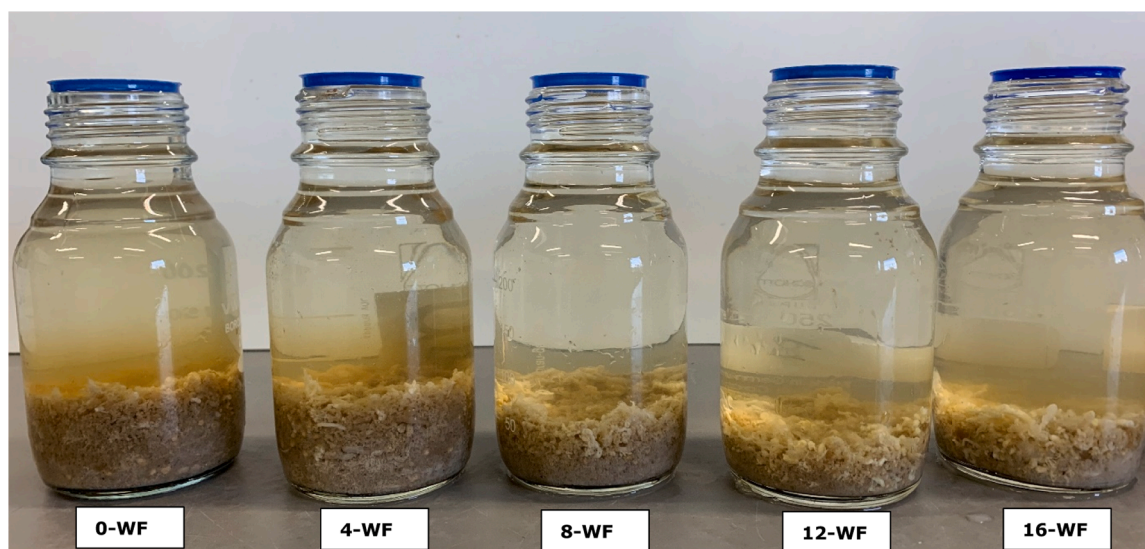


Fig. 6. Overnight collected faeces (each collection bottle represents one replicate per treatment) of yellowtail kingfish fed the 0-gelatinised wheat flour (WF), 4-WF, 8-WF, 12-WF, and 16-WF diet (left to right) to apparent satiation for 14 days.

Table 5

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

	Feeding period	Diet					SEM	P-value		
		0-WF	4-WF	8-WF	12-WF	16-WF		Diet	F	Diet x F
Bile acid intake ($\mu\text{mol kg}^{-1} \text{BW d}^{-1}$)	R	3.3 ^c	2.8 ^a	3.1 ^b	2.9 ^a	3.0 ^{ab}	0.04	*** ^a		
	S	4.3 ^{bc}	3.7 ^a	4.3 ^{bc}	4.2 ^b	4.4 ^c	0.04	***		
	R+S							***	***	**
Faecal bile acid content ($\mu\text{mol g}^{-1} \text{OM}$)	R	6.6 ^b	5.3 ^{ab}	6.0 ^{ab}	5.9 ^{ab}	4.6 ^a	0.32	*		
	S	6.0	6.0	5.8	6.2	6.4	0.55	ns ^a		
	R+S							ns	ns	ns
Faeces production ($\text{g OM kg}^{-1} \text{BW d}^{-1}$)	R	1.8 ^a	1.8 ^a	2.2 ^b	2.6 ^{bc}	2.8 ^c	0.08	***		
	S	4.0 ^a	3.8 ^a	4.2 ^a	4.7 ^b	5.5 ^c	0.09	***		
	R+S							***	***	**
Faecal bile acid losses ($\mu\text{mol kg}^{-1} \text{BW d}^{-1}$)	R	11.8 ^{ab}	9.4 ^a	13.4 ^b	15.1 ^b	12.9 ^{ab}	0.81	**		
	S	24.1 ^a	21.7 ^a	24.8 ^a	29.2 ^{ab}	38.5 ^b	2.20	** ^a		
	R+S							**	***	**
Bile acid balance ($\mu\text{mol kg}^{-1} \text{BW d}^{-1}$)	R	-8.4 ^a	-6.6 ^a	-10.3 ^{ab}	-12.2 ^b	-9.9 ^{ab}	0.79	**		
	S	-19.8 ^a	-17.9 ^a	-20.5 ^a	-25.0 ^{ab}	-34.1 ^b	2.22	** ^a		
	R+S							**	***	*

