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Dietary starch, non-starch polysaccharides and their interactions affect nutrient digestibility, faecal waste production and characteristics differentially in three salmonids: Rainbow trout, Atlantic salmon and Arctic charr

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ABSTRACT

Waste management has emerged as a critical issue in aquaculture. In this study, we examined the impact of dietary starch and non-starch polysaccharides (NSP) content on nutrient digestibility, faecal waste production, faecal removal efficiency and the faecal characteristics in three salmonid species, namely rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*) and Arctic charr (*Salvelinus alpinus*). Four diets were formulated according to a 2 × 2 factorial design. The first factor, starch, was tested by including 0% gelatinised wheat flour (low starch) or 20% gelatinised wheat flour (high starch) in a plant-based basal diet. The second factor, NSP, was tested by adding 0% NSP source (low NSP) or 10% NSP source (high NSP). High NSP level was achieved by adding an equal mixture of soya hull (5%) and wheat bran (5%). Diets were tested in triplicates for each species and feeding was done restrictively. Experimental duration was 42 days for rainbow trout and Atlantic salmon and 49 days for Arctic charr. Among the three species investigated, Arctic charr had the lowest digestibility values for most nutrients, whereas rainbow trout and Atlantic salmon values were comparable. High starch level in the diet reduced the macronutrient (protein, fat and ash) digestibility in all three species. High starch and high NSP levels in the diet increased faecal waste production, with the effect being more pronounced for the NSP content of the diet. High dietary starch levels increased the proportion of smaller-sized particles, while high NSP content increased the ability of faecal particles to withstand mechanical stress. The high starch level in the diet lowered faecal removal efficiency but increased by high NSP content. The highest and lowest faecal removal efficiency was recorded for Atlantic salmon and Arctic charr, respectively. The amount of non-removed faeces accumulating in the system was increased by the high starch levels in rainbow trout and Atlantic salmon but remained unaffected by the NSP content of the diet across the species. No interaction effect between starch and NSP content of diets was observed for faecal removal efficiency and non-removed faeces. Among the three species investigated, Arctic charr had the maximum amount of non-removed faeces per kilogram of dry matter feed.

1. Introduction

Salmonid aquaculture production has increased manifold in the last few decades. The current global production of the two most widely farmed salmonids, Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) is estimated at 2.72 and 0.96 million tons, respectively (FAO, 2022). Arctic charr (*Salvelinus alpinus*) is another salmonid species considered ideal for aquaculture due to its ability to

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perform better at lower temperature and higher densities (Jobling et al., 1993; Summerfelt et al., 2004; Carlberg et al., 2018). The global production of Arctic charr is estimated close to 8000 tons (Helgadóttir et al., 2021). It is expected that the growing demand for seafood, coupled with the limited availability of land and water resources, will result in intensification of production methods (Oddsson, 2020). Additionally, changing trends in fish feed formulation towards increasing inclusion levels of plant-based ingredients will increase the level of starch and dietary fibres in aquafeeds. Adopting intensive production methods and including raw materials of plant origin in the diet would increase waste generation, raising concerns regarding their removal from the system or effluent discharge in recipient aquatic ecosystems (Dauda et al., 2019). Waste from an aquaculture production system can be broadly categorised as solid and dissolved waste. Solid waste accumulation in the production system can potentially impact the growth and welfare of cultured fish species. Efficient solid waste removal is also essential for achieving optimal functioning of the production system and keeping the operational cost minimal (Davidson et al., 2013; Pedersen et al., 2017; Van Rijn, 2013). The primary source of solid waste from aquaculture is faeces, which is directly linked to diet, hence manipulating dietary factors presents potential avenues for solid waste management. However, it is still unknown if the same diet-based intervention will equally suite all the species in terms of solid waste management.

Faecal waste accumulating in the system depends on the quantity of faecal waste produced (dry matter digestibility of diet) and its removal efficiency (Amirkolaie et al., 2006; Letelier-Gordo et al., 2015; Meriac et al., 2014; Prabhu et al., 2019; Tran-Tu et al., 2018). Removal efficiency of solid waste in recirculating aquaculture system (RAS) is determined by the characteristics of faeces, such as size, stability (i.e. the ability of faecal particles to withstand mechanical stress) and density. Size and stability of faeces determine the potential conversion of solid waste into a dissolved form, affecting the performance of other system components such as biofilters and disinfection systems (Schumann and Brinker, 2020). Despite several years of research and significant advances in understanding the nutrient requirement of salmonids, our knowledge of the effect of dietary interventions on faecal characteristics is limited. For instance, no information (i.e., no quantitative data) is available to date regarding the impact of dietary interventions on faecal characteristics (size, stability etc.) and faecal removal efficiency of Atlantic salmon and Arctic charr. Information on faecal characteristics of Atlantic salmon is crucial as there is a strong tendency in the industry to extend the land-based phase of production by using indoor RAS based systems (Bjørndal and Tusvik, 2019., Meriac, 2019). Simultaneously, in the sea-water phase, the industry is gearing up to develop and embrace technological interventions to efficiently remove solid waste from open cages (Krogli, 2023). For Arctic charr, there is limited information available regarding the nutrient digestibility and changes in faecal characteristics in response to dietary factors. This complicates drawing conclusions about the quantity of waste produced, removal efficiency, and composition of faecal waste produced by Arctic charr. Thus, a systematic investigation into the impact of dietary interventions on waste production and faecal characteristics of candidate salmonid species' is warranted.

