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Time-related changes in nutrient digestibility and faecal bile acid loss of rainbow trout (*Oncorhynchus mykiss*) as affected by dietary fat level and non-starch polysaccharide level

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Abstract

Time-related changes in apparent digestibility coefficients (ADC) and faecal bile acid loss as affected by dietary fat level and non-starch polysaccharide (NSP) level were studied in rainbow trout (*Oncorhynchus mykiss*). Low-Fat versus High-Fat and Low-NSP versus High-NSP diets were formulated. Fish were fed for 6 weeks to apparent satiation and faeces were sampled in week 2, 4 and 6. Most nutrient ADC increased with time and increases were consistently larger for the High-Fat and High-NSP diets. Nevertheless, time of adaptation was equal between diets/nutrients and steady-state digestion was reached at week 3. Although faecal bile acid loss was affected by diet, the observed time-related decrease in faecal bile acid loss was mainly driven by a decrease in feed intake and faeces production. Fat ADC improved over time regardless of NSP level, which does not support the hypothesis that enhanced faecal bile acid loss (related to satiation feeding of high-NSP diets) causes a depletion of the total body bile acid pool size that is critical for proper fat digestion. The high-fat diets consistently resulted in the lowest Fat ADC, which might have been related to a lower availability of bile acids relative to the level of dietary fat.

KEYWORDS

bile acids, diet adaptation, faeces production, feed intake, lipid digestion, steady-state digestion

1 | INTRODUCTION

In order to formulate diets that fulfil the nutritional and energetic requirements of fish, it is imperative to have accurate data on the digestibility of their constituting nutrients and energy (Glencross et al., 2007). In general, time-related changes in nutrient apparent digestibility coefficients (ADC) occur after exposure of fish to a new diet (i.e., diet adaptation), and such changes have been linked to acclimation of the digestive physiology (e.g., changes in microbial and enzymatic activity) to the chemical composition of a new diet (Anderson et al., 1991; Kihara & Sakata, 1997; Mohapatra et al., 2003). In order to have more accurate estimates of digestibility, most studies deal with the issue of diet adaptation by allowing for a certain period of time before the collection of faecal samples.

The applied adaptation time varies greatly between studies, ranging from 1 week to more than 6 weeks for several fish species (Dias et al., 1998; Glencross et al., 2012; Refstie et al., 2001; Robaina et al., 1999). Although the usefulness of an adaptation period before

measuring digestibility is widely recognized, few studies have actually assessed the time that is needed to reach a steady state of digestion in fish. The available studies show that the minimal adaptation time is highly dependent on fish species. For example, Blyth et al. (2015) found a minimal adaptation period of 4 days for barramundi (*Lates calcarifer*), while Amirkolaie and Schrama (2015) found an adaptation period of 5–6 weeks for Nile tilapia (*Oreochromis niloticus*). Furthermore, the time for diet adaptation can be dependent on diet composition. For example, the study by Amirkolaie and Schrama (2015) showed that harder-to-digest carbohydrate types result in a longer adaptation period. This effect of carbohydrate type on diet adaptation time is of value in the context of the ongoing replacement of fish meal by plant ingredients in aquafeeds, since plant ingredients often containing significant amounts of non-starch polysaccharides (NSP) (Sinha et al., 2011).

Dietary inclusion of NSP generally results in a decrease of the dry matter (DM) ADC, and this because the enzymes that are needed for their digestion (e.g., β-glucanases and β-xylanases) are largely lacking in fish (Maas et al., 2020). Some types of NSP result in a decrease in DM ADC that is significantly higher than what can be caused by their undigestible nature alone, which can be explained by a detrimental effect on the digestion of other nutrients (e.g., protein and fat) (Irvin et al., 2016). The latter is in particular the case for soluble types of NSP (e.g., pectins), which increase chymal viscosity and can reduce mixing of nutrients and digestive enzymes in the intestine (Amirkolaie et al., 2005; Glencross et al., 2012; Hossain et al., 2003; Kumar et al., 2011; Øverland et al., 2009; Refstie et al., 1999; Schneider et al., 2004; Storebakken, 1985). The effect of NSP-rich ingredients on nutrient ADC is not always straightforward, which most likely is related to existence of interaction effects between different types of NSP on nutrient ADC (Irvin et al., 2016). Several studies, both with mammals and fish, linked the decrease of in particular fat ADC to the capacity of NSP to sequester bile acids and thus to enhance faecal bile acid loss (Ikegami et al., 1990; Moundras et al., 1997; Øverland et al., 2009; Staessen, Verdegem, Weththasinghe, & Schrama, 2020). Bile acids aid both fat digestion and transport in the intestine through their detergent-like properties (Maldonado-Valderrama et al., 2011; Rust, 2003), and furthermore activate certain lipases in the intestine (Romarheim et al., 2006). Bile acids are efficiently recycled between the liver and the intestine through the process of enterohepatic circulation. Only a small amount of bile acids normally escapes absorption in the distal intestine and is thus lost through the faeces. Under homeostasis, faecal bile acid loss is compensated by de novo bile acid synthesis in the liver (Hofmann, 2009). Staessen, Verdegem, Weththasinghe, et al. (2020) and Staessen, Verdegem, Koletsi, and Schrama (2020) observed enhanced faecal bile acid loss in rainbow trout (*Oncorhynchus mykiss*) that was fed NSP-rich diets to satiation. Enhanced faecal bile acid loss correlated negatively with fat ADC in those studies, and this inverse relationship disappeared with dietary bile acid supplementation (Staessen, Verdegem, Koletsi, et al., 2020). The outcome of these 2 studies suggests that enhanced faecal bile acid loss of rainbow trout results in a lack of bile acids that are available for fat digestion in the intestine. This lack of bile acids is hypothesized to have occurred either instantly through formation of insoluble NSP-bile acid complexes (Ebihara & Schneeman, 1989; Sinha et al., 2011; Vahouny et al., 1981), or to have occurred over time as enhanced bile acid loss gradually depleted the bile acid pool (Dowling et al., 1970). Unfortunately, the studies by Staessen, Verdegem, Weththasinghe, et al. (2020) and Staessen, Verdegem, Koletsi, et al. (2020) measured fat ADC using faecal samples that were collected during the last week of the experimental period, and therefore, information on the time-related relationship between faecal bile acid loss and fat ADC is not available.

Besides the effects of dietary NSP level, there are relatively few studies, which investigated the effects of dietary fat level on fat ADC in fish, and the few studies reporting such effects show highly variable results (Austreng et al., 1979; Bendiksen et al., 2002; Berge & Storebakken, 1991; Refstie et al., 2001). Most differences between studies on the effect of fat level on fat ADC are likely related to differences in fatty acid profile of the fat sources used. However, as the level of emulsification determines the level of fat digestion and absorption (Golding & Wooster, 2010), changes in concentration of available bile acids will alter the quantity of fat solubilized (Gallaher & Schneeman, 1986) and might affect fat ADC.