WF - gelatinized wheat flour, the number linked to WF indicates the inclusion level (%); F – feeding level; R – restricted feeding; S – satiation feeding; R+S – restricted feeding and satiation feeding used in statistical analyses; BW – mean body weight; OM – organic matter. Values are means (n = 3) and the pooled 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, $p > 0.05$; * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$.

^a The parameter did not meet the criteria for normality and/or homogeneity, but the statistical effect (larger or smaller than $p = 0.05$) did not change with a non-parametric test.

loss was more pronounced during satiation feeding. Under normal conditions, these faecal losses are most likely compensated by the synthesis of bile acids from cholesterol in the liver and/or directly being supplied by diets containing fishmeal. The diets used in this study, which included 20 % fish meal and 45 % plant protein ingredients, had on average $0.123 \mu\text{mol/g DM}$ of bile acid content. This value is relatively low compared to the bile acid content ($0.712 \mu\text{mol/g DM}$) of rainbow trout diets with 48 % fishmeal in [Staessen et al., \(2020a\)](#), and likely much lower than in their natural food sources of yellowtail kingfish. The results showed that yellowtail kingfish lost a considerable amount of bile acids through their faeces, and that our experimental diets may be not sufficient to maintain the body's bile acid pool. The bile acid balance was negative during both the restricted and satiation feeding period, which could be related to a depletion of body bile. if dietary intake endogenous synthesis is insufficient, which may ultimately impair fat digestion. However, the fish body bile acid pool was not quantified in

the current study, further research is required to confirm this hypothesis. Substantial efforts have been made to replace fishmeal with plant protein in aquafeeds, with findings showing that replacement levels of up to 100 % in carnivorous species are possible without significant negative effects on growth ([Oliva-Teles et al., 2015](#)). Nevertheless, when considering the replacement of fishmeal with plant protein in aquafeeds, it is important to take our findings regarding bile acid disturbances into account and seek appropriate solutions to maintain fish growth and performance in long run.

The effect of increasing dietary starch level on fat ADC coincided with a negative bile acid balance and increased faecal bile acid losses. Previous studies have related the increased faecal bile acid loss in fish fed diets containing plant ingredients to the presence of anti-nutritional factors (ANF), as well as to their capacity to bind/entrap bile acids ([Li et al., 2017](#)), and/or increased faecal waste production ([Staessen, et al., 2020](#)). In the current study, bile acid content of faeces was not affected

by dietary starch level during satiation feeding. Therefore, our data opposed the concept of the binding/entrapment theory which suggested that matrix structure of starch molecules can sequester bile acids. During restricted feeding, there was a diet effect, whereby the faecal bile acid content was lowest for the high starch diets, while the faecal waste production increased. Therefore, the observed enhanced faecal bile acid loss with feeding level and dietary starch level were mainly caused by increased faecal waste production for both feeding periods. This is in line with the findings of [Staessen et al. \(2020a\); \(2020b\)](#) in rainbow trout. Additionally, feed intake increased with starch level during satiation feeding, as explained by literature that fish adjust for a dilution of energy intake by increasing feed intake ([Jobling, 1983; Sinha et al., 2011](#)). This higher feed intake along with decreased nutrient digestibility, resulted in higher faeces production and consequently, a greater amount of faecal bile acid loss. The interaction effects between diet and feeding level indicated that the increase in faecal bile acid loss was larger for the high starch diets (the 12 % and 16 % WF diets) compared to the low starch diets (the 0 % and 4 % WF diets). Furthermore, gelatinized wheat flour used in the current study as starch source might also have introduced small amounts of NSP, which are well known as ANF that can negatively interfere with the digestion of other dietary macronutrients and fish endogenous enzymes, and also cause changes in intestinal physiology ([Sinha et al., 2011](#)). However, the NSP content of the five diets were almost equal ([Table 1](#)), therefore it is likely that the undigested starch in the intestine caused the effect on faecal bile acid loss and fat ADC similarly as NSP ([Fountoulaki et al., 2005; Hemre et al., 1995](#)).