Dietary factors investigated for its impact on faecal characteristics include (but not limited to) ingredient composition (Prabhu et al., 2019; Prakash et al., 2023), diet viscosity (Brinker, 2007; Tran-Tu et al., 2019), inclusion of non-starch polysaccharides (NSP) (Meriac et al., 2014) and starch (Horstmann et al., 2023b; Prakash et al., 2024) sources. The impact of various carbohydrate fractions on faecal characteristics was shown to be dose-dependent and species-specific. Improvement in faecal removal efficiency was noted in the case of Nile tilapia (*Oreochromis niloticus*) with increased starch levels in the diet (Amirkolaie et al., 2006). In contrast, high dietary starch levels increased faecal waste production and reduced faecal removal efficiency in yellowtail kingfish (*Seriola lalandi*) (Horstmann et al., 2023b), African catfish, (*Clarias gariepinus*) (Phan et al., 2022) and rainbow trout (Prakash et al., 2024). In a study comparing diets with high starch and high non-starch polysaccharides (NSP) content, the diet with high NSP content, despite resulting in increased faecal waste production, reduced amount of non-removed faeces accumulating in the system (Meriac et al., 2014). In our previous investigation evaluating dietary ingredients for rainbow trout, we showed that diets with high carbohydrate content resulted in lower faecal removal efficiency (Prakash et al., 2023). However, it was unclear whether this outcome was due to high starch content, high NSP content, type of NSP or associated with presence of other unknown components in used ingredients. In a subsequent study, we demonstrated that increasing dietary starch levels reduced faecal removal efficiency in rainbow trout (Prakash et al., 2024). Plant-based ingredients used in fish diets present a complex mixture of starch and NSP, and the impact of their interactions on faecal characteristics has not yet been explored. Additionally, it remains unknown whether different salmonid species would respond similarly to these dietary factors (starch, NSP and their interactions).

Within this context, the current study evaluated the effect of dietary starch, NSP and their interactions on nutrient digestibility, faecal waste production and faecal removal efficiency in three salmonid species: rainbow trout, Atlantic salmon and Arctic charr.

2. Materials and methods

2.1. Diets

Four diets were formulated according to a 2 × 2 factorial design to investigate the effect of dietary starch, non-starch polysaccharides and their interactions on nutrient digestibility, faecal waste production and faecal characteristics of rainbow trout, Arctic charr and Atlantic salmon parrs. The diet was produced as a single batch to ensure that batch-to-batch variation in production parameters did not influence the physical characteristics of feed and, consequently, the measured variables (nutrient digestibility and faecal characteristics). A basal mixture was prepared consisting of wheat gluten, pea protein concentrate, soy protein concentrate, krill meal and fish oil (Table 1). The basal diet was supplemented with crystalline amino acids DL-methionine, L-threonine,

Table 1
The amounts (in g/kg) of ingredients used in the basal mixture.

Ingredients	Inclusion (g/kg as is)
Wheat gluten	103
Pea protein concentrate	220
Soya protein concentrate	220
Fish oil	150
Krill meal	20
Monocalcium phosphate	30
Chalk ($CaCO_3$)	20
DL-methionine	6.0
L-threonine	2.0
Histidine	2.0
Casein	143
Pellet binders[a]	62.5
Premix[b]	21.4
Yttrium oxide	0.25

[a] Pellet binders – in house composition.