Based on the foregoing, the current study investigated in rainbow trout the time-related changes in ADC (with a focus on fat ADC) during adaptation to diets differing in both fat level and NSP level. Furthermore, this study looked at time-related changes in faecal bile acid loss as affected by diet to determine if this is related to changes in fat ADC over time. Concerning the latter, it was hypothesized that conditions that enhance faecal bile acid loss (i.e., high-NSP level and high feed intake) gradually deplete the total bile acid pool, thus leading to a gradual decrease of fat ADC with time.

2 | MATERIALS AND METHODS

This experiment was approved by the Animal Welfare Body of Wageningen University, The Netherlands. All procedures applied to the animals were in line with the Dutch legislation (Act on Animal Experiments) and were classified as not being an animal experiment according to Dutch legislation.

2.1 | Feed formulation

All experimental diets were formulated to meet or exceed the minimum recommended nutrient requirements of rainbow trout (*O. mykiss*) according to the National Research Council (NRC, 2011).

The experiment was set up according to a two-by-two factorial design. Four diets differing in fat level (Low-Fat vs. High-Fat) and non-starch polysaccharide level (Low-NSP vs. High-NSP) were tested. Because this study aimed at establishing the relationship between enhanced faecal bile acid loss and fat ADC, diets were formulated to keep the supply of bile acids and bile acid precursors to

a minimum. Table 1 shows the composition of the basal ingredient mixture, the NSP-rich ingredient mixture and the plant oil mixture used during feed manufacturing. Contrasts in dietary NSP and fat level were obtained by combining these ingredient mixtures according to the scheme shown in Table 2. The basal ingredient mixture, either with or without addition of the NSP-rich ingredient mixture, was pelleted by extrusion (3 mm; Diet Research Services). The plant oil mixture was added to the pellets by vacuum coating (Wageningen University). Pea protein concentrate and soy protein concentrate were used as main protein sources. Methionine and histidine were supplemented to meet amino acid recommendations. The plant oil mixture, consisting of equal parts of palm oil and rapeseed oil, was used as main fat source. Salmon oil was added to provide enough n-3 long chain-polyunsaturated fatty acids to meet fatty acid requirements. Wheat meal was the main starch source. The NSP-rich ingredient mixture, consisting of equal parts of wheat bran and soy hulls, provided the majority of NSP. Actual NSP-rich ingredients were chosen over purified NSP sources since the NSP purification process alters physico-chemical characteristics which in turn might alter the potential of NSP to interact with bile acids (Kritchevsky, 1978). Krill meal and fish soluble concentrate were added to increase palatability of the diets. A vitamin/mineral premix, chalk ($CaCO_3$) and monocalciumphosphate were added to meet dietary requirements for phosphorus, vitamins and other minerals. Yttrium oxide was used as inert marker for determination of fat digestibility and faecal bile acid loss. Feed was stored at 4°C throughout the experiment.

2.2 | Housing facilities

The study was carried out in the aquatic research facility of Wageningen University in The Netherlands. The experimental setup comprised twelve glass tanks (90 × 60 × 45 cm; 200 L) connected to a common water recirculation system. Water flow to individual tanks was controlled by a water flow meter (MAGFLO MAG 5000, Danfoss A/S) and kept constant at 7.0 ± 0.05 L/min. A trickling filter, sump and drum filter (Hydrotech 500, Hydrotech Engineering) helped in maintaining water quality parameters within a set range. Allowed values for NH_4^+-, NO_2-concentrations and conductivity of the outlet water were <4 mg/L, <1.5 mg/L and <4,000 μS/cm respectively. A partial water exchange was done when these parameters surpassed their allowed maximum. The pH of the inlet water was kept within

TABLE 1 Composition of the ingredient mixtures

	Ingredient mixture		
Ingredients (g/kg)	Basal ingredient mixture	Plant oil mixture	NSP-rich ingredient mixture
Pea protein concentrate	322.6	-	-
Wheat meal	274.0	-	-
Soy protein concentrate	161.3	-	-
Krill meal	64.5	-	-
Fish soluble concentrate	64.5	-	-
Salmon oil[†]	40.3	-	-
Vitamin/mineral premix[‡]	16.1	-	-
Monocalciumphosphate	40.3	-	-
Chalk ($CaCO_3$)	8.1	-	-
DL-Methionine	4.8	-	-
L-Histidine	3.2	-	-
Yttrium oxide	0.3	-	-
Palm oil	-	500	-
Rapeseed oil	-	500	-
Soy hulls	-	-	500
Wheat bran	-	-	500

Note: Vitamins (IU or g/kg premix): thiamin, 1 g; riboflavin, 1 g; pyridoxine, 1 g; panthotenic acid, 4 g; niacin, 2 g; biotin, 0.02 g; cyanocobalamin, 0.0015 g; folic acid, 0.2 g; ascorbic acid, 10 g; DL-alpha tocopherol acetate, 10,000 IU; retinyl palmitate, 300,000 IU; DL-cholecalciferol, 200,400 IU; sodium menadione bisulfite (51%), 1 g; inositol, 40 g; choline, 200 g; butylhydroxytolueen, 10 g; calcium propionate, 100 g; anti-oxidant BHT (E300-321), 10 g.

Minerals (g/kg premix): iron (as $FeSO_4 \cdot 7H_2O$), 5 g; zinc (as $ZnSO_4 \cdot 7H_2O$), 3 g; cobalt (as $CoSO_4 \cdot 7H_2O$), 0.01 g; copper (as $CuSO_4 \cdot 5H_2O$), 1 g; selenium (as Na_2SeO_3), 0.05 g; manganese (as $MnSO_4 \cdot 4H_2O$), 2 g; magnesium (as $MgSO_4 \cdot 7H_2O$), 50 g; chromium (as $CrCl_3 \cdot 6H_2O$), 0.1 g; calcium (as $CaIO_3 \cdot 6H_2O$), 0.2 g.

[†]INVE Salmon oil, Belgium.

[‡]Vitamin/mineral premix.