Faeces (the non-digestible fraction of feed), upon excretion, are removed by sedimentation and/or drum filtration in RAS to maintain good water quality. In this study, the inclusion of starch reduced nutrient digestibility, leading to a substantial increase in the amount of faeces produced. For instance, during restricted feeding, a decrease of 3.8 % OM digestibility by 16 % WF inclusion resulted in a 46 % increase in faecal production compared to the 0 % WF diet. Similar decreases in nutrient digestibility related to increasing levels of dietary starch levels have been reported in other carnivorous species such as rainbow trout, Atlantic salmon ([Krogdahl et al., 2004](#)), and gilthead sea bream ([Couto et al., 2012](#)). It should be noted that the decreases found in the previous studies occurred at a 20 % difference in starch inclusion level ([Couto et al., 2012; Krogdahl et al., 2004](#)), while in this study, even a 6 % difference in starch level caused a significant drop of digestibility in yellowtail kingfish. This strong negative effect of starch level on overall macronutrient digestibility and faecal waste production, to our knowledge, has not been reported in other species. The FCR (0.65–0.98) was within the range of earlier research with yellowtail kingfish conducted at the same facilities ([Horstmann et al., 2023a, 2023c; Zhang et al., 2024](#)), but slightly better (lower) than other studies with fish of comparable size ([Booth et al., 2013; Booth and Pirozzi, 2022](#)). The FCR decreased as dietary starch levels increased, and corresponds to the reported decrease in energy digestibility and lower protein content in diets with increasing starch levels. Similarly, [Horstmann et al. \(2023c\)](#) showed that as dietary starch levels increased, so did FCR. The increased FCR with increasing feeding level (satiation feeding) can primarily be explained by the lower digestibility of all measured nutrients compared to the restricted feeding period.

When it comes to farming yellowtail kingfish in RAS, a major challenge is their poor faecal integrity. This was also shown by low faecal removal efficiencies by settling ranging from 23 % to 45 % ([Horstmann et al., 2023a, 2023b, 2023c](#)). In this study, a comparably poor faecal removal efficiency was observed, with an average of 34 %. Faecal removal efficiency can be influenced by diet composition. Literature showed that physical properties such as density and/or particle size of the faeces determined the removal efficiency ([Schumann et al., 2017; Unger and Brinker, 2013; Amirkolaie et al., 2005; Leenhouders et al., 2007](#)). Our study found that the inclusion of starch had a negative effect on faecal removal efficiency, however, starch did not affect PSD of

collected faeces. One possible explanation could be the density of the faeces, of which starch has a low density and its high water-binding capacity further reduce its density. Changes in faecal composition may have influenced the overall density of faeces, affecting the faecal removal efficiency. For example, fish fed the 16 % WF diet had the highest starch content and lowest protein content in the faeces, which also coincided with the lowest faecal removal efficiency. Furthermore, increasing feeding level tended to have a positive impact on faecal removal efficiency. This could be attributed to the large proportion of large particle size resulting from an increased feeding level, along with the aforementioned factors related to faecal composition and density. The larger proportion of large particle size during satiation feeding compared to restricted feeding may be related to the less pronounced negative effect of starch at high feeding level. Or it could be a result of fish adapting to the experimental diets, or simply due to the larger size of fish during the satiation feeding period. Among all the diets, faecal starch content decreased and protein content increased during satiation feeding, potentially influencing faecal density. Similar patterns were also reported in salmonids, where faecal density was found to be associated with sinking velocity ([Chen et al., 2003; Ogunkoya et al., 2006](#)).