[b] Premix Vitamins (IU or g/kg premix): thiamin, 1 g; riboflavin, 1 g; pyridoxine, 1 g; pantothenic acid, 4 g; niacin, 6.5 g; biotin, 0.02 g; cyanocobalamin, 0.017 g, folic acid, 0.33 g; ascorbic acid (as ascorbic acid phosphate), 15 g; DL-alpha tocopherol acetate, 20,000 IU; retinyl palmitate, 300,000 IU; DL-cholecalciferol, 240,000 IU; sodium menadione bisulfite (51%), 1 g; inositol, 40 g; choline, 200 g (given as choline chloride). Minerals (g kg^{-1} premix): iron (as $FeSO_4.7H_2O$), 5 g; zinc (as $ZnSO_4.7H_2O$); 10 g; cobalt (as $CoSO_4.7H_2O$), 0.01 g; copper (as $CuSO_4.5H_2O$), 1 g; Selenium (as Na_2SeO_3), 0.02 g; manganese (as $MnSO_4.4\ H_2O$), 2 g; magnesium (as $MgSO_4.7H_2O$), 50 g; chromium (as Cr Cl3.6H2O), 0.1 g; iodine (as $CaIO_3.6H_2O$) 0.2 g. Preservatives (g kg^{-1} premix): Anti-oxidant BHT (E300–321), 10 g; calcium propionate, 100 g.

and histidine. Additionally, monocalcium phosphate was added to ensure that phosphorus was not a limiting factor. Yttrium oxide (Y_2O_3) was added (at 0.02%) as an inert marker for digestibility determination. The first factor, starch, was tested by including 0% gelatinised wheat flour (LS-low starch) or 20% gelatinised wheat flour (HS-high starch) in the basal diet. The second factor, NSP, was tested by adding 0% NSP source (low NSP) or 10% NSP source (high NSP). High NSP level was achieved by adding an equal mixture of soya hull (5%) and wheat bran (5%). The diets were produced by cold extrusion by Research Diet Services (Wijk bij Duurstede, The Netherlands), according to Kals et al. (2019). Diets were produced at the Animal science group (Wageningen University & Research, The Netherlands) facility using a co-rotating double screw extruder (M.P.F.50, Baker Perkins, Peterborough, United Kingdom) with a 3 mm die, resulting in 3 mm sinking pellets. Following pelleting, the pellets were first dried for 2 h at 45 °C and followed by drying at 70 °C for 3 h. Afterwards, the pellets were cooled to room temperature. Diets were produced approximately one week before the start of the first phase of experiment and stored at 4 °C throughout the whole experiment. The analysed nutrient content of experimental diets is provided in Table 2.

2.2. *Fish, rearing conditions and housing facilities*

The experiment followed the Dutch and European legislation governing the use of experimental animals. According to Dutch legislation, the Animal Welfare Body of Wageningen University and Research (The Netherlands) classified this experiment as non-invasive rather than an animal experiment. Fish of mixed sex were obtained from commercial farms- Mohnen, GmbH, Stolberg, Germany (rainbow trout); Viskwekerij Streekvis, Dodewaard, The Netherlands (Arctic charr); and Zalmboerderij Maashorst, Volkel, The Netherlands (Atlantic salmon). The experiment was conducted at the Aquaculture Research Facility (ARF) of Wageningen University, the Netherlands. Trials were conducted in two phases due to the unavailability of juveniles of all three species simultaneously and their different photoperiod requirements. In phase I (12 h light: 12 h dark), rainbow trout and Arctic charr, each were evaluated in 12 experimental tanks (same RAS). In phase II (24 h light: 0 h dark), the impact of the identical four diets was tested with Atlantic salmon in 12 experimental tanks. A 24-h light phase was followed for Atlantic salmon to avoid their smoltification during the course of the experiment (Handeland and Stefansson, 2001; Strand et al., 2018). Considering the lower feed intake of Arctic charr and to have adequate amount of faecal samples collected for analytical purposes, the experimental duration was kept at seven weeks for Arctic charr, while for rainbow trout and Atlantic salmon, it was six weeks. To ensure adequate amount of faecal samples for analytical purposes, the number of animals stocked in each experimental tank was 25 for rainbow trout, and 30 for Arctic charr and Atlantic salmon. At the start of the experiment, 300 fish with an average weight of 81 ± 1.9 g (mean ± SEM) in the case of rainbow trout and 360 fish with an average weight of 70 ± 1.2 g (Arctic charr) and 39 ± 0.9 g (Atlantic salmon) were randomly distributed to circular tanks (24 in first phase and 12 in second phase) of 0.99 m diameter and 0.48 m depth, resulting in three replicates per dietary treatment per species. The water volume in each tank was maintained at 360 L. All fish were acclimatized to experimental conditions for at least three weeks before the trial. Fish were weighed (Mettler-Toledo ICS429) at the experiment's beginning and end to determine the initial and final weight and calculate growth performance. Feeding was stopped one day before weighing to allow emptying of gastrointestinal tracts. Since all 24 experimental tanks in phase I (rainbow trout and arctic charr) were connected to a common RAS, each experimental tank's inlet water quality parameters were similar. Similarly, in phase II, all 12 experimental tanks with Atlantic salmon were connected to a common RAS. The common RAS facility consisted of a sump, settling tank, drum filter, protein skimmer, trickling filter and a UV treatment unit identical to that described in Prakash et al. (2023). An air stone was provided to each tank. The outlet of each experimental tank was connected to a swirl separator (Aqua Optima AS, column height 44 cm; diameter 24.5 cm), with a detachable glass bottle at the bottom to collect faeces and quantify the spilled feed pellets from each tank. The water flow rate to each experimental unit was regulated at 7 ± 0.05 L/min using a magnetic inductive flow sensor (SM 6000, IFM electronic, Essen, Germany).