TABLE 2 Composition and analysed nutrient content of the experimental diets

	Diets			
	Low-fat		High-fat	
	Low-NSP	High-NSP	Low-NSP	High-NSP
Ingredient mixtures (parts by weight)				
Basal ingredient mixture†	62	62	62	62
NSP-rich ingredient mixture‡	0	16	0	16
Plant oil mixture§	7	7	22	22
Ingredient mixtures (g/kg)				
Basal ingredient mixture†	898.6	729.4	738.1	620.0
NSP-rich ingredient mixture‡	0.0	188.2	0.0	160.0
Plant oil mixture§	101.4	82.4	261.9	220.0
Analysed nutrient content (g/kg DM)				
DM (g/kg)	937	934	953	949
Ash	85	77	69	66
Crude protein (N × 6.25)	457	389	362	336
Crude fat	179	154	323	275
Starch + sugars	226	204	185	172
NSP¶	53	176	61	151
Gross energy (kJ/g DM)	22	22	26	25
Calcium	14	12	11	10
Phosphorus	16	15	13	12
Yttrium	0.18	0.15	0.15	0.13
Bile acids (μmol/kg DM)	BDL	BDL	BDL	BDL

Abbreviations: BDL, Below detection limit (<30 μmol/kg DM); DM, dry matter; N, nitrogen.

†, ‡ and § Compositions of the ingredient mixtures are given in Table 1.

¶NSP = 1,000 − ash − crude protein − crude fat − (starch + sugars).

the range of 7.0–7.8. A cooling system maintained the water temperature at 14.0 ± 0.5°C. Each tank was drained separately, and each outlet located at the lower point of the sloping tank bottom was connected to a swirl separator. (44 cm in height, 24.5 cm in diameter; Aqua Optima A/S). A photoperiod of 12 hr light–12 hr dark was maintained for the entire duration of the experiment.

2.3 | Experimental procedures and sampling

Each diet was randomly assigned to the tanks in triplicate. Rainbow trout (*O. mykiss*) were obtained from a commercial trout farm (Mohnen Aquaculture GmbH, Germany). Before the start of the experiment, all fish were fed the same commercial trout diet. At the start of the experiment, trout were randomly stocked at a density of 30 fish tank^{-1}, and batch-weighed to determine total biomass. Fish were subsequently hand-fed the experimental diets to apparent satiation (9:00 and 15:30 hr) for 6 weeks. The fish were considered to have reached satiation when about 10 pellets remained floating on the water surface for more than 10 min, or when the feeding time exceeded 1 hr per feeding moment. Uneaten feed pellets that were flushed out from the tanks were collected using the swirl separators for accurate determination of feed intake. Before each feeding moment, tanks were checked for mortality. During week 2, 4 and 6 of the experiment, faeces were collected overnight in detachable 250 ml bottles placed below each swirl separator. The tank bottoms were cleaned each day by syphoning prior to faeces collection (after feeding). During collection, collection bottles were submerged into a mixture of water and ice to minimize bacterial decomposition of the faeces. Faeces were pooled per week per tank and stored at −20°C. Fish were starved for 24 hr before batch weighing at the beginning and end of the experiment to allow emptying of the gastro-intestinal tract and accurate determination of total biomass.

2.4 | Analytical methods

Analyses were performed on the 4 experimental diets and on the faeces collected during week 2, 4 and 6 of the experiment. Faecal samples were dried at 70°C until constant weight. Dried faeces were subsequently pulverized using a mixer mill with a fixed screen opening of 1 mm set at 12,000 RPM (MM 200 Retch, Brinkmann). Feed and faeces were analysed for dry matter (DM), ash, yttrium, calcium, phosphorus, crude protein, crude fat, starch + sugars, energy content and bile acid content.

DM was determined gravimetrically by drying samples at 103°C until constant weight (ISO 6496, 1999). Ash content was determined gravimetrically by incineration in a muffle furnace at 550°C (ISO 5984, 2002). Yttrium content was analysed using ICP-AES (NEN 15510, 2007). Crude protein content (N × 6.25) was measured according to Kjeldahl's method (ISO 5983-2, 2009). Crude fat was determined gravimetrically using acid hydrolysis (Hydrotherm, C. Gerhardt GmbH & Co. KG) and Soxhlet extraction using petroleum–ether (Soxtherm, C. Gerhardt GmbH & Co. KG) (ISO 6492, 1999). Starch and free sugars were determined using an enzymatic digestion method (Nutrilab). Starch was determined after washing away free sugars with 40% v/v ethanol. The dried residue was digested with Termamyl®, after which starch was hydrolysed using amyloglucosidase. The formed glucose units were determined using the Luff-Schoorl reagent. Starch + sugars was measured as described above, leaving out the washing step with 40% v/v ethanol. Gross energy was measured using a bomb calorimeter (C7000 IKA, IKA-Werke GmbH & Co. KG) (ISO 9831, 1998). Bile acid content was determined enzymatically after extraction. Extraction was performed according to Porter et al. (2003) and consisted of alkaline hydrolysis in ethylene

glycol–KOH followed by extraction with diethyl ether. Diethyl ether extracts were evaporated at room temperature 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 (Dialab®, Vienna, Austria), using methanol as a reagent blank.

2.5 | Calculations

Mortality (%) was calculated as $(N_0 - N_t)/N_0 \times 100\%$, where N_0 and N_t are the number of fish at the beginning and end of the experiment respectively. Feed conversion ratio on DM basis (FCR; g/g) was calculated as $(FI \times (dmF/1000))/(W_t - W_0)$, where FI is feed intake (g), dmF is the DM content of the feed (g/kg), and W_0 and W_t are the initial and final BW (g), respectively.

Feed intake (FI; g/kg BW/d) was calculated as $(FI \times (dmF/1000))/t/W_g$, where F is feed intake (g), dmF is the DM content of the feed (g/kg) , t is the number of days, and W_g is the geometric mean BW (kg) of each week, respectively. The W_g was calculated as $e^{((\ln Wt + \ln W0)/2)}$, where W_0 and W_t are the initial and final BW (kg) for each week, respectively. Faeces production on DM basis (g/kg BW/d) was calculated as $FI \times (dmF/1000) \times (D_i/F_i)/t/W_g$, where FI is feed intake (g), dmF is the DM content of the feed (g/kg), D_i is the content inert marker of the diet (g/kg), F_i is the content inert marker of the faeces (g/kg), t is the number of days, and W_g is the geometric mean BW (kg) of each week, respectively. Specific growth rate (SGR; % BW/d) was calculated as $(\ln W_t - \ln W_0)/t \times 100\%$, where W_0 and W_t are the initial and final BW (g), and t is the number of days of each week, respectively.

NSP content (g/kg DM) was calculated as 1,000 − ash − (N-content × 6.25) − crude fat − (starch + sugars), with ash, N-content, crude fat and starch + sugars expressed as g/kg DM.

The apparent digestibility coefficient (ADC; %) of each nutrient was calculated using the formula described by Halver and Hardy (2002): $100 - (100 \times (F/D) \times (D_i/F_i))$, where D is the percentage nutrient (or kJ/g gross energy) of the diet, F the percentage nutrient (or kJ/g gross energy) of the faeces, D_i the percentage inert marker of the diet and F_i the percentage inert marker of the faeces.

