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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*)

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ABSTRACT

This study investigated in rainbow trout (*Oncorhynchus mykiss*) if dietary bile acid supplementation is effective in restoring hampered fat digestibility related to conditions that enhance fecal bile acid loss (i.e., high dietary non-starch polysaccharide (NSP) level and high feeding level). Four diets were formulated according to a two-by-two factorial design. A Low- and High-NSP level (0 vs. 160 g kg^{-1} inclusion of a NSP-rich ingredient mixture) and two bile acid supplementation levels (0 vs. 2 g kg^{-1} inclusion of sodium taurocholate) were tested. A contrast in feeding level (i.e., feed intake) was created by subsequently feeding fish restrictively (1.1% BW d^{-1}) for four weeks and to satiation for three weeks. The apparent digestibility coefficient (ADC) of all nutrients was affected by feeding period (i.e., restricted vs. satiation feeding), but the effect was dependent on diet composition with the ADC decline between feeding periods being larger for the High-NSP diets. The ADC of all macronutrients decreased alongside dietary NSP level, but this decrease was much more pronounced for fat compared to starch and protein, especially during satiation feeding (6.7%). This large drop in fat ADC during satiation feeding of NSP-rich diets occurred alongside enhanced fecal bile acid loss and correlated with a negative bile acid balance. The correlation between the bile acid balance and fat ADC found during satiation feeding of diets without bile acid supplementation was not present in fish fed diets with bile acid supplementation. In contrast to fat ADC, the ADC of protein and starch were independent from bile acid supplementation. In conclusion, dietary bile acid supplementation is an effective way to remediate decreased fat ADC related to enhanced fecal bile acid loss in rainbow trout, but is not effective in improving protein and starch ADC.

1. Introduction

Current aquafeed formulations encompass a variety of plant ingredients to replace fishmeal (FAO, 2018; Gatlin III et al., 2007; Naylor et al., 2009). However, the use of plant ingredients can interfere with growth, apparent nutrient digestibility (ADC) and health, especially in carnivorous fish as their digestive systems are not well-adapted (Kaushik et al., 1995; Krogdahl et al., 2010; Øverland et al., 2009). These negative effects on growth, ADC and health are ascribed to a range of characteristics inherent to plant ingredients (e.g., unbalanced amino acid profiles, absence of growth factors, low bioavailability of minerals and vitamins, presence of anti-nutritional factors, etc.) (Gatlin III et al., 2007; Hardy, 2010; Naylor et al., 2009; Sinha et al., 2011).

Hampered fat digestion in fish fed plant-based diets often co-occurs with an altered bile acid metabolism. Bile acids are essential for fat digestion as their amphipathic nature aids both fat hydrolysis and transport through micelle formation (Maldonado-Valderrama et al., 2011; Rust, 2003). Furthermore, bile acids activate certain lipases in the intestine (Romarheim et al., 2006; Thirstrup et al., 1994).

Several mechanisms exist by which plant-based diets hamper fat digestion and the bile acid metabolism. Bile acid synthesis in the liver requires both cholesterol as precursor, and taurine for conjugation (Hagey et al., 2010). Contrary to marine-based diets, the supply of taurine (De Moura et al., 2019; Kim et al., 2015; Nguyen et al., 2015; Salze and Davis, 2015) and cholesterol (Deng et al., 2013; Kortner et al., 2013, Kortner et al., 2014) coming from plant-based diets might be insufficient to support proper bile acid synthesis and fat digestion in some species.

Furthermore, many studies investigated the effect of plant-based diets on fat ADC and the bile acid metabolism in the context of fishmeal

Abbreviations: ADC, apparent digestibility coefficient; BAS, bile acid supplementation; BW, body weight; DM, dry matter; FI, feed intake; RFP, relative feces production; NSP, non-starch polysaccharide

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replacement by soybean meal (SBM). The growth supressing effect of SBM, and of soybean saponins in particular, co-occurred in several cases with a decrease in fat ADC, bile acid levels and symptoms of SBM-induced enteritis (Gu et al., 2014; Iwashita et al., 2009; Iwashita et al., 2008; Kortner et al., 2013; Krogdahl et al., 2015; Murashita et al., 2013; Nguyen et al., 2011; Romarheim et al., 2006; Romarheim et al., 2008; Yamamoto et al., 2008). In particular interest to the current study, also non-starch polysaccharides (NSP) can explain adverse effect of plant ingredients on fat ADC and the bile acid metabolism.

The effect of viscous NSP on fat ADC has among others been ascribed to reduced mixing of nutrients and digestive enzymes as shown in rainbow trout (*Oncorhynchus mykiss*) (Kumar et al., 2011; Storebakken, 1985), Atlantic salmon (*Salmo salar*) (Øverland et al., 2009; Refstie et al., 1999) and Nile tilapia (*Oreochromis niloticus*) (Amirkolaie et al., 2005; Hossain et al., 2003; Schneider et al., 2004). In addition, reduced fat ADC has been linked to the capacity of NSP to sequester bile acids and enhance fecal bile acid loss in rats (Ikegami et al., 1990; Moundras et al., 1997), Atlantic salmon (Øverland et al., 2009) and rainbow trout (Staessen et al., 2019), thus reducing the bile acid efficiency in solubilising fats (Ebihara and Schneeman, 1989; Sinha et al., 2011; Vahouny et al., 1981). The study by Staessen et al. (2019) found a significant inverse relationship between fecal bile acid loss and fat ADC, but only at higher levels of feed intake (i.e., satiation feeding). As the impact of dietary NSP level on fat ADC and fecal bile acid loss was dependent on the level of feed intake, the authors suggested that fecal bile acid loss was dependent on the amount of feces produced (which in turn depended on feed intake and dry matter ADC).

Dietary bile acid supplementation was shown to be effective at preventing reduced growth, fat ADC, bile acid levels, and intestinal/hepatic abnormalities caused by soybean meal or soybean antinutrients in rainbow trout (Iwashita et al., 2008, 2009; Yamamoto et al., 2007). Staessen et al. (2019) showed that also dietary NSP level reduced fat ADC, and although the increase in NSP level in that study was realized by increasing the inclusion of among others soy hulls, the authors estimated that the level of soy saponins in the final diets was too low to induce symptoms of enteritis. Furthermore, recent observations with similar diets did not show histological signs of enteritis in rainbow trout (Staessen et al., unpublished data). This suggests that the hampered fat ADC in the study by Staessen et al. (2019) was indeed related to the effect of NSP. Information on possible remediation of adverse NSP effects on fat ADC by bile acid supplementation is lacking for fish.

Based on the foregoing, the current study aimed at investigating the effect of dietary bile acid supplementation to both a Low-NSP and High-NSP diet on fat ADC and the bile acid balance in rainbow (*Oncorhynchus mykiss*). Because Staessen et al., (2019) showed that the impact of dietary NSP level on fat ADC and fecal bile acid loss was dependent on the level of feed intake, the current study included both a restricted and satiation feeding period. If conditions that enhance fecal bile acid loss (i.e., high NSP level and high feeding level) hamper fat digestion by reducing the availability of bile acids in the small intestine, then bile acid supplementation was hypothesized to be effective in remediating such situation, and the efficacy of bile acid supplementation should be predominantly present for the High-NSP diet during satiation feeding.

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

The ingredient composition and analyzed nutrient content of the experimental diets are given in Table 1. All diets fulfilled known nutrient requirements of rainbow trout (*Oncorhynchus mykiss*) according to recommendations of the National Research Council (NRC, 2011). Four diets were formulated according to a two-by-two factorial design. The first factor was dietary non-starch polysaccharide (NSP) level (Low-NSP vs. High-NSP), and the second factor was bile acid supplementation (BAS) level (0% BAS vs. 0.2% BAS). The NSP level was altered by inclusion of either 0 or 160 g kg^{-1} feed of a NSP-rich ingredient mixture consisting of wheat bran and soy hull (1:1). Natural NSP-rich ingredients were chosen over purified NSP, since the latter are not widely used in practice. BAS level was set at either 0 or 2 g kg^{-1} feed of sodium taurocholate (≥98%, Carl Roth®, The Netherlands). Sodium taurocholate was chosen as bile acid supplement since it is the most abundant bile acid naturally synthesized in rainbow trout (Hagey et al., 2010). Levels were chosen to provide compensation for fecal bile acid

Table 1
Ingredient composition, analyzed nutrient content and viscosity of the experimental diets.