Gelatinized wheat flour was used as starch source in this study, which is commonly used as binder for making stable feed pellets. A previous study showed that gelatinized starch increased faecal particle size and faecal removal efficiency in Nile tilapia ([Amirkolaie et al., 2006](#)). In contrast, the results of this study found that increased starch level reduced faecal removal efficiency in yellowtail kingfish, which is in consistent with the study in African catfish ([Phan et al., 2022](#)). It is important to note that the effect of starch on faecal removal efficiency can vary among different species. Therefore, the reasons behind this discrepancy cannot be merely explained by the physical properties of the faecal waste, as one would expect a similar pattern across species if physical properties were the determining factors. Other factors, such as fish species physiology, can play a role in exerting the response to dietary starch. The differences in faecal characteristics seem to primarily arise from the species differences, however diet can still steer it and subsequently affect faecal removal efficiency.

Increased dietary starch inclusion level decreased OM digestibility as well as faecal removal efficiency, resulting in more non-removed faecal waste. This non-removed faecal waste could become a source of suspended and dissolved solid waste ([Timmons et al., 2018](#)). [Patterson and Watts \(2003\)](#) did find that diet did influence faecal quality, with the majority of suspended solids originating from undigested cellulose and gelatinized starches. As a result, reducing dietary starch levels could be an effective waste management tool.

Based on the linear plateau models, the inflection points of dietary starch content were around 50 g/kg DM for yellowtail kingfish under restricted feeding, and around to 60 g/kg DM under satiation feeding for fat digestibility, faecal removal efficiency and non-removed faeces. It should be noted that these break point levels are close to the minimal used level (38 g/kg DM starch); therefore, if the response is linear or levels off, the estimated minimal dietary starch levels could be underestimated. In terms of nutrient digestibility and faecal waste management, these parameters are crucial for this species cultured in RAS. Above these starch levels, those parameters were strongly negatively affected. As the aquafeed industry strives for sustainability, plant-based ingredients, which often have considerable quantities of starch, are replacing animal-based ingredients, resulting in the dietary starch level expected to increase.

5. Conclusion

In yellowtail kingfish, both starch inclusion level and feeding level (restricted vs. satiation) had negative effects on macronutrient digestibility in yellowtail kingfish, with fat digestibility being the most strongly affected. The estimated maximal inclusion levels of dietary starch content ranges between 50 and 60 g/kg DM (depending on

feeding level) above which dietary starch started to influence fat digestibility and the amount of non-removed faeces. However, because the break points were close to the minimum level (38 g starch/kg DM diet), the maximal dietary starch level may have been overestimated if the response is linear or levels off. Under satiation feeding conditions, yellowtail kingfish increased feed intake with increasing dietary starch levels. High feeding level reduced nutrient digestibility (restricted vs. satiation). Notably, starch had a more pronounced negative effect during restricted feeding on nutrient digestibility and faecal waste production than satiation feeding. All diets used in the current study, which contained a mixture of fish meal and plant protein ingredients, resulted in a negative bile acid balance. This potentially resulted in a decrease of the body bile acid pool, which may account for the observed low fat digestibility in this study. Furthermore, faecal bile acid loss increased with both increasing dietary starch level and feeding level, mainly caused by the increased faecal waste production. In terms of faecal characteristics, higher starch inclusion levels resulted in increased faecal waste production, and higher amounts of non-removed faeces by settling due to reduced faecal removal efficiency. As a result, the dietary starch levels for RAS-grown yellowtail kingfish should be less than 6 %.