Faecal bile acid loss (μmol/kg BW/d) was calculated as $((FI \times (D_i/F_i))/t)/W_g) \times F_b$, where FI is feed intake (g), D_i is the percentage of inert marker in the diet, F_i is the percentage of inert marker (i.e., yttrium) in the faeces, t is the number of days, W_g is the geometric mean BW (kg), and F_b is the faecal bile acid content on wet weight (μmol/g) for each week, respectively.

2.6 | Statistical analysis

Tanks ($n = 12$) were considered as experimental units. Combined data of week 2, 4 and 6 were first analysed by repeated measurement analysis to look at the effect of week, fat level, NSP level, their interaction effects and the random effect of tank nested within fat level and NSP level. The effect of fat level, NSP level and their interaction was tested against the random effect of tank nested within fat level and NSP level. The effect of week and its interactions with fat level and NSP level was tested against the random error of the whole model (i.e., random variation within tanks between weeks). During the repeated measurement analysis, data were tested for sphericity using Mauchly's test, and in case of violation of sphericity the Greenhouse–Geisser correction was applied. Since, for many parameters, interaction effects between week and both fat level and NSP level were present, data of the different weeks were also analysed separately using a two-way ANOVA for the effect of fat level, NSP level and their interaction. All data were tested for homogeneity of variance by Levene's test prior to ANOVA. Normal distribution of residuals was checked using the Kolmogorov–Smirnov test. ANOVA was followed by a Tukey test for pairwise comparison of means. Statistical significance was tested at the 0.05 probability level. p-values between .1 and .05 ($.1 > p \geq .05$) were defined as close to statistical significance and as indicative for tendencies in the data. All tests were performed using the statistical program SPSS statistics 23, IBM Statistics Inc., USA.

3 | RESULTS

3.1 | Fish performance

Mean fish performance during the 6-week experimental period is given in Table S1. The average initial body weight (BW) was 64 g/fish and not statistically different between treatments ($p \geq .1$). Mortality was low (1.1% averaged over treatments) and unaffected by diet ($p \geq .1$). Feed conversion ratio (FCR) deteriorated with increasing dietary non-starch polysaccharide (NSP) level ($p < .001$; Low-NSP: 0.80 vs. High-NSP: 0.89 g/g). Furthermore, the deterioration of the FCR with NSP level was dependent on the dietary fat level, being more pronounced for the low-fat diets ($p < .01$; Low-Fat: +0.14 vs. High-Fat: +0.05 g/g). Despite these significant effects on FCR, neither NSP level nor fat level had a clear effect on growth ($p \geq .05$) and final BW was not different between diets ($p \geq .1$; 166 g/fish averaged over diets).

Figure 1a,b,d,e and Table S2 show the time-related changes in both feed intake and faeces production related to dietary fat and NSP level. A strong time-related decrease in feed intake was observed for all diets ($p < .001$; week 2: 25.3 vs. week 4: 18.4 vs. week 6: 13.0 g/kg BW/d averaged over diets; Figure 1a), and this decrease with time was independent from diet ($p \geq .1$). Data analysed separately per week show both main effects of fat level ($p < .001$) and NSP level ($p < .001$) on feed intake. Increasing the fat level led to a reduction in feed intake (Low-Fat: 20.0 vs. High-Fat: 17.7 g/kg BW/d averaged over weeks), while increasing the NSP level led to an increase in feed intake (Low-NSP: 17.8 vs. High-NSP: 20.0 g/kg BW/d averaged over weeks) (Figure 1d). In accordance with feed intake, faeces production decreased over time for all diets

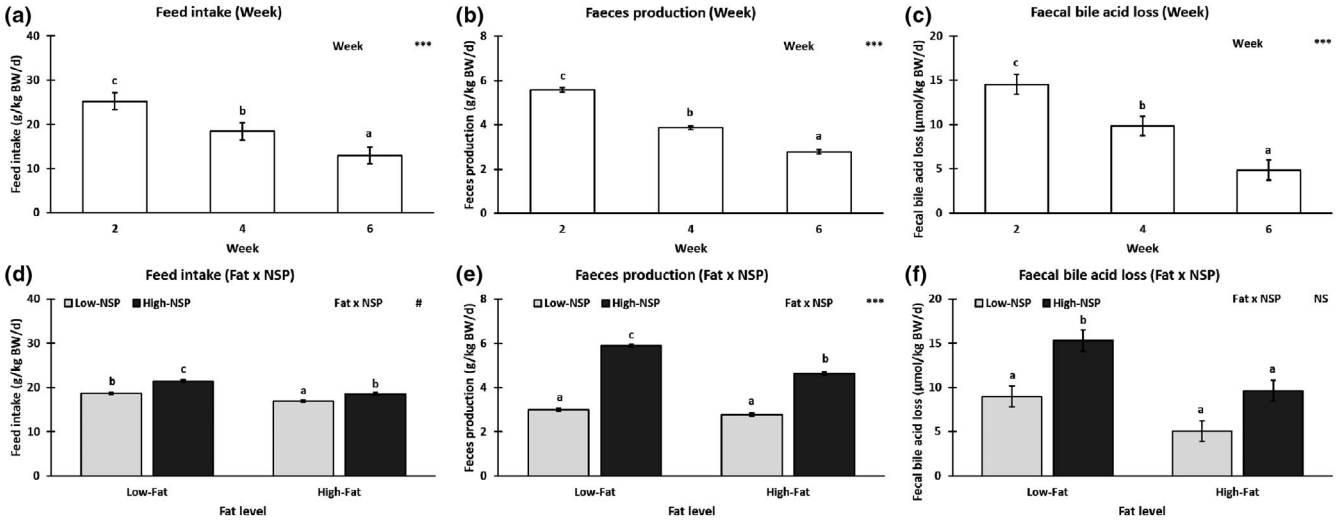

FIGURE 1 Effect of week on (a) feed intake, (b) faeces production and (c) faecal bile acid loss of rainbow trout during 6 weeks of satiation feeding averaged over diets; interaction effect between dietary fat and non-starch polysaccharide level (Fat × NSP) on (d) feed intake, (e) faeces production and (f) faecal bile acid loss of rainbow trout during 6 weeks of satiation feeding averaged over weeks; BW: body weight; NS, not significant: $p \geq .1$; #: $p < .1$; ***: $p < .001$; Error bars indicate standard error of means; treatments in the same panel lacking a common letter are statistically different ($p < .05$) according to Tukey's multiple comparison test

TABLE 3 Time-related changes in apparent nutrient digestibility coefficients of rainbow trout during satiation feeding averaged over diets

	Week				
ADC (%)	2	4	6	*SEM*	*p*-value
Dry matter	78.1^{a}	79.2^{b}	79.2^{b}	0.11	***
Organic matter	81.8^{a}	83.1^{b}	82.8^{b}	0.12	***
Ash	32.9^{b}	30.4^{a}	34.1^{c}	0.30	***
Crude protein	94.1^{a}	94.5^{b}	94.2^{a}	0.07	**
Crude fat	87.1^{a}	89.4^{b}	89.4^{b}	0.19	***
Total carbohydrates	64.3^{a}	65.9^{b}	65.5^{b}	0.24	***
Energy	83.3^{a}	84.9^{b}	84.8^{b}	0.11	***

Note: Values in the same row lacking common superscripts are statistically different ($p < .05$) according to Tukey's multiple comparison test.