	Diets			
	Low-NSP		High-NSP	
	0% BAS	0.2% BAS	0% BAS	0.2% BAS
Ingredients (g kg^{-1})				
Fishmeal[a]	480.0	480.0	480.0	480.0
Plant oil mixture	220.0	220.0	220.0	220.0
Palm oil	*110.0*	*110.0*	*110.0*	*110.0*
Rapeseed oil	*110.0*	*110.0*	*110.0*	*110.0*
Wheat feed flour	254.8	252.8	94.8	92.8
NSP-rich ingredient mixture	-	-	160.0	160.0
Soy hulls	-	-	*80.0*	*80.0*
Wheat bran	-	-	*80.0*	*80.0*
Monocalciumphosphate	30.0	30.0	30.0	30.0
Vitamin/mineral premix[b]	10.0	10.0	10.0	10.0
Chalk ($CaCO_3$)	5.0	5.0	5.0	5.0
Yttrium oxide	0.2	0.2	0.2	0.2
Bile acid (sodium taurocholate)[c]	-	2.0	-	2.0
Analyzed nutrient content (g kg^{-1}DM)				
DM (g kg^{-1})	958	983	971	986
Ash	102	97	109	115
Crude protein (N × 6.25)	404	377	398	412
Crude fat	274	311	288	278
Starch + sugars	203	190	99	98
NSP[d]	12	23	102	96
Gross energy (kJ g^{-1} DM)	24	25	24	24
Calcium	25	23	25	26
Phosphorous	20	18	20	21
Yttrium	0.2	0.2	0.2	0.2
Bile acids (μmol kg^{-1} DM)	676	2300	747	2475
Cholesterol	1.6	1.5	1.6	1.4
Taurine	2.5	2.4	2.5	2.5
Methionine	11.3	10.6	11.2	11.5
Cysteine	3.8	3.6	3.8	3.9
Dietary viscosity (cP)	1.7	1.6	1.9	2.0

x% NSP y% BAS: fish meal-based diet with x% of the NSP-rich ingredient mixture and y% bile acid supplementation (sodium taurocholate).

[a] LT fishmeal – crude protein 72%, Triple Nine Fish protein, Esbjerg, Denmark.

[b] Vitamin/mineral premix: Vitamins (IU or mg kg^{-1} diet): thiamin, 10 mg; riboflavin, 10 mg; pyridoxine, 10 mg; panthotenic acid, 40 mg; niacin, 20 mg; biotin, 0.2 mg; cyanocobalamin, 0.015 mg; folic acid, 2 mg; ascorbic acid, 100 mg; DL-alpha tocopherol acetate, 100 IU; retinyl palmitate, 3000 IU; DL-cholecalciferol, 2400 IU; sodium menadione bisulphate (51%), 10 mg; inositol, 400 mg; choline, 2000 mg; butylhydroxytolueen, 100 mg; calcium propionate, 1000 mg; anti-oxidant BHT (E300–321), 100 mg. Minerals (mg kg^{-1} diet): iron (as $FeSO_4 \cdot 7H_2O$), 50 mg; zinc (as $ZnSO_4 \cdot 7H_2O$), 30 mg; cobalt (as $CoSO_4 \cdot 7H_2O$), 0.1 mg; copper (as $CuSO_4 \cdot 5H_2O$), 10 mg; selenium (as Na_2SeO_3), 0.5 mg; manganese (as $MnSO_4 \cdot 4H_2O$), 20 mg; magnesium (as $MgSO_4 \cdot 7H_2O$), 500 mg; chromium (as $CrCl_3 \cdot 6H_2O$), 1 mg; calcium (as $CaIO_3 \cdot 6H_2O$), 2 mg.

[c] Sodium taurocholate (≥98%), Carl Roth®, The Netherlands.

[d] NSP = 1000 – ash – (N-content × 6.0) – crude fat – (starch + sugars).

loss in fish fed the High-NSP diet as calculated from the results of Staessen et al. (2019). Both the inclusion level of the NSP-rich ingredient mixture and sodium taurocholate were altered by exchanging wheat feed flour with the respective components. Fishmeal was used as main protein source. A plant oil mixture consisting of rapeseed oil and palm oil (1:1) was chosen as main fat source. Both the inclusion levels of fishmeal and the plant oil mixture were kept constant between diets to avoid creating contrasts in the dietary supply of taurine and cholesterol, which otherwise could have confounded with the results of the study. Wheat feed flour was included as the main carbohydrate source. A mineral premix, calcium carbonate and monocalciumphosphate were added to each diet to meet nutrient requirements. Yttrium oxide was included as inert marker for determination of nutrient apparent digestibility (ADC), fecal bile acid loss and fecal cholesterol loss. Extruded pellets of each diet (3 mm; Research and Diet Services B.V., The Netherlands) were vacuum coated with the plant oil mixture at the aquatic research facility of Wageningen University. Diets were stored at 4 °C throughout the duration of the experiment.

2.2. Housing facilities

This study was carried out in the metabolic unit of the aquatic research facility of Wageningen University in The Netherlands. The metabolic unit comprised twelve glass tanks (90 × 60 × 45 cm; 200 L) connected to a common water recirculation system. Water flow into each tank was controlled by a water flow meter (MAGFLO® MAG 5000, Danfoss A/S, Denmark) and was kept constant at 7.0 ± 0.05 L min^{-1}. A trickling filter, sump and drum filter (Hydrotech 500®, Hydrotech Engineering, Italy) helped in maintaining water quality parameters within a set range. Maximum allowed values for NH_4^+-, NO_2-concentrations, and conductivity of the outlet water were < 4 mg L^{-1}, < 1.5 mg L^{-1} and < 4000 μS cm^{-1}, respectively. A partial water change was done when these parameters surpassed their allowed maximum. The pH of the inlet water was kept within the range of 7.0–7.8 by addition of either $NaHCO_3$ or HCl. A cooling system maintained the water temperature at 14.0 ± 0.5 °C. The concentration of dissolved oxygen in the outlet water was maintained at a level > 4.5 mg L^{-1} by an oxygenator, the latter injecting pure oxygen into the common inlet and controlled by a mass flow controller (Brooks® Model 5850S, Brooks Instruments, USA) and a microprocessor (Brooks® Read Out and Control Electronics Model 0154, Brooks Instruments, USA). The outlet of each tank was located at the lower point of the sloping tank bottom and was connected to a swirl separator (44 cm in height, 24.5 cm in diameter; Aqua Optima A/S, UK) to collect feces. A photoperiod of 12 h light – 12 h dark was maintained for the entire duration of the experiment.