CRediT authorship contribution statement

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

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European Maritime
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During the preparation of this work the author(s) used ChatGPT 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.2025.102664](https://doi.org/10.1016/j.aqrep.2025.102664).

Data availability

Data will be made available on request.

References

- Amirkolaie, A.K., Leenhouders, J.I., Verreth, J.A.J., Schrama, J.W., 2005. Type of dietary fibre (soluble versus insoluble) influences digestion, faeces characteristics and faecal waste production in Nile tilapia (*Oreochromis niloticus* L.). *Aquac. Res.* 36, 1157–1166. <https://doi.org/10.1111/j.1365-2109.2005.01330.x>.
- Amirkolaie, A.K., Verreth, J.A.J., Schrama, J.W., 2006. Effect of gelatinization degree and inclusion level of dietary starch on the characteristics of digesta and faeces in Nile tilapia (*Oreochromis niloticus* (L.)). *Aquaculture* 260, 194–205. <https://doi.org/10.1016/j.aquaculture.2006.06.039>.
- Badiola, M., Mendiola, D., Bostock, J., 2012. Recirculating aquaculture systems (RAS) analysis: main issues on management and future challenges. *Aquac. Eng.* 51, 26–35. <https://doi.org/10.1016/j.aquaeng.2012.07.004>.
- Booth, M.A., Moses, M.D., Allan, G.L., 2013. Utilisation of carbohydrate by yellowtail kingfish *Seriola lalandi*. *Aquaculture* 376–379 151–161. <https://doi.org/10.1016/j.aquaculture.2012.11.024>.
- Booth, M.A., Pirozzi, I., 2021. The interaction of feeding regime and dietary specification on growth and nutrient utilisation in Yellowtail Kingfish *Seriola lalandi*. *Aquaculture* 544, 737094.
- Booth, M.A., Pirozzi, I., 2022. The digestible histidine requirement of juvenile yellowtail kingfish *Seriola lalandi*. *Aquaculture* 548, 737543.
- Bromley, P.J., 1994. The role of gastric evacuation experiments in quantifying the feeding rates of predatory fish. *Rev. Fish. Biol. Fish.* 4, 36–66. <https://doi.org/10.1007/BF00043260>.
- Bureau, D.P., Hua, K., 2010. Towards effective nutritional management of waste outputs in aquaculture, with particular reference to salmonid aquaculture operations. *Aquac. Res.* 41, 777–792. <https://doi.org/10.1111/j.1365-2109.2009.02431.x>.
- Chen, Y.S., Beveridge, M.C.M., Telfer, T.C., Roy, W.J., 2003. Nutrient leaching and settling rate characteristics of the faeces of Atlantic salmon (*Salmo salar* L.) and the implications for modelling of solid waste dispersion. *J. Appl. Ichthyol.* 19, 114–117. <https://doi.org/10.1046/j.1439-0426.2003.00449.x>.
- Cheng, Z.J., Hardy, R.W., 2002. Apparent digestibility coefficients and nutritional value of cottonseed meal for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 212, 361–372. [https://doi.org/10.1016/S0044-8486\(02\)00260-0](https://doi.org/10.1016/S0044-8486(02)00260-0).
- Couto, A., Enes, P., Peres, H., Oliva-Teles, A., 2012. Temperature and dietary starch level affected protein but not starch digestibility in gilthead sea bream juveniles. *Fish. Physiol. Biochem.* 38, 595–601. <https://doi.org/10.1007/s10695-011-9537-5>.
- Dias, J., Christine Huelvan, I., Dinis, M.T., 1998. Influence of dietary bulk agents (silica, cellulose and a natural zeolite) on protein digestibility, growth, feed intake and feed transit time in European seabass (*Dicentrarchus labrax*) juveniles. *Aquat. Living Resour.* 11, 219–226. [https://doi.org/10.1016/S0990-7440\(98\)89004-9](https://doi.org/10.1016/S0990-7440(98)89004-9).
- Elesho, F.E., Kröckel, S., Sutter, D.A.H., Nuraini, R., Chen, L.J., Verreth, J.A.J., Schrama, J.W., 2021. Effect of feeding level on the digestibility of alternative protein-rich ingredients for African catfish (*Clarias gariepinus*). *Aquaculture* 544, 737108. <https://doi.org/10.1016/j.aquaculture.2021.737108>.
- El-Sayed, A.F.M., 2002. Effects of stocking density and feeding levels on growth and feed efficiency of Nile tilapia (*Oreochromis niloticus* L.) fry. *Aquac. Res.* 33, 621–626. <https://doi.org/10.1046/j.1365-2109.2002.00700.x>.
- EUMOFA, 2020. Recirc. Aquac. Syst. <https://doi.org/10.2771/66025>.
- Fountoulaki, E., Alexis, M.N., Nengas, I., Venou, B., 2005. Effect of diet composition on nutrient digestibility and digestive enzyme levels of gilthead sea bream (*Sparus aurata* L.). *Aquac. Res.* 36, 1243–1251. <https://doi.org/10.1111/j.1365-2109.2005.01232.x>.
- Geurden, I., Borchert, P., Balasubramanian, M.N., Schrama, J.W., Dupont-Nivet, M., Quillet, E., Kaushik, S.J., Panserat, S., Médale, F., 2013. The positive impact of the early-feeding of a plant-based diet on its future acceptance and utilisation in rainbow trout. *PLoS One* 8, e83162. <https://doi.org/10.1371/journal.pone.0083162>.