Abbreviations: ADC, apparent digestibility coefficient; *SEM*, standard error of means.

**$p < .01$.

***$p < .001$.

($p < .001$; week 2: 5.6 vs. week 4: 3.9 vs. week 6: 2.8 g/kg BW/d averaged over diets; Figure 1b). Furthermore, this time-related decrease was dependent on NSP level ($p < .001$), being more pronounced for the high-NSP diets (Low-NSP: −2.1 vs. High-NSP: −3.7 g/kg BW/d from week 2 to 6). Data averaged over weeks show that faeces production increased when fish were fed the high-NSP diets ($p < .001$; Low-NSP: 2.9 vs. High-NSP: 5.3 g/kg BW/d). However, the significant interaction between fat level and NSP level shows that the increase with NSP level was dependent on the dietary fat level. The effect of NSP was more pronounced for the low-fat diets ($p < .001$; Figure 1e).

3.2 | Apparent nutrient digestibility

Apparent nutrient digestibility coefficients (ADC) were calculated for week 2, 4 and 6 of the experimental period. The time-related changes in ADC averaged over diets are shown in Table 3. Time-related changes in ADC of all nutrients occurred after exposure to the experimental diets ($p < .01$). The ADC of most nutrients increased with time, and a steady-state digestion was reached during week 3, as post hoc analysis did not longer reveal differences in ADC between week 4 and 6 ($p \geq .1$). Comparing week 2 and 4, the increase in ADC of dry matter (DM), organic matter (OM), crude fat, total carbohydrates and energy was 1.1%, 1.3%, 2.3%, 1.6% and 1.6% averaged over diets, respectively. The time-related changes in ash ADC did not show a consistent trend, but ash ADC was significantly higher comparing week 6 to week 2 ($p < .05$). The ADC of protein did not show consistent time-related changes and was not statistically different between week 2 and 6 ($p \geq .1$).

Table S3 shows that the time-related changes for most ADC were dependent on diet, as indicated by several significant interaction effects between week and fat level, and between week and NSP level. Despite the presence of these interaction effects, post hoc analysis did not reveal significant differences in the time needed to reach steady-state digestion between diets for any of the ADC ($p \geq .1$). In other words, the significant interaction effects between week and diet reflect differences in the strength of the time-related increase in ADC between diets. The increase in ADC between week 2 and 4 was smaller for the low-fat diets compared to the high-fat diets (DM: +0.8 vs. +1.4%; OM: +1.2 vs. +1.6%; crude fat: +2.1 vs. +2.5%; energy: +0.8 vs. +1.4%) and smaller for the low-NSP diets compared to the high-NSP diets (DM: +0.7 vs. +1.4%; OM: +1.0 vs. +1.7%; crude protein: +0.3 vs. +0.4%; total carbohydrates: +0.9 vs. +2.5%; energy: +1.3 vs. +2.0%).

The effect of dietary fat level and NSP level on nutrient ADC of individual and combined weeks is shown in Table S3. Most clearly was the adverse effect of increasing NSP levels on the ADC of all nutrients (except calcium) ($p < .01$). The decrease in ADC with NSP level was for DM, OM, ash, protein, carbohydrates and energy 10.0%, 10.8%, 3.9%, 2.1%, 22.5% and 8.1%, respectively. For DM, OM and energy, this decrease in ADC with NSP level was dependent on dietary fat level ($p < .01$), and the decrease in ADC with NSP level was consistently more pronounced for the low-fat diets. No interpretable effect of fat level on protein ADC was found.

Because of the focus of the study, results on fat ADC are shown in Figure 2. Of the macronutrients (protein, fat and carbohydrates), the time-related increase in ADC was strongest for fat (Figure 2a). Moreover, the time-related increase in fat ADC tended to interact with dietary fat level ($p < .1$; Figure 2c), showing that the increase was more pronounced for the high-fat diets compared to the low-fat diets (Low-Fat: +2.1 vs. High-Fat: +2.5%). Furthermore, data of the individual weeks show that fat ADC was adversely affected by increasing dietary fat level ($p < .001$; Low-Fat: 90.6 vs. High-Fat 86.7% averaged over weeks; Figure 2b). No significant main effects of NSP level on fat ADC were found ($p \geq .1$; Figure 2d). However, significant interaction effects between fat level and NSP level show that an increasing dietary NSP level led to a decrease in fat ADC for the low-fat diets, while it did not affect fat ADC of the High-NSP diets ($p \geq .05$).

3.3 | Faecal bile acid content and faecal bile acid loss

Data on the time-related changes in faecal bile acid content and faecal bile acid loss as affected by dietary fat and NSP level are shown in Table 4. No differences in faecal bile acid content were found between weeks ($p > .1$). Furthermore, diet did not have a large impact on faecal bile acid content. Dietary fat level did affect overall faecal bile acid content ($p < .05$); however, the data of individual weeks show that the effect of fat level was only statistically significant for week 2. The faecal bile acid content was lower for fish fed the high-fat diets (Low-Fat: 2.73 vs. High-Fat: 2.03 μmol/g DM averaged over weeks). NSP level did not affect the faecal bile acid content ($p \geq .1$). Faecal bile acid loss expressed per unit of body weight (Figure 1c,f; Table 4) significantly decreased over time ($p < .001$; week 2: 15 vs. week 4: 10 vs. week 6: 5 μmol/kg BW/d averaged over diets). The time-related decrease in faecal bile acid loss was independent from dietary fat level ($p \geq .1$) In contrast, there was a tendency for interaction between week and NSP level ($p < .1$), which indicated that the time-related decrease in faecal bile acid loss was stronger for fish fed the high-NSP diets (Low-NSP: −6 vs. High-NSP: −14 μmol/kg BW/ d comparing week 2 and 6). Overall data show that increasing the dietary fat level resulted in a decrease in faecal bile acid loss ($p < .001$; Low-Fat: 12 vs. High-Fat: 7 μmol/kg BW/d). Furthermore, an increase in the dietary NSP level resulted in an increase of the faecal bile acid loss ($p < .001$; Low-NSP: 7 vs. High-NSP: 13 μmol/kg BW/d). These effects of fat and NSP level were numerically present for all weeks, but only significant for week 2 ($p < .01$). Opposite to faecal bile acid loss expressed per unit of body weight, week did not significantly affect faecal bile acid loss expressed per unit of feed intake ($p \geq .1$; Table 4). Moreover, also the effects of fat level and NSP level become less strong when faecal bile acid loss is expressed per unit of feed intake ($p < .01$; Table 4).