2.3. Experimental procedures and sampling

Each diet was tested in triplicate and assigned randomly to the experimental units. Rainbow trout (*Oncorhynchus mykiss*) were obtained from a commercial fish farm (Mohnen Aquaculture GmbH, Germany), and transferred to the aquatic research facilities of Wageningen University. Fish (73 ± 0.4 g) were randomly distributed over the experimental units at a stocking density of 30 fish $tank^{-1}$, and subsequently batch weighed per tank to determine the total biomass. The fish were fed restrictively for 4 weeks, followed by 3 weeks of satiation feeding. During restricted feeding, the aim was to provide daily equal amounts of feed per fish for all diets (expressed as g d^{-1}). This was done to rule out effects of feed intake on ADC and fecal bile acid loss. The calculated daily feed ration was set at 1.1% BW d^{-1}, except in the first week when it was increased stepwise from 20% to 100% of the intended feed ration to allow habituation to the diets. Throughout the restricted feeding period, the ration was daily increased. This increase was equal for all diets and based on the expected growth using the expected average FCR of 0.75. The daily feed ration was divided into two equal portions, which were hand-fed at 9:00 and 15:30 h. During satiation feeding, fish were hand-fed twice daily at 9:00 and 15:30 h until they stopped feeding, with a maximum of 1 h per feeding moment. Tanks were checked for mortality twice daily and dead fish were removed. In case of mortality during restricted feeding, the feeding list for the respective tank(s) was adjusted to maintain equal relative feed intake for all treatments. While feeding, a set of bottles connected to each swirl separator was used to collect the uneaten feed pellets flushed out from the tanks, which allowed accurate determination of feed intake. The last week of the restricted (week 4) and satiation (week 7) feeding period, feces were collected in detachable 250 mL bottles placed at the bottom of each swirl separator. Before the start of fecal collection, the tank bottoms were thoroughly siphoned to prevent contamination by 'old faces' or uneaten feed pellets. Feces collection bottles were submerged into a mixture of water and ice to minimize bacterial decomposition of the feces. Feces were pooled per week per tank and stored at −20 °C awaiting analysis. At both the end of the restricted and satiation feeding period, fish were batch weighed for determination of total biomass. Fish were starved each time for 24 h before batch weighing to allow emptying of the gastro-intestinal tract.

2.4. Analytical methods

Analyses were performed on the 4 experimental diets and on the feces collected during week 4 (restricted feeding) and week 7 (satiation feeding). Fecal samples were first dried at 70 °C until constant weight. Subsequently, dried feces were pulverized using a mixer mill with a 1 mm fixed screen opening set at 12,000 RPM (MM 200 Retch®, Brinkmann, Germany). Pulverized fecal samples could acclimatize to ambient air in the lab for one week before analysis. Feed and feces were analyzed in triplicate for dry matter (DM) content, ash, yttrium, crude protein, crude fat, starch + sugars, energy, bile acid content and cholesterol content. NSP content (g kg^{-1} DM) was calculated as 1000 – ash – (N-content × 6.0) – crude fat – (starch + sugars), with ash, N-content, crude fat and starch + sugars expressed as g kg^{-1} DM. Additionally, diets were analyzed for taurine, methionine, cysteine and viscosity.

Dry matter was determined gravimetrically by drying samples at 103 °C until constant weight (ISO 6496, 1999). Ash content was determined gravimetrically, following the dry matter determination, by incineration in a muffle furnace at 550 °C until constant weight (ISO 5984, 2002). Yttrium content of the samples was analyzed after transferring ashed samples to volumetric flasks and dissolving minerals in a concentrated sulphuric acid solution by autoclaving. Samples were subsequently diluted in water and filtered using a syringe filter (45 μm pores). Yttrium concentrations were finally measured by ICP-OES (NEN 15510, 2007). Crude protein content was measured according to Kjeldahl's method (ISO 5983-2, 2009), using a protein conversion factor of 6.25. Crude fat was determined gravimetrically using acid hydrolysis (Hydrotherm®, C. Gerhardt GmbH & Co. KG, Germany) paired with Soxlet extraction (Soxtherm®, C. Gerhardt GmbH & Co. KG, Germany) (ISO 6492, 1999). Starch and free sugars were determined using an enzymatic digestion method (Nutrilab, The Netherlands). Starch was determined after washing away free sugars with 40% ethanol. The dried residue was digested with Termamyl®, after which starch was hydrolyzed 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% ethanol. Gross energy was measured using a bomb calorimeter (C7000 IKA®, IKA analysentechnik, Weitershem, Germany) (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) with the use of methanol as a reagent blank. Cholesterol was determined enzymatically on the fat extract, using a commercially available kit (Cholesterol liquid color®, Human GmbH, Wiesbaden, Germany). Taurine, methionine and cysteine in the diets were analyzed by Evonik Nutrition and Care GmbH (AMINOLab®, Germany), using ion exchange chromatography and postcolumn derivatization with ninhydrin. Taurine, methionine and cysteine were oxidized with performic acid, which was neutralized with sodium metabisulfite. Amino acids were liberated from the protein by hydrolysis with 6 N HCl for 24 h at 110 °C and quantified with the internal standard by measuring the absorption of reaction products with ninhydrin at 570 nm. Tryptophan was determined by HPLC with fluorescence detection (extinction 280 nm, emission 356 nm), after alkaline hydrolysis with barium hydroxide octahydrate for 20 h at 110 °C. Viscosity of the diets was measured using a Brookfield LVDV-I + cone/plate viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, U.S.A.) as described by Leenhouwers et al. (2007) with some minor modifications. Firstly, 1 g of ground diet was mixed with 4 mL of distilled water, and the mixture was incubated at 15 °C for 30 min. Secondly, viscosity was measured on the supernatant after centrifugation (10 min; 10,000*g*). Viscosity measurements were done at 15 °C and expressed in centipoise (cP) at a shear rate of 750 s^{-1}.

2.5. Calculations

Calculations of performance indicators, apparent digestibility coefficients (ADC), bile acid balance and cholesterol balance were done separately for the restricted feeding and the satiation feeding period.

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 each feeding period, respectively. Feed conversion ratio on dry matter basis (FCR; g g^{-1}) was calculated as $(FI \times dmF)/(W_t - W_0)$, where FI is feed intake (g), dmF is the dry matter content of the feed (%), and W_0 and W_t are the initial and final BW (g) for each feeding period, respectively. Feed intake (FI; % BW d^{-1}) was calculated as $FI/t/W_g \times 100\%$, where FI is feed intake (g), t is the number of days, and W_g is the geometric mean BW (g) of each feeding period, 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 (g) for each feeding period, respectively. Feces production on dry matter basis (RFP; % BW d^{-1}) was calculated as $FI \times (dmF) \times (D_i/F_i)/t/W_g \times 100\%$, where FI is feed intake (g), dmF is the dry matter content of the feed (%), D_i is the percentage inert marker of the diet, F_i is the percentage inert marker of the feces, t is the number of days, and W_g is the geometric mean BW (g) of each feeding period, respectively. Specific growth rate (SGR; % BW d^{-1}) 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 day of each feeding period, respectively.

The apparent digestibility coefficient (%) 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^{-1} gross energy) of the diet, F the percentage nutrient (or kJ g^{-1} gross energy) of the feces, D_i the percentage inert marker of the diet and F_i the percentage inert marker of the feces.

Bile acid intake (μmol kg^{-1} BW d^{-1}) was calculated as $(FI/t/W_g) \times D_b$, where FI is feed intake (g), t the number of days, W_g the geometric mean body weight (kg) and D_b the content bile acids in the diet. Fecal bile acid loss (μmol kg^{-1} BW d^{-1}) 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 in the feces, t is the number of days, W_g is the geometric mean BW (kg), and F_b is the fecal bile acid content on wet weight (μmol g^{-1}) for each feeding period, respectively. The bile acid balance was calculated as bile acid intake – bile acid loss.

Cholesterol intake, cholesterol loss and cholesterol balance were calculated in the same way as for the bile acids and units are expressed as g kg^{-1} BW d^{-1}.