- Hemre, G., Sandnes, I., Lie, K., Torrissen, O., Waagbø, R., 1995. Carbohydrate nutrition in Atlantic salmon, *Salmo salar* L.: growth and feed utilization. *Aquac. Res.* 26, 149–154. <https://doi.org/10.1111/j.1365-2109.1995.tb00896.x>.
- Henken, A.M., Kleingeld, D.W., Tijssen, P.A.T., 1985. The effect of feeding level on apparent digestibility of dietary dry matter, crude protein and gross energy in the African catfish *Clarias gariepinus* (Burchell, 1822). *Aquaculture* 51, 1–11. [https://doi.org/10.1016/0044-8486\(85\)90235-2](https://doi.org/10.1016/0044-8486(85)90235-2).
- Horstmann, P., Maas, R.M., de Boer, X.V., Staessen, T.W.O., Kokou, F., Schrama, J.W., 2023b. Faecal waste characteristics of yellowtail kingfish (*Seriola lalandi*) fed with pelleted and natural feed. *Anim. Feed Sci. Technol.* 299, 115625. <https://doi.org/10.1016/j.anifeedsci.2023.115625>.
- Horstmann, P., Maas, R.M., de Boer, X.V., de Jong, T.M.B., Staessen, T.W.O., Kokou, F., Schrama, J.W., 2023a. Effect of dietary protein source and ingredient grinding size on fish performance, faecal waste production and characteristics of yellowtail kingfish (*Seriola lalandi*) fed restrictively and to apparent satiation. *Aquaculture* 562, 738875. <https://doi.org/10.1016/j.aquaculture.2022.738875>.
- Horstmann, P., Maas, R.M., Kals, J., Prakash, S., Staessen, T.W.O., Kokou, F., Schrama, J.W., 2023c. Dietary starch level affects nutrient digestibility, faecal waste production and characteristics in yellowtail kingfish (*Seriola lalandi*) depending on the level of fish meal replacement. *Aquaculture* 577, 739915. <https://doi.org/10.1016/j.aquaculture.2023.739915>.
- Horstmann Zuther, P.H., Maas, R.M., Blok, T., Kals, J., Nederlof, M.A., Prakash, S., Schols, H.A., Staessen, T.W., Zhang, Y., Kokou, F., Schrama, J.W., 2024. Effect of dietary starch on digestion kinetics and nutrient utilization in yellowtail kingfish (*Seriola lalandi*). *Aquaculture* 591, 741149.
- Jobling, M., 1983. A short review and critique of methodology used in fish growth and nutrition studies. *J. Fish. Biol.* 23, 685–703. <https://doi.org/10.1111/j.1095-8649.1983.tb02946.x>.
- Jobling, M., 1986. Gastrointestinal overload—a problem with formulated feeds? *Aquaculture* 51, 257–263. [https://doi.org/10.1016/0044-8486\(86\)90317-0](https://doi.org/10.1016/0044-8486(86)90317-0).
- Kals, J., Blonk, R.J.W., van der Mheen, H.W., Schrama, J.W., Verreth, J.A.J., 2019. Effect of vitamin B12 and taurine on the alleviation of nutritional anaemia in common sole (*Solea solea*). *Aquac. Nutr.* 25, 456–465. <https://doi.org/10.1111/anu.12871>.