4 | DISCUSSION

4.1 | Length of the adaptation period in digestibility trials

The current study shows that important time-related changes in nutrient apparent digestibility occur after introducing rainbow trout to a new diet (i.e., diet adaptation). The apparent digestibility coefficient (ADC) of most nutrients improved over time. Different mechanism can explain these time-related improvements of nutrient ADC during diet adaptation. Animals, including fish, can adapt their digestive enzyme activity in response to a new diet (Anderson et al., 1991;

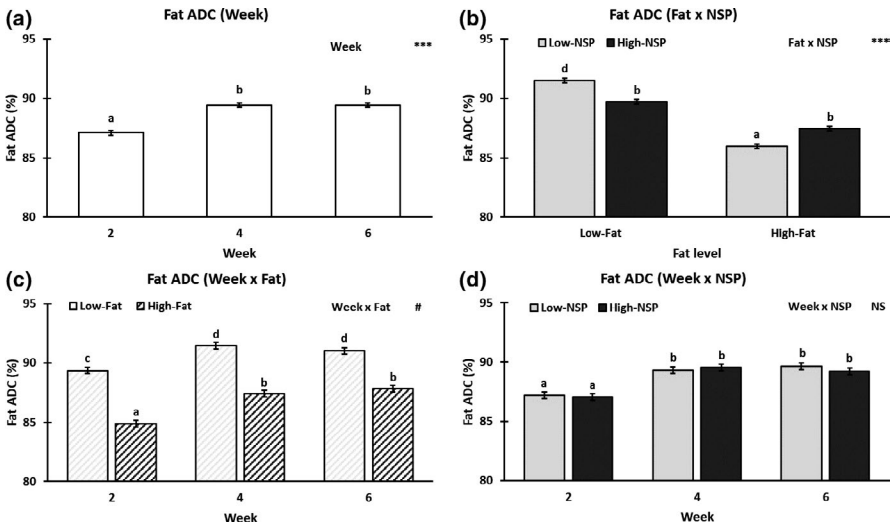

FIGURE 2 Effect of week (a), interaction effect between dietary fat and non-starch polysaccharide level (Fat × NSP) (b), interaction effect between week × dietary fat level (week × fat) (c) and interaction effect between week and dietary NSP level (week × NSP) (d) on the fat apparent digestibility coefficient (ADC) of rainbow trout during 6 weeks of satiation feeding; BW: body weight; NS, not significant: $p \geq .1$; #: $p < .1$; ***: $p < .001$; Error bars indicate standard error of means; Treatments in the same panel lacking a common letter are statistically different ($p < .05$) according to Tukey's multiple comparison test

TABLE 4 Time-related changes in faecal bile acid content and faecal bile acid loss of rainbow trout during satiation feeding of diets differing in fat level and non-starch polysaccharide level

		Diets					*p*-value							
		Low-fat		High-fat										
	Week	Low-NSP	High-NSP	Low-NSP	High-NSP	*SEM*	Fat	NSP		Fat × NSP	Week	Week × Fat	Week × NSP	Week × Fat × NSP
Faecal bile acid content (μmol/g DM)	2	3.2	2.9	1.5	2.5	0.43	*	NS	NS		-	-	-	-
	4	3.6	2.4	1.9	2.2	0.62	NS	NS	NS		-	-	-	-
	6	2.1	2.2	2.7	1.4	0.48	NS	NS	NS		-	-	-	-
	2 + 4 + 6						*	NS	NS		NS	NS	NS	NS
Faecal bile acid loss (μmol/kg BW/d)	2	13^{a}	24^{b}	6^{a}	15^{ab}	2.3	**	**	NS		-	-	-	-
	4	10	14	5	10	2.9	NS	NS	NS		-	-	-	-
	6	4	8	4	4	1.0	NS	#	#		-	-	-	-
	2 + 4 + 6						***	***	NS		***	NS	#	NS
Faecal bile acid loss (μmol/kg diet/d)	2	534^{ab}	803^{b}	265^{a}	636^{ab}	95	#	**	NS		-	-	-	-
	4	572	690	301	517	137	NS	NS	NS		-	-	-	-
	6	324	616	410	342	83	NS	NS	#		-	-	-	-
	2 + 4 + 6						**	**	NS		NS	NS	NS	NS

Note: Week 2, 4 and 6:2 factorial ANOVA; Week 2 + 4 + 6: mixed model ANOVA with fat level and NSP level as between-subject factors and week as within-subject factor.

Values in the same row lacking a common superscript are statistically different ($p < .05$) according to Tukey's multiple comparison test.

Abbreviations: BW, body weight; DM, dry matter; Fat, fat level; NSP, non-starch polysaccharide level; *SEM*, standard error of means.

NS, not significant: $p \geq .1$; #: $p < .1$; *: $p < .05$; **: $p < .01$; ***: $p < .001$.