2.6. Statistical analysis

Tanks ($n = 12$) were considered as experimental units. Combined data of restricted and satiation feeding were first analyzed using a mixed model ANOVA to look at the effect of feeding period (restricted vs. satiation feeding), non-starch polysaccharide (NSP) level, bile acid supplementation (BAS) level, their interaction effects and the random effect of tank nested within NSP level and BAS level. The effect of NSP level, BAS level and their interaction was tested against the random effect of tank nested within NSP and BAS level. The effect of feeding period and its interactions with NSP and BAS level were tested against the random error of the whole model (i.e., random variation within tanks between feeding periods). Data were tested for sphericity using Mauchly's test. Preliminary analysis showed that the initial body weight during both the restricted and satiation feeding period were affected by the effect of dietary NSP level. Therefore, data of restricted and satiation feeding were also analyzed separately using a two-way ANOVA for the effect of NSP level, BAS level and their interaction with the inclusion of initial body weight at each period as covariable. This covariance analysis showed that for all most all parameters (except for final BW during satiation feeding, and bile acid intake and cholesterol intake during restricted feeding) the covariable, initial body weight, was not significant (data not shown). Compared to a two-way ANOVA without the inclusion of a covariable, the covariance analysis did not alter the observed effects of the dietary treatments. Therefore, the analysis of data separately during the restricted and satiation feeding period were done without initial body weight as covariable. All data were tested for homogeneity of variance by Levene's test prior to ANOVA. Normal distribution of residuals was checked using Kolmogorov-Smirnov test. ANOVA was followed by a Tukey test for pairwise comparison of means. The correlation between the bile acid balance and fat ADC was tested using Pearson's correlation test. Statistical significance was tested at the 0.05 probability level. *P*-values between 0.1 and 0.05 ($.1 > P \geq .05$) were defined as close to statistical significance and as indicative for tendencies in the data. All statistical tests were performed using the program SPSS statistics 23, IBM Statistics Inc., USA.

3. Results

3.1. Performance

Performance indicators for both feeding periods (restricted vs. satiation) are shown in Table 2. The experiment ran well as shown by low mortality (1.1% averaged over treatments). Furthermore, mortality was unaffected by both feeding period and diet ($P \geq .1$). Feed intake was affected by both the interactions between feeding period and non-starch polysaccharide (NSP) level ($P < .001$) as well as feeding period and bile acid supplementation (BAS)($P < .01$). For all diets feed intake was higher during satiation feeding. The difference in feed intake over feeding periods increased with increasing NSP level and was lower for the diets receiving BAS. During restricted feeding, feed intake was similar for all diets (ranging between 1.07 and 1.11% BW d^{-1}). During satiation feeding, feed intake was affected by both dietary NSP level ($P < .001$) and BAS ($P < .01$). Increasing the NSP level led to an increase of the feed intake (from 1.94 to 2.25% BW d^{-1}), while BAS led to a decrease of the feed intake during satiation feeding (from 2.15 to 2.05% BW d^{-1}). Relative feces production (RFP, % BW d^{-1}) followed feed intake to a large extent. Independently from diet, more feces were produced during the satiation feeding period, being 0.20 and 0.44% BW d^{-1} averaged over diets during restricted and satiation feeding, respectively. For both feeding periods, the lower DM ADC (Supplemental Table 1) of fish fed the High-NSP diets resulted in more feces compared to the Low-NSP diets ($P < .001$). However, due to an increase in feed intake, the increase in feces production alongside NSP level was more

pronounced during satiation feeding (0.30 and 0.58% BW d^{-1} for the Low-NSP and High-NSP diets, respectively) as shown by the significant interaction effect between feeding period and NSP level ($P < .001$). For both feeding periods, BAS led to a lower DM ADC, which resulted in decreased feces production ($P < .001$). The decrease in feces production when supplementing diets with bile acids was largest during satiation feeding (0.48 and 0.41% BW d^{-1} for the 0% BAS and 0.2% BAS diets, respectively) because in that period also feed intake was lower for those diets ($P < .01$). Fish growth was higher for all diets during satiation feeding, however, the effect of feeding period on growth interacted with dietary NSP level ($P < .01$), showing that the difference in growth between feeding periods was in particular large for fish fed the High-NSP diets. NSP level did not affect growth during restricted feeding ($P \geq .1$), while a higher NSP level led to a better growth during satiation feeding related to an increase in feed intake (SGR, 2.69 and 2.84% BW d^{-1} for the Low-NSP and High-NSP diets, respectively) ($P < .05$). BAS did not affect growth ($P \geq .1$).

3.2. Apparent nutrient digestibility

Details on apparent nutrient digestibilities (ADC) are summarized in Supplemental Table 1. Data for macronutrient (crude protein, crude fat and starch) ADC are visualized in Fig. 1. Table 3 shows the effect of feeding period on the mean dietary ADC. Satiation feeding resulted in lower ADC of all nutrients ($P < .01$) and the decrease between feeding periods of the ADC of protein, fat and starch was −1.4%, −3.9%

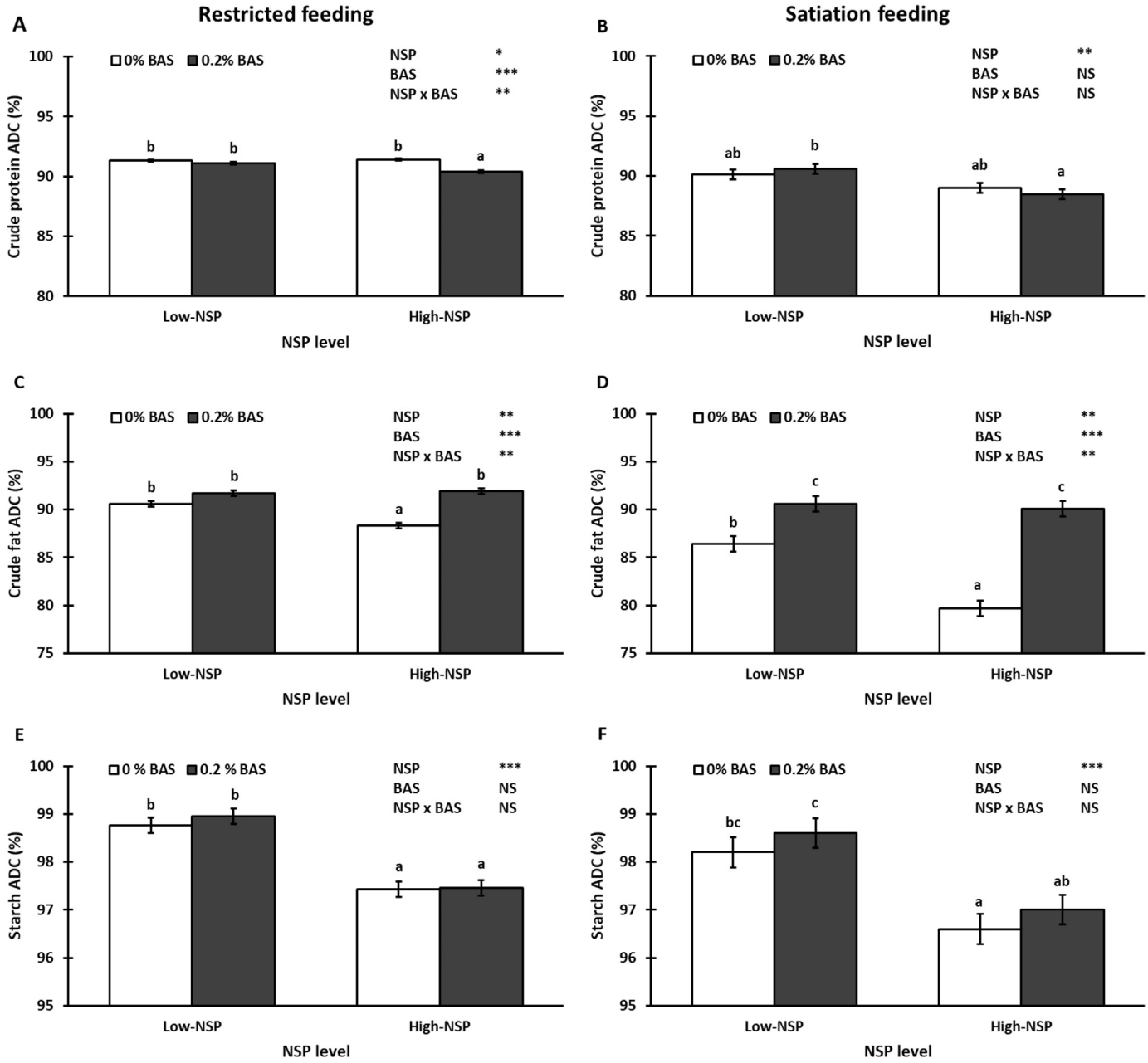

Fig. 1. Effect of dietary non-starch polysaccharide level (Low-NSP vs. High-NSP) and bile acid supplementation (0% BAS vs. 0.2% BAS) on the apparent nutrient digestibility (ADC) during restricted feeding (panel A, C and E) and satiation feeding (panel B, D and F); Crude protein ADC in panel A and B; Crude fat ADC in panel C and D; Starch ADC in panel E and F; Statistical data within each panel are derived from two-way ANOVA for the effect of BAS, NSP and their interaction (NS, not significant: $P \geq .1$; #: $P < .1$; *: $P < .05$; **: $P < .01$; ***: $P < .001$); Error bars indicate standard error of means; Means of the four experimental diets within panels lacking a common letter are statistically different ($P < .05$) according to Tukeys' multiple comparison test.