- Krogdahl, Å., Sundby, A., Olli, J.J., 2004. Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) digest and metabolize nutrients differently. Effects of water salinity and dietary starch level. *Aquaculture* 229, 335–360. [https://doi.org/10.1016/S0044-8486\(03\)00396-X](https://doi.org/10.1016/S0044-8486(03)00396-X).
- Leenhouwers, J.J., Ortega, R.C., Verreth, J.A.J., Schrama, J.W., 2007. Digesta characteristics in relation to nutrient digestibility and mineral absorption in Nile tilapia (*Oreochromis niloticus* L.) fed cereal grains of increasing viscosity. *Aquaculture* 273, 556–565. <https://doi.org/10.1016/j.aquaculture.2007.10.044>.
- Li, C.Y., Mense, A.L., Brewer, L.R., Lau, C., Shi, Y.C., 2017. In vitro bile acid binding capacity of wheat bran with different particle sizes. *Cereal Chem.* 94, 654–658. <https://doi.org/10.1094/CCHEM-08-16-0211-R>.
- National Research Council (NRC), 2011. *Nutrient Requirements of Fish and Shrimp*. The National Academies Press, Washington DC.
- Ogunkoya, A.E., Page, G.I., Adewolu, M.A., Bureau, D.P., 2006. Dietary incorporation of soybean meal and exogenous enzyme cocktail can affect physical characteristics of faecal material egested by rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 254, 466–475. <https://doi.org/10.1016/j.aquaculture.2005.10.032>.
- Oliva-Teles, A., Enes, P., Peres, H., 2015. Replacing fishmeal and fish oil in industrial aquafeeds for carnivorous fish. Feed and feeding practices in aquaculture. Feed and Feeding Practices in Aquaculture. Woodhead Publishing Limited, Waltham, MA, pp. 203–233. <https://doi.org/10.1016/B978-0-08-100506-4.00008-8>.
- Patterson, R.N., Watts, K.C., 2003. Micro-particles in recirculating aquaculture systems: microscopic examination of particles. *Aquac. Eng.* 28, 115–130. [https://doi.org/10.1016/S0144-8609\(03\)00028-1](https://doi.org/10.1016/S0144-8609(03)00028-1).
- Phan, L.T.T., Kals, J., Masagounder, K., Mas-Muñoz, J., Schrama, J.W., 2022. Energy utilisation efficiencies of digestible protein, fat and carbohydrates for African catfish (*Clarias gariepinus*). *Aquac. Rep.* 23, 101051. <https://doi.org/10.1016/j.aqrep.2022.101051>.
- Porter, J.L., Fordtran, J.S., Santa Ana, C.A., Emmett, M., Hagey, L.R., Macdonald, E.A., Hofmann, A.F., 2003. Accurate enzymatic measurement of fecal bile acids in patients with malabsorption. *Journal of Laboratory and Clinical Medicine* 141 (6), 411–418. [https://doi.org/10.1016/S0022-2143\(03\)00040-4](https://doi.org/10.1016/S0022-2143(03)00040-4).
- Refstie, S., Svihus, B., Shearer, K.D., Storebakken, T., 1999. Nutrient digestibility in Atlantic salmon and broiler chickens related to viscosity and non-starch polysaccharide content in different soyabean products. *Anim. Feed Sci. Technol.* 79, 331–345. [https://doi.org/10.1016/S0377-8401\(99\)00026-7](https://doi.org/10.1016/S0377-8401(99)00026-7).