Corring, 1980; Mohapatra et al., 2003). Also an increased fermentation rate by the intestinal microbiota can lead to better nutrient ADC (Kihara & Sakata, 1997; Tulung et al., 1987). Furthermore, increased feed retention time can positively affect nutrient ADC (Van Soest et al., 1983). Increased feed retention time in the current experiment might have occurred because fish increased more than twice in size (resulting in an increase of the length of the intestine) and because of the time-related decrease in feed intake (Figure 1a; Table S2). Limited information is available about time-related changes in nutrient ADC during diet adaptation in fish, especially with regard to individual nutrients. De Silva and Perera (1983) observed a time-related increase in the protein ADC of a cichlid during diet adaptation. Amirkolaie and Schrama (2015) reported an increase in organic matter (OM) and dry matter (DM) ADC of Nile tilapia, results that are in line with those of the current study. In contrast to Amirkolaie and Schrama (2015), Tran-Ngoc et al. (2017) did not find any time-related changes in ADC of Nile tilapia. The difference between these studies in time-related changes of ADC during diet adaptation might be explained by differences in diet composition. For example, Blyth et al. (2015) found an interaction effect between time-related measurements of energy ADC and diet composition (i.e., a basal fishmeal-based diet, and this basal diet diluted with either gelatinized starch or lupin kernel meal) for barramundi. Furthermore, in the study by Amirkolaie and Schrama (2015) with Nile tilapia, the time needed for stabilization of DM and OM ADC was dependent on diet composition, with the optimal length of the adaptation period being longer for native starch diets compared to gelatinized starch diets. Similarly, Brunsgaard et al. (1995) found that the length of the adaptation period in rats is longer for NSP-rich diets. In the current study, also several significant interactions between time (i.e., week) and diet (i.e., fat level and/or NSP level) were found, suggesting that the length of the adaptation period was dependent on the diet composition. However, opposite to the studies of Brunsgaard et al. (1995) and Amirkolaie and Schrama (2015), post hoc analysis for the interactions between week and diet in the current study did not reveal differences between diets in time needed to reach a steady-state digestion. For example, the significant interaction effect between week and dietary NSP level on DM and OM ADC was mainly attributed to changes of total carbohydrate ADC. Post hoc analysis of the interaction effect between week and dietary NSP level did however not show further improvement in carbohydrate ADC for the low- and high-NSP diets after week 4. The interaction effects between week and diet seem to be mainly ascribed to differences in the size of the ADC increase (rather than the time of the ADC increase) between diets. The difference between the current study and the study of Amirkolaie and Schrama (2015) in effect of NSP level on time-related changes might be related to the fact that the potential for NSP fermentation in cold-water species (i.e., rainbow trout) is much lower compared to warm-water species (e.g., Nile tilapia) (Wilson, 1994). Furthermore, also differences in characteristics between types of NSP (i.e., viscosity, solubility, etc.) affect the rate of fermentation by intestinal bacteria (Macfarlane & Macfarlane, 2003) and might explain differences between studies. The observations from the current study on time-related changes in nutrient ADC indicate that the time needed to reach a steady-state digestion in trout is 3 weeks. The time required for diet adaptation in rainbow trout is longer than the 4 days proposed for barramundi (Blyth et al., 2015), and shorter than the observed 5–6 weeks in Nile tilapia (Amirkolaie & Schrama, 2015). Consideration on the length of the adaptation period in digestibility trials thus needs to be made at the level of species.

4.2 | Time- and diet-related changes in faecal bile acid loss

Besides changes in nutrient ADC, the current study also looked at time-related changes in faecal bile acid loss as affected by dietary fat and NSP level. Results show that faecal bile acid loss (μmol/kg BW/d) was affected by both diet and time. Increasing the dietary fat level resulted in a decrease of faecal bile acid loss. To our knowledge, no studies are available for fish assessing faecal bile acid loss in response to the dietary fat level. However, several studies with humans and rats showed that faecal bile acid loss was either unaffected by fat level (Antonis & Bersohn, 1962; Gordon et al., 1957; Moore et al., 1968) or increased with fat level (Cummings et al., 1978; Reddy et al., 1977). An increase in faecal bile acid loss with fat level could be due to an absolute increase of faecal fat (triglyceride) loss, as some triglycerides were suggested to inhibit reabsorption of bile acids in the ileum (Ammon & Phillips, 1974). Differences in dietary triglyceride content could also explain the differences in the effect of dietary fat level on faecal bile acid loss between studies. Furthermore, Cummings et al. (1978) suggested that enhanced faecal bile acid loss with increasing dietary fat level could be related to an increase of the total bile acid pool size, assuming that a constant portion of bile acids is lost via the faeces. Nevertheless, a decrease in faecal bile acid loss with increasing dietary fat level like in the current study has to our knowledge not been reported. The effect of fat level on faecal bile acid loss in the current study was most likely caused indirectly by the effect of fat level on feed intake and faeces production (explained later in the discussion). Besides dietary fat level, data show that faecal bile acid loss was larger comparing the high-NSP diets to the low-NSP diets. High-NSP diets were used to stimulate faecal bile acid loss as NSP have a known capacity to sequester bile acids (Matin et al., 2016; Overton et al., 1994; Walters et al., 1975). Our previous studies which used an NSP-rich ingredient mixture with the same formulation as in the current study showed enhanced faecal bile acid loss with dietary NSP level only when fish had a high feed intake (Staessen, Verdegem, Koletsi, et al., 2020; Staessen, Verdegem, Weththasinghe, et al., 2020). These results are in line with those of the current study. Opposite to most studies, enhanced faecal bile acid loss in our previous and current studies was not caused by NSP-bile acid binding, as indicated by the absence of a NSP level effect on faecal bile acid content. Instead, enhanced faecal bile acid loss was related to higher feed intake and faeces production of fish fed the high-NSP diets compare to the low-NSP diets. Finally, the current results also showed a significant time-related decrease in faecal

bile acid loss that was independent from diet (although there was a tendency for interaction between week and NSP level). Information about time-related changes in faecal bile acid loss is to our knowledge not reported. Romarheim et al. (2008) found a time-related decrease of the bile acid concentration in the intestine of rainbow trout fed soybean meal-based diets. In the current study, there was no time-related change in the faecal bile acid content, but the time-related decrease in faecal bile acid loss seems to follow the strong time-related decrease in feed intake and faeces production. The effects of both diet (i.e., fat level and NSP level) and time on faecal bile acid loss thus are believed to stem from differences in feed intake and faeces production between diets and over time. The latter can be nicely demonstrated by comparing faecal bile acid loss expressed per unit of body weight (μmol/kg BW/d) with faecal bile acid loss expressed per unit of feed intake (μmol/kg diet/d). The strong effect of week on faecal bile acid loss disappears when expressing faecal bile acid loss per unit of feed intake. Similarly, to the effect of week, also the effects of diet (i.e., fat level and NSP level) on faecal bile acid loss become less significant comparing faecal bile acid loss expressed per unit of feed intake with faecal bile acid loss per unit of body weight. The effect of both dietary fat level and NSP level on faecal bile acid loss can thus partly be explained by their effect on feed intake. Fat level and NSP level affected feed intake because fish were fed to satiation and feed intake can be compensated to get equal digestible energy intake in fish (Dias et al., 1998; Lekva et al., 2010; Sinha et al., 2011). However, the effects of fat level and NPS level on faecal bile acid loss did not completely disappear when expressing the latter per unit of feed intake. The remaining effects are most likely related to DM ADC and amount of faeces produced. DM ADC of high-NSP diets was lower (and faeces production higher) due to the indigestible nature of NSP in most monogastrics (Choct et al., 2010). Nevertheless, results of the current study still show that changes in faecal bile acid loss in relation to diet composition and time were mostly determined by feed intake and faeces production.