and 0.8%, respectively. Furthermore, Fig. 1 (and Supplemental Table 1) show that the decrease in ADC of protein, fat and starch between feeding periods was dependent on dietary NSP level ($P < .05$), being larger for the High-NSP diets. Increasing the dietary NSP level led to a decrease in the ADC of −0.9%, −2.3% and −1.5% for protein, fat and starch, respectively averaged over feeding periods. Fat ADC was thus most strongly affected by dietary NSP level. Of the macronutrients, only fat benefitted from BAS and this during both feeding periods ($P < .001$). This translated also in a positive effect of BAS on the ADC of DM and energy ($P < .05$). Furthermore, a significant three-way interaction effect between feeding period, NSP level and BAS ($P < .05$) was found for fat ADC. Post hoc analysis of this effect showed that significant decreases in fat ADC over feeding period and dietary NSP level were not present for fish fed the 0.2% diets. In other words, regardless of feeding period and NSP level, fat ADC of the 0.2% diets (ranging between 90.1 and 91.9%) were not significantly different from the fat ADC found for the Low-NSP 0% BAS diet fed restrictively (90.6%).

3.3. Bile acid parameters

Details on the bile acid parameters are summarized in Table 4. Higher feed intake during satiation feeding resulted in an overall lower fecal NSP content compared to restricted feeding (0.24 and 0.21 g g^{-1} DM for the restricted and satiation feeding period, respectively), but this difference was almost entirely attributed to the High-NSP diets as is shown by the significant interaction effect between feeding period and NSP level ($P < .001$). Fecal NSP content increased with 0.29 g g^{-1} DM averaged over feeding periods with dietary NSP level ($P < .05$). Also, fecal bile acid content was higher during satiation feeding for all diets (1.7 and 5.8 μmol g^{-1} DM averaged over diets for the restricted and satiation feeding period, respectively). Moreover, the effect of feeding period on fecal bile acid content was dependent on the diet ($P < .01$). Averaged over feeding periods, fecal bile acid content decreased with 2.4 μmol g^{-1} DM with increasing NSP level ($P < .001$), and increased with 1.5 μmol g^{-1} DM with BAS ($P < .001$). Post hoc analysis on data of the satiation feeding period showed no differences in fecal bile acid

Table 3
Effect of feeding period (restricted vs. satiation feeding) on apparent nutrient digestibility of dry matter, ash, protein, fat, starch and energy in rainbow trout.

ADC (%)	Feeding period		SEM	*P*-value
	Restricted	Satiation		
DM	81.6	79.3	1.51	***
Ash	37.2	32.6	0.52	***
Crude protein	91.0	89.6	0.23	***
Crude fat	90.6	86.7	1.01	***
Starch	98.2	97.4	0.25	**
Energy	88.3	85.8	1.21	***

ADC: apparent digestibility coefficient; DM: dry matter. SEM: standard error of means.
** $P < .01$.
*** $P < .001$.

Table 2
Effect of dietary non-starch polysaccharide level and bile acid supplementation on performance of rainbow trout during restricted feeding (28 days) and satiation feeding (21 days).

Performance indicator	Feeding period	Diets				SEM	*P*-value						
		Low-NSP		High-NSP			NSP	BAS	NSP × BAS	F	F × NSP	F × BAS	F × NSP × BAS
		0% BAS	0.2% BAS	0% BAS	0.2% BAS								
Initial BW (g $fish^{-1}$)	R	73	75	72	73	0.4	**	NS	NS				
	S	116	118	114	115	1.0	*	NS	NS				
	R + S						*	NS	NS	***	NS	NS	NS
Final BW (g $fish^{-1}$)	R	116	118	114	115	1.0	*	NS	NS				
	S	206	205	206	207	1.9	NS	NS	NS				
	R + S						NS	NS	NS	***	**	NS	NS
BW gain (g $fish^{-1}$)	R	42	43	41	42	0.8	NS	NS	NS				
	S	90	87	93	93	1.4	*	NS	NS				
	R + S						NS	NS	NS	***	**	NS	NS
Mortality (%)	R	2.2	0.0	0.0	4.4	1.57	NS	NS	#				
	S	0.0	0.0	1.1	1.2	0.83	NS	NS	NS				
	R + S						NS	NS	NS	NS	NS	NS	*
FCR (g g^{-1})	R	0.67	0.65	0.68	0.67	0.013	NS	NS	NS				
	S	0.71	0.72	0.79	0.77	0.007	***	NS	#				
	R + S						***	#	NS	***	**	NS	NS
Feed intake													
$FI_{absolute}$ (g DM d^{-1})	R	1.01	1.01	1.01	1.01	0.001	NS	NS	NS				
	S	3.06^{a}	2.95^{a}	3.50^{b}	3.39^{b}	0.032	***	**	NS				
	R + S						***	*	NS	***	***	**	NS
$FI_{relative}$ (% BW d^{-1})	R	1.09^{ab}	1.07^{a}	1.11^{b}	1.11^{b}	0.007	**	NS	NS				
	S	1.98^{b}	1.90^{a}	2.29^{d}	2.20^{c}	0.013	***	***	NS				
	R + S						***	**	NS	***	***	***	NS
RFP (% BW d^{-1})	R	0.16^{a}	0.15^{a}	0.25^{b}	0.25^{b}	0.003	***	*	#				
	S	0.33^{a}	0.27^{a}	0.62^{c}	0.54^{b}	0.013	***	**	NS				
	R + S						***	***	NS	***	***	**	NS
SGR (% BW d^{-1})	R	1.63	1.64	1.61	1.63	0.024	NS	NS	NS				
	S	2.75^{a}	2.62^{ab}	2.84^{b}	2.83^{b}	0.034	**	#	NS				
	R + S						*	NS	NS	***	**	NS	NS

x% NSP y% BAS: fish meal-based diet with x% of the NSP-rich ingredient mixture and y% bile acid supplementation (sodium taurocholate). BW: body weight; DM: dry matter; FCR: feed conversion ratio; FI: feed intake; RFP: relative feces production; SGR: specific growth rate. SEM: standard error of means; NSP: non-starch polysaccharide level; BAS: bile acid supplementation; F: feeding period (restricted vs. satiation feeding). R: restricted feeding (two-way ANOVA); S: satiation feeding (two-way ANOVA); R + S: restricted + satiation feeding (mixed model ANOVA with NSP level and BAS as between-subject factors and feeding period as within-subject factor). NS, not significant: $P \geq .1$; #: $P < .1$; *: $P < .05$; **: $P < .01$; ***: $P < .001$. Values in the same row lacking common superscripts are statistically different ($P < .05$) according to Tukeys' multiple comparison test.

Table 4
Effect of dietary non-starch polysaccharide level and bile acid supplementation on fecal NSP content, fecal bile acid content, bile acid intake, fecal bile acid loss and bile acid balance of rainbow trout during restricted feeding (28 days) and satiation feeding (21 days).