- Schrama, J.W., Saravanan, S., Geurden, I., Heinsbroek, L.T.N., Kaushik, S.J., Verreth, J.A.J., 2012. Dietary nutrient composition affects digestible energy utilisation for growth: a study on Nile tilapia (*Oreochromis niloticus*) and a literature comparison across fish species. *Br. J. Nutr.* 108, 277–289. <https://doi.org/10.1017/S0007114511005654>.
- Schumann, M., Unger, J., Brinker, A., 2017. Floating faeces: effects on solid removal and particle size distribution in RAS. *Aquac. Eng.* 78, 75–84. <https://doi.org/10.1016/j.aquaceng.2016.10.007>.
- Sinha, A.K., Kumar, V., Makkar, H.P.S., De Boeck, G., Becker, K., 2011. Non-starch polysaccharides and their role in fish nutrition - a review. *Food Chem.* 127, 1409–1429. <https://doi.org/10.1016/j.foodchem.2011.02.042>.
- Staessen, Thomas W.O., Verdegem, M.C.J., Koletsi, P., Schrama, J.W., 2020a. The effect of dietary protein source (fishmeal vs. plant protein) and non-starch polysaccharide level on fat digestibility and faecal bile acid loss in rainbow trout (*Oncorhynchus mykiss*). *Aquac. Res.* 51, 1170–1181. <https://doi.org/10.1111/are.14467>.
- Staessen, T.W.O., Verdegem, M.C.J., Nederlof, M.A.J., Eding, E.H., Schrama, J.W., 2021. Effect of type of dietary non-protein energy source (starch vs. fat) on the body bile acid pool size and composition, faecal bile acid loss and bile acid synthesis in rainbow trout (*Oncorhynchus mykiss*). *Aquac. Nutr.* 27, 865–879. <https://doi.org/10.1111/anu.13231>.
- Staessen, T.W.O., Verdegem, M.C.J., Schrama, J.W., 2022. Effect of dietary NSP level and bile acid supplementation on nutrient digestibility and the bile acid metabolism in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 561, 738724.
- Staessen, T.W.O., Verdegem, M.C.J., Wethasinghe, P., Schrama, J.W., 2020b. The effect of dietary non-starch polysaccharide level and bile acid supplementation on fat digestibility and the bile acid balance in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 523, 735174. <https://doi.org/10.1016/j.aquaculture.2020.735174>.
- Timmons, M.B., Guerdat, T., Brian, V.J., 2018. *Recirculating Aquaculture*, 4th edition. Ithaca Publishing Company LLC, Ithaca, p. 775.
- Unger, J., Brinker, A., 2013. Feed and treat: What to expect from commercial diets. *Aquac. Eng.* 53, 19–29. <https://doi.org/10.1016/j.aquaceng.2012.11.012>.
- Zhang, Y., Maas, R.M., Horstmann, P., Prakash, S., Staessen, T.W., Kals, J., Kokou, F., Schrama, J.W., 2024. 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*). *Aquac. Rep.* 36, 102179.
- Zhu, L., Jones, C., Guo, Q., Lewis, L., Stark, C.R., Alavi, S., 2016. An evaluation of total starch and starch gelatinization methodologies in pelleted animal feed. *J. Anim. Sci.* 94, 1501–1507. <https://doi.org/10.2527/jas.2015-9822>.