4.3 | Time- and diet-related changes in fat ADC

The current study was designed to assess time-related changes in fat ADC as affected by dietary fat level and NSP level. Dietary fat level consistently affected fat ADC, with higher fat levels resulting in lower fat ADC. Relatively seen, there are few studies investigating the level of dietary fat on fat ADC in fish. Furthermore, the effect of fat level on fat ADC is highly variable between studies. The fat ADC was unaffected by dietary fat level in Atlantic halibut (*Hippoglossus hippoglossus*) (Berge & Storebakken, 1991) and in rainbow trout (Austreng et al., 1979). In contrast, for Atlantic salmon (*Salmo salar*) (Bendiksen et al., 2003) found an increase in fat ADC while (Refstie et al., 2001) found a decrease in fat ADC with increased dietary fat level. Differences between studies might be related to different sizes of the contrast in fat level(s) applied, but also to differences in fatty acid profile between fat source(s) used. Concerning the latter, Hua and Bureau (2009) developed a model to predict digestible fat content in salmonid feeds, which showed that variations in fat ADC can be primarily explained by the ratio of saturated fatty acids to total fatty acids. In the current study, slight differences of fatty acid profile between diets should have occurred because the main 2 fat sources, salmon oil (as part of the basal ingredient mixture) and the vegetable oil mixture (consisting of palm oil and rapeseed oil), were combined in different ratios for each diet, especially comparing the low-fat and high-fat diet groups (Table 2). Based on the fatty acid profile of palm oil, rapeseed oil and salmon oil (Kahveci et al., 2010; Zambiazi et al., 2007) and the formula of (Hua & Bureau, 2009), the estimated fat ADC for the low-fat and high-fat diets was 90.0% and 90.2%, respectively. The observed fat ADC for the low-fat and high-fat diets was 90.6% and 86.7% averaged over weeks. The difference trend in fat ADC alongside dietary fat level between the calculated and observed fat ADC suggests that the observed lower fat ADC of the high-fat diets compared to the low-fat diets was not caused by a difference in fatty acid profile. Possibly, assuming equal bile acid pool sizes in fish receiving the different treatments at the start of the experiment, the lower fat ADC in fish fed the high-fat diets compared to the low-fat diets might be related to less bile acid available per unit of dietary fat. The level of emulsification determines the level of fat digestion and absorption (Gallaher & Schneeman, 1986; Golding & Wooster, 2010), thus the higher ratio of dietary fat to bile acids might have led to a smaller quantity of fat being solubilized and thus lower fat ADC for the high-fat diets.

Our previous studies showed that conditions that stimulate enhanced faecal bile acid loss (i.e., NSP level and feed intake) coincide with a reduction of fat ADC in rainbow trout (Staessen, Verdegem, Koletsi, et al. (2020); Staessen, Verdegem, Weththasinghe, et al., 2020). A reduction of bile acid availability in the intestine was proposed to cause this reduction in fat ADC. Opposite our previous studies, the relationship between faecal bile acid loss and fat ADC in the current study was less clear. Faecal bile acid loss increased with dietary NSP level (Figure 1f; Table 4) independently from the dietary fat level. In contrast, fat ADC reduced with dietary NSP level only in fish fed the low-fat diets (Figure 2b). In other words, faecal bile acid loss showed an inverse relationship with fat ADC for the low-fat diets which is in line with those previous studies, but did not relate with fat ADC in fish fed the high-fat diets. A clear explanation for this is not available. Possibly, the lower feed intake (and thus faecal bile acid loss) of fish fed the high-fat diets compared to the low-fat diets could explain the presence of an NSP effect on fat ADC in the low-fat diets while absent for the high-fat diets. The same explanation holds when comparing the effects of NSP level on fat ADC and faecal bile acid loss of the current study with those of our previous studies (Staessen, Verdegem, Koletsi, et al. (2020); Staessen, Verdegem, Weththasinghe, et al., 2020). The overall NSP effects in the current study were weaker compared to those previous studies, and most likely these NSP effects were dampened by the strong time-related decrease in feed intake (i.e., faecal bile acid loss). Faecal bile acid loss in the current study (ranging from 3.6 to 23.6 μmol/kg BW/d) might have been too low compared to previous studies (ranging from 4.1 to 30.5 μmol/kg$^{/}$ BW/d) to evoke strong effects

on fat ADC. In addition, although the NSP-rich ingredients in the current study were the same as in the previous studies, natural variation in their composition (e.g., composition NSP fraction, presence of saponins, etc.) might have played a role in the different outcome of the current experiment.

Figure 2c,d show the time-related increase in fat ADC for diets grouped based on dietary fat and NSP level. The time-related change in fat ADC was independent from dietary NSP level, but tended towards an interaction with dietary fat level. As discussed earlier, the high-NSP diets resulted in more faecal bile acid loss compared to the low-NSP diets. However, opposite our hypothesis, enhanced faecal bile acid loss of fish fed the high-NSP diets did not lead to a time-related decrease in fat ADC. This suggests direct shortage of bile acids in the intestine of fish fed high-NSP diets rather than a time-related decrease of fat ADC caused by a gradual depletion of the total bile acid pool size. The time-related improvement in fat ADC did tend to interact with dietary fat level. The latter shows that the time-related increase in fat ADC was stronger for the high-fat diets compared to the low-fat diets, resulting in the fat ADC difference between both dietary groups becoming smaller over time. Dietary fat level can influence the bile acid metabolism (Bisschop et al., 2004). Bertolotti et al. (1995) reported that dietary fat level can affect bile acid synthesis in rats. An increase of the total bile acid pool size at higher dietary fat level was suggested and the latter might explain the stronger increase in fat ADC for the high-fat diets in the current study. However, this is speculative as information about effects of diets composition on total bile acid pool size in fish is lacking. Further research quantifying the total bile acid pool in response to dietary changes could help in better understanding the effects on fat ADC observed in the current study.

5 | CONCLUSION

This study shows that rainbow trout (*O. mykiss*) need a minimal adaptation period of 3 weeks to reach a steady-state digestion after shifting diets. The time needed to reach steady-state digestion was independent of dietary composition in the current study. Faecal bile acid loss was affected by both the dietary fat and NSP level, but the main factor driving faecal bile acid loss was feed intake (i.e., faeces production). The large time-related decrease in faecal bile acid loss was mainly due to the time-related reduction in feed intake. The time-related increase in fat ADC of rainbow trout fed both the low-NSP and high-NSP diets does not support the hypothesis that enhanced faecal bile acid loss in fish fed high-NSP diets causes a critical depletion of the total bile acid pool size that is needed for proper fat digestion. Assuming an equal total bile acid pool size for all fish at the start of the experiment, the lower fat ADC of the high-fat compared to the low-fat diets directly after exposure to the experimental diets might be related to less bile acid available per unit of dietary fat; however more, research is needed to determine the exact relationship between fat digestion and total bile acid pool size.

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DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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