Bile acid parameters	Feeding period	Diets				SEM	*P*-value						
		Low-NSP		High-NSP			NSP	BAS	NSP × BAS	F	F × NSP	F × BAS	F × NSP × BAS
		0% BAS	0.2% BAS	0% BAS	0.2% BAS								
Fecal NSP content (g g^{-1} DM)	R	0.08^{a}	0.08^{a}	0.39^{b}	0.40^{c}	0.003	***	*	#				
	S	0.08^{a}	0.07^{a}	0.33^{b}	0.36^{c}	0.004	***	*	***				
	R + S						*	**	**	***	***	NS	*
Fecal bile acid content (μmol g^{-1} DM)	R	2.1^{bc}	2.7^{c}	0.6^{a}	1.1^{ab}	0.22	***	*	NS				
	S	4.9^{a}	9.8^{b}	4.3^{a}	4.1^{a}	0.51	***	**	**				
	R + S						***	**	**	***	**	**	**
Bile acid intake (μmol kg^{-1} BW d^{-1})	R	7.4^{a}	24.7^{c}	8.3^{b}	27.4^{d}	0.13	***	***	***				
	S	13.4^{a}	43.6^{c}	17.1^{b}	54.5^{d}	0.24	***	***	***				
	R + S						***	***	***	***	***	***	***
Fecal bile acid loss (μmol kg^{-1} BW d^{-1})	R	3.3^{ab}	3.9^{b}	1.6^{a}	2.7^{ab}	0.39	**	*	NS				
	S	15.9^{a}	26.5^{ab}	26.8^{b}	22.3^{ab}	2.39	NS	NS	*				
	R + S						NS	NS	*	***	*	NS	**
Bile acid balance (μmol kg^{-1} BW d^{-1})^{a}	R	4.1^{a}	20.7^{c}	6.7^{b}	24.6^{d}	0.43	***	***	NS				
	S	-2.5^{a}	17.2^{b}	-9.7^{a}	32.1^{c}	2.40	NS	***	**				
	R + S						*	***	**	***	NS	**	**

x% NSP y% BAS: fish meal-based diet with x% of the NSP-rich ingredient mixture and y% bile acid supplementation (sodium taurocholate). BW: body weight; DM: dry matter. SEM: standard error of means; NSP: non-starch polysaccharide level; BAS: bile acid supplementation; F: feeding period (restricted vs. satiation feeding). R: restricted feeding (two-way ANOVA); S: satiation feeding (two-way ANOVA); R + S: restricted + satiation feeding (mixed model ANOVA with NSP level and BAS as between-subject factors and feeding period as within-subject factor). NS, not significant: $P \geq .1$; #: $P < .1$; *: $P < .05$; **: $P < .01$; ***: $P < .001$. Values in the same row lacking common superscripts are statistically different ($P < .05$) according to Tukeys' multiple comparison test.[a] Bile acid balance = bile acid intake – fecal bile acid loss.

content between the diets, except a higher content for the 0% NPS 0% BAS diet ($P < .05$). Bile acid intake was clearly higher comparing the 0.2% BAS to the 0% BAS diets ($P < .001$). Furhtermore as feed intake increased over feeding period and with NSP level during satiation feeding, bile acid intake increased. Overall, fecal bile acid loss was unaffected by NSP level and BAS level, but satiation feeding did significantly increase bile acid loss compared to restricted feeding. No difference in fecal bile acid loss were found for the restricted feeding period. Satiation feeding showed an increase in bile acid loss alongside dietary NSP level. BAS increased loss only for the Low-NSP diet.

Results on the bile acid balance show an accumulation of dietary bile acids in the body for all diets during restricted feeding. Both increasing the dietary NSP level and BAS let to an increase in the bile acid balance during restricted feeding ($P < .001$). Especially BAS led to a strong increase of the bile acid balance of 17.25 μmol kg^{-1} BW d^{-1}. Satiation feeding resulted in a negative bile acid balance in fish fed the diets without bile acid supplementation (regardless of the NPS level). NSP level did not affect the bile acid balance during satiation feeding ($P \geq .1$). BAS resulted in positive bile acid balances (5.4 and 22.7 μmol kg^{-1} BW d^{-1} for the 0% BAS and 0.2% BAS diets, respectively), but the effect of BAS on the bile acid balance was stronger for the High-NSP diet.

3.4. Cholesterol parameters

Details on cholesterol parameters are given in Table 5. Both the cholesterol balance and cholesterol ADC increased going from restricted to satiation feeding (+9.1 mg kg^{-1} BW d^{-1} and +5.7% averaged over diets for the cholesterol balance and fat ADC, respectively). The overall increase over feeding period was for both parameters dependent on dietary NSP level, and especially for cholesterol ADC attributed to the Low-NSP diets ($P < .05$). Both during restricted and satiation feeding, the cholesterol balance and ADC decreased with increasing NSP level ($P < .01$). This decrease with NSP level was 4.6 mg kg^{-1} BW d^{-1} and 19.4% averaged over feeding periods for the cholesterol balance and cholesterol ADC, respectively. BAS did not affect cholesterol ADC ($P \geq .1$), but did lead to a significant decrease in the cholesterol balance of 2.5 mg kg^{-1} BW d^{-1} averaged over feeding periods ($P < .05$).

3.5. Correlation between the bile acid balance and fat digestibility

For both the restrictive and satiation feeding period, Fig. 2 shows the correlation between the bile acid balance and fat ADC of fish fed either the 0% BAS or the 0.2% BAS diets. The bile acid balance during restricted feeding of both the 0% BAS and 0.2% BAS diets did not correlate significantly with fat ADC ($P \geq .1$). In contrast to restricted feeding, satiation feeding of the 0% BAS diets did result in a significant correlation between the bile acid balance and fat ADC ($P < .05$; Pearson correlation coefficient: 0.841). This correlation showed that a decrease in the bile acid balance of 10 μmol kg^{-1} BW d^{-1} corresponded with a decrease in fat ADC of 6.6%. Furthermore, the hampered fat ADC of fish fed the 0% BAS diets to satiation occurred together with a negative bile acid balance. This correlation between the bile acid balance and fat ADC was not present during satiation feeding for the 0.2% BAS diets ($P \geq .1$) and compared to the 0% BAS diets the bile acid balance was not negative for the 0.2% BAS diets.

4. Discussion

Our previous study hypothesized that reduced fat ADC in rainbow trout (*Oncorhynchus mykiss*) under conditions that enhance fecal bile acid loss (i.e., high dietary NSP level and high feeding level) is at least partly caused by limited emulsification capacity in the small intestine (Staessen et al., 2019). This hypothesis is confirmed in the current study by recovery of decreased fat ADC in rainbow trout with enhance fecal bile acid loss and the disappearance of a significant correlation between the bile acid balance and fat ADC when feeding diets supplemented with bile acids.

In the current study, fish were fed restrictively for four weeks and to satiation for three weeks. Results show that higher feed intake (i.e., feeding level) during satiation feeding adversely affected the ADC of all nutrients (Table 3). Because fish were subsequently fed restrictively and to satiation, the decrease in ADC over feeding period might partially be explained by a change in weight or age of the fish. If an effect of body weight or age on ADC occurs, one would normally expect an increase in ADC because adaptation to the diet (Keramat Amirkolaie and Schrama, 2015). However, no studies report a decrease in ADC with increasing

Table 5
Effect of dietary non-starch polysaccharide level and bile acid supplementation on fecal cholesterol content, cholesterol intake, fecal cholesterol loss, cholesterol balance and apparent cholesterol digestibility of rainbow trout during restricted (28 days) and satiation (21 days) feeding.

Cholesterol parameters	Feeding period	Diets				SEM	*P*-value						
		Low-NSP		High-NSP			NSP	BAS	NSP × BAS	F	F × NSP	F × BAS	F × NSP × BAS
		0% BAS	0.2% BAS	0% BAS	0.2% BAS								
Fecal cholesterol content (g kg^{-1} DM)	R	5.0^{b}	5.2^{b}	3.9^{a}	3.7^{a}	0.19	***	NS	NS				
	S	2.8	3.9	3.4	3.4	0.32	NS	NS	NS				
	R + S						*	NS	#	***	**	NS	NS
Cholesterol intake (mg kg^{-1} BW d^{-1})	R	18.0^{b}	16.1^{a}	17.6^{b}	15.8^{a}	0.10	**	***	NS				
	S	32.6^{c}	28.6^{a}	36.2^{d}	31.5^{b}	0.20	***	***	NS				
	R + S						***	***	NS	***	***	***	#
Fecal cholesterol loss (mg kg^{-1} BW d^{-1})	R	7.9a	7.6a	10.0^{b}	9.2^{ab}	0.44	**	NS	NS				
	S	8.97^{a}	10.59^{ab}	21.49^{c}	18.55^{bc}	1.77	***	NS	NS				
	R + S						***	NS	NS	***	**	NS	NS
Cholesterol balance (mg kg^{-1} BW d^{-1})^{a}	R	10.1^{c}	8.6^{bc}	7.6^{ab}	6.6^{a}	0.39	***	*	NS				
	S	23.6^{b}	18.0^{ab}	14.7^{a}	12.9^{a}	1.74	**	#	NS				
	R + S						**	*	NS	***	*	NS	NS
Cholesterol ADC (%)	R	56.1^{c}	53.1^{bc}	43.1^{ab}	41.9^{a}	2.34	**	NS	NS				
	S	72.4^{b}	62.9^{ab}	40.7^{a}	41.1^{a}	4.98	**	NS	NS				
	R + S						***	NS	NS	*	*	NS	NS

x% NSP y% BAS: fish meal-based diet with x% of the NSP-rich ingredient mixture and y% bile acid supplementation (sodium taurocholate). ADC: apparent digestibility coefficient; BW: body weight; DM: dry matter. SEM: standard error of means; NSP: non-starch polysaccharide level; BAS: bile acid supplementation; F: feeding period (restricted vs. satiation feeding). R: restricted feeding (two-way ANOVA); S: satiation feeding (two-way ANOVA); R + S: restricted + satiation feeding (mixed model ANOVA with NSP level and BAS as between-subject factors and feeding period as within-subject factor). NS, not significant: $P \geq .1$; #: $P < .1$; *: $P < .05$; **: $P < .01$; ***: $P < .001$. Values in the same row lacking common superscripts are statistically different ($P < .05$) according to Tukeys' multiple comparison test. [a] Cholesterol balance = cholesterol intake – fecal cholesterol loss.

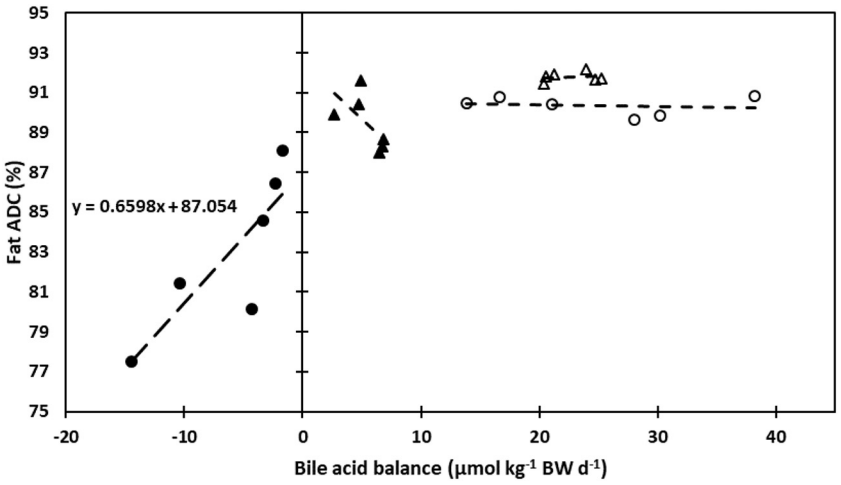

Fig. 2. Relationship between the bile acid balance and fat apparent digestibility coefficient (ADC) for diets with and without bile acid supplementation (BAS) during restricted feeding and satiation feeding; ▲ Restricted feeding of diets with 0% BAS, ● Satiation feeding of diets with 0% BAS, Δ Restricted feeding of diets with 0.2% BAS, ○ Satiation feeding of diets with 0.2% BAS; Only satiation feeding of diets with 0.2% BAS resulted in a significant Pearson correlation coefficient between bile acid balance and fat ADC ($r = 0.84$; $P < .05$).

weight or age like in the current study, making it unlikely that the decrease in ADC was related to changes in weight or age. If an age or weight effect was present, it will most likely have led to an underestimation of the feeding period effect. Literature shows that the effect of feeding level on nutrient ADC of fish is quite variable (Olsen and Ringø, 1997). Storebakken and Austreng (1987) and Cho and Kaushik (1990) stated that feeding level has no or very little effect on nutrient ADC in rainbow trout. In contrast, other studies with rainbow trout (Bergot and Breque, 1983; Windell et al., 1978), African catfish (*Clarias gariepinus*) (Henken et al., 1985) and Nile tilapia (*Oreochromis niloticus*) (Haidar et al., 2016; Schrama et al., 2012) described decreased ADC of one or more nutrients with increasing feed intake, and this likely due to overloading of the digestive enzyme and nutrient absorption capacity. Differences between studies in the effect of feeding level on nutrient ADC can stem from both differences in the range of applied feeding levels or differences in diet formulation. It was shown in both our previous study (Staessen et al., 2019) and the current study that the effect of feeding period on nutrient ADC is dependent on diet formulation. Significant interaction effects between feeding period and NSP level were found for all nutrient ADC, except for starch (Supplemental Table 1). These interactions show that the decrease in nutrient ADC between both feeding periods was consistently larger for the High-NSP diets compared to the low-NSP diets. The latter might be partly explained by an increase in feed intake alongside NSP level during satiation feeding (Table 2), overloading the digestive enzyme and nutrient absorption capacity and resulting in lower nutrient ADC for the High-NSP diets. In addition, results show that cholesterol retention (cholesterol balance) and cholesterol ADC increased over feeding period (Table 5). This increase in cholesterol ADC is in contrast with the effect of feeding period on fat ADC, which shows that cholesterol digestion/uptake is regulated independently from fat digestion. Kortner et al. (2014) suggested for Atlantic salmon (*Salmo salar*) that altering uptake of dietary cholesterol by the intestinal lumen is crucial in maintaining cholesterol homeostasis. Comparing macronutrient ADC, the decrease in fat ADC over feeding period was much more pronounced compared to protein and starch ADC (Table 3). This suggests that factors other than the difference in feed intake over feeding period (e.g., dietary NSP level) should be considered as well in evaluating the

impact on nutrient ADC in the current study, especially for fat ADC.

Dietary NSP level adversely affected the ADC of all macronutrients, and consequently the ADC of DM and energy (Fig. 1). Comparing macronutrients, NSP level most strongly affected fat ADC, and this during both restricted and satiation feeding. Inevitably, using a NSP-rich ingredient mixture, rather than purified NSP, altered the source of part of the nutrients in the final diet (estimated share in final diet coming for the NSP-rich ingredient mixture for protein ± 20, fat ± 5 and ± 75 starch g kg^{-1} DM). For protein and starch, difference in nutrient source could explain some differences in the ADC between the Low-NSP and High-NSP diets. The ingredients used to increase dietary NSP content, soy hulls and wheat bran, have a very low fat content (23.1 and 42.7 g kg^{-1} respectively; Staessen et al., unpublished data). Therefore, it is unlikely that the large effect of NSP level on fat ADC is related to the change in fat source.

Several studies reported adverse effects of dietary NSP level on nutrient ADC in fish. Storebakken (1985) and Amirkolaie et al. (2005) showed that the increase in digesta viscosity caused by the soluble NSP guar gum reduced nutrient ADC in rainbow trout and Nile tilapia, respectively. In contrast, other studies showed no effect of increasing levels of the insoluble NSP cellulose on nutrient ADC in rainbow trout (Amirkolaie et al., 2005; Glencross, 2009; Glencross et al., 2012; Hansen and Storebakken, 2007). Comparing studies shows that the effect of dietary NSP level on nutrient ADC is dependent on type of NSP and their capacity to increase viscosity. Soluble NSP can increase digesta viscosity, which reduces mixing of nutrients and digestive enzymes in the intestine (Knudsen, 2001). In contrast, insoluble NSP do have little impact on digesta viscosity (Glencross, 2009; Sinha et al., 2011). The NSP-rich ingredient mixture used in the current study contained both soluble and insoluble NSP (based on Knudsen (1997) and Knudsen (2014); 505 g NSP kg^{-1} DM of which 74 g was soluble). The relatively low amount of soluble NSP in the NSP-rich ingredient mixture resulted in a somewhat higher dietary viscosity of the High-NSP diets compared to the Low-NSP diets (Table 1), and the latter might be partly responsible for the decrease in nutrient ADC. The minor effect of NSP level on protein and starch ADC might be explained by differences in feed intake (feed load on the digestive enzyme and nutrient absorption capacity), differences in source of protein and starch between the 0 and High-NSP diets and/or an increase in diet viscosity. In contrast, the decrease of fat ADC alongside dietary NSP level, especially during satiation feeding, was numerically much larger compared to the other macronutrients and another explanation than those mentioned earlier is believed to have played a major role in this.

Literature showed adverse effects of dietary NSP on fat ADC, since some have the capacity to enhance fecal bile acid loss (Ikegami et al., 1990; Romarheim et al., 2008). Increased fecal bile acid loss may reduce the total bile acid pool if de novo synthesis reaches its maximum capacity (Divakaran et al., 1992; Kortner et al., 2013), possibly limiting availability of bile acids in the small intestine for proper fat digestion. Like our previous study (Staessen et al., 2019), the current study showed a significant increase in fecal bile acid loss alongside dietary NSP level during satiation feeding (Table 4). This increase in fecal bile acid loss was not caused by NSP-bile acid binding, since fecal bile acid content for diets without bile acid supplementation was unaffected by dietary NSP level. The increase in fecal bile acid loss can be explained by an increase in feces production caused by a lower overall DM ADC (Supplemental Table 1) and by an increase in feed intake alongside NSP level during satiation feeding (Table 2). Another explanation for the increase in fecal bile acid loss of fish fed the High-NSP diets could be the presence of saponins coming from the soy hulls in the NSP-rich ingredient mixture. Saponins can form non-absorbable complexes with bile acids (Kregiel et al., 2017) and cause enteritis (Gu et al., 2014; Iwashita et al., 2009; Iwashita et al., 2008; Kortner et al., 2013; Krogdahl et al., 2015; Murashita et al., 2013; Nguyen et al., 2011; Romarheim et al., 2006; Romarheim et al., 2008; Yamamoto et al., 2007; Yamamoto et al., 2008), which both can result in an increase of bile acid loss. However, if enhanced fecal bile acid loss was caused by formation of saponin-bile acid complexes, an increase in the fecal bile acid concentration would be expected. This is not the case for fish with enhanced fecal bile acid loss in the current study. Furthermore, we do not expect that fish developed enteritis in the current study. Based on the content of saponins in soy hulls (Shi et al., 2004), the maximum level of saponins was estimated to be around 1.6 g kg^{-1} diet, which is lower than the porposed minimum level of 2 g soy saponins kg^{-1} diet needed in the more inflammation sensitive Atlantic salmon (Krogdahl et al., 2015). Furthermore, recent observations with similar diets like in the current study did not show signs of SBM-induced enteritis in rainbow trout (Staessen et al., unpublished data). Results of the current study show positive effects of dietary bile acid supplementation on fat ADC, while no effect occurred for ADC of protein and starch. Decreased fat ADC of the High-NSP diets during both feeding periods improved after bile acid supplementation to levels similar to those found for the Low-NSP diets. Bile acid supplementation to the Low-NSP diet fed restrictively did not further improve fat ADC. Positive effects of bile acid supplementation on fat ADC were reported for fish (Iwashita et al., 2008, 2009; Kortner et al., 2016; Yamamoto et al., 2007, 2008). Yamamoto et al. (2008) found, besides improved fat ADC, also an improved starch ADC after dietary supplementation of bovine bile acids. The improvement of fat ADC after bile acid supplementation could be related to a decrease in feed intake, the latter reducing the load on the digestive system. Literature shows both positive and negative effects of bile acids on palatability and feed intake in fish (Kasumyan and Vinogradskaya, 2019; Rolen and Caprio, 2008). However, the decrease in feed intake of the 0.2% BAS diets in the current study is believed to be related to an overall higher DM ADC and the capacity of fish to regulate feed intake based on the level of digestible nutrients in a diet (Lekva et al., 2010). If the reduction in feed intake is responsible for the improved fat ADC, a positive effect of bile acid supplementation on other nutrient ADC would be expected. This was not the case and the positive effect of BAS on fat ADC was most likely directly related to a higher bile acid availability in the small intestine. Both literature and the current study show that bile acid supplementation mainly affects fat ADC. This is because bile acids play a crucial role in digestion and absorption of dietary fat, forming micelles which increase the surface area on which digestive enzymes can act and aid in transportation to the brush border for absorption (Pasquier et al., 1996). Furthermore, bile acids can increase both pancreatic lipase and protease activities (Robic et al., 2011; Romarheim et al., 2006; Thirstrup et al., 1994), although a positive effect of bile acid supplementation on protein ADC was not present in this study. In contrast to aforementioned studies, Kortner et al., 2016 did not find effects of bile acid supplementation on any of the macronutrient ADC in Atlantic salmon. The difference in effect of bile acid supplementation on nutrient ADC between the last and aforementioned studies might be related to differences in diet composition. Also in the current experiment, bile acid supplementation was dependent on diet composition (i.e., NSP level), as bile acid supplementation to the Low-NSP diet did not further improve fat ADC. Additionally, in the case of cholesterol homeostasis we expected a decrease in cholesterol ADC with bile acid supplementation, as less cholesterol is needed for de novo synthesis of bile acids (Hofmann and Hagey, 2008; Kortner et al., 2016). However, we did not see any effect of bile acid supplementation on cholesterol ADC in this study and do not have an explanation for this.

To compensate bile acid intake in the bile acid supplemented diets, the bile acid balance was calculated (Table 4). In this study, a correlation between the bile acid balance and fat ADC in fish fed diets without bile acid supplementation was found for the satiation feeding period (Fig. 2). These results show that if the bile acid balance becomes more negative, Fat ADC is adversely affected. A negative bile acid balance indicates either an upregulation of endogenous bile acid synthesis to compensate fecal bile acid loss, or a decrease in the total bile acid pool. A lowered total bile acid pool would reduce micelle

formation, therefore impairing lipid digestion and absorption (Ebihara and Schneeman, 1989; Pasquier et al., 1996). There was no significant correlation for the restrictive feeding period and in fish fed diets supplemented with bile acids. Results are similar to those reported for the fishmeal-based diets in Staessen et al. (2019), but in that study fecal bile acid loss was plotted against fat ADC. Results of the current study also show that bile acid supplementation resulted in a more positive bile acid balance, indicating an increase in the total bile acid pool. Data on total bile acid pool in fish are limiting, Yamamoto et al. (2007) found increased bile acid concentrations in the gallbladder of rainbow trout fed diets supplemented with bile acids, which might suggest an increase in total bile acid pool.

5. Conclusion

Nutrient ADC is adversely affected by increased feed intake in rainbow trout (*Oncorhynchus mykiss*), but the effect of feeding level, especially on fat ADC, is dependent on diet quality being more pronounced for High-NSP diets. Conditions (i.e., high feed intake and dietary NSP level) that result in a significant increase of fecal bile acid loss and a negative bile acid balance seem to correlate with a strong decrease in fat ADC. Bile acid supplementation results in the absence of this correlation between fat ADC and bile acid balance. This suggests that lower fat ADC in trout with enhanced fecal bile acid loss is at least partly related to a lack of bile acid availability in the small intestine. Dietary bile acid supplementation is an effective way to remediate decreased fat ADC related to enhanced fecal bile acid loss in rainbow trout, but is not effective in improving protein and starch ADC.

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Declaration of Competing Interest

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

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