Table 2
Diet composition and analysed nutrient content of experimental diets.

	Low starch		High starch	
	Low NSP	High NSP	Low NSP	High NSP
Inclusion level of test ingredients (in g/kg as is)				
Gelatinised wheat flour	0	0	200	200
Soya hulls	0	50	0	50
Wheat bran	0	50	0	50
Basal mixture (see Table 1)	1000	900	800	700
Analysed nutrient content (in g/kg DM)				
Dry matter (DM; g/kg)	954	963	925	936
Crude protein	585	544	474	428
Crude fat	190	179	154	142
Ash	113	107	90	85
Total carbohydrate	113	170	283	345
Starch + sugar	30	49	237	249
Non-starch polysaccharides	82	121	46	96
Phosphorus	17.4	16.2	14.1	12.9
Calcium	17.6	16.2	14.0	12.8
Magnesium	2.1	2.2	1.6	1.8
Yttrium	0.19	0.18	0.16	0.14
Energy (kJ/g DM)	23.2	22.7	22.2	21.6

DM- dry matter; NSP- non-starch polysaccharides.

Water quality parameters were monitored daily. The conditions regarding the water quality parameters were identical in both phases except for the photoperiod. Temperature and dissolved oxygen were measured by hand-held digital probe (WTW Multi 3630 IDS - FDO 925) in the outlet water of randomly selected tanks (swirl separator connected to holding tanks). pH and electrical conductivity (WTW Multi 3630 IDS – Sentix 940) were measured in the water sample taken from the sump. During phase I of the experiment with rainbow trout and Arctic charr water quality parameters were as follows: temperature (mean 14.0 °C, range 13.5 to 14.6 °C), dissolved oxygen (mean 8.6 mg/L, range 7.0 to 10.1 mg/L), pH (mean 7.5, ranging from 7.2 to 7.9) and electrical conductivity (3.5 milisiemens/cm (mS/cm), ranging between 2.3 and 4.5 mS/cm). During phase II of the experiment with Atlantic salmon water quality parameters were as follows: temperature (mean 13.7 °C, range 13.2–14.5 °C), dissolved oxygen (mean 9.9 mg/L, range 9.4 to 10.3 mg/L), pH (mean 7.7, ranging from 7.2 to 7.9) and electrical conductivity (3.0 mS/cm, ranging between 2.7 and 3.3 mS/cm). Other water quality parameters such as total ammonia nitrogen (TAN), (Merck, Aquamerck Colorimetric Ammonium test), NO_2-N (Merck Aquamerck, Colorimetric Nitrite test), NO_3-N concentrations (Merck MQuant Nitrate test strips) were maintained below the pre-set range <2 mg/L, <1 mg/L, <80 mg/L, respectively. Water was refreshed to keep the nitrate levels <80 mg/L. Light intensity was set at 200 lx, and a photoperiod regime of 12:12 h (light: dark), with lights turning on and off at 8:00 and 20:00 h, was followed in phase I for rainbow trout and Arctic charr, while in phase II for Atlantic salmon, a 24 h light period of 200 lx was maintained.

2.3. *Experimental procedures and sampling*

Feeding was done restrictively to keep the amount of feed delivered on dry matter (DM) basis per fish within a species equal. A feeding level at about 90% of expected satiation was applied based on the mean initial weight per species over all diets. Due to species-specific variation in feed intake, feeding at 90% of expected satiation corresponded to a predicted feeding rate of 12 $g/kg^{0.8}$ BW/day, 10 $g/kg^{0.8}$ BW/day and 8 $g/kg^{0.8}$ BW/day for rainbow trout, Atlantic salmon and Arctic charr respectively. Daily feed ration per tank was increased based on an expected growth using an FCR of 0.9 for all diets. The daily feed portions were split into two equal portions and fed at 9:00 h and 15:00 h. On the first day of the experiment, feeding was done at 50% of the intended ratio and was increased to 100% over the next two days. Fish were hand-fed, and feeding was completed within 1 h. Fifteen minutes after feeding, uneaten/spilled feed pellets were determined by checking bottles attached to the swirl separators. Mortality was checked twice daily before feeding.

For quantifying the dry matter digestibility, the apparent digestibility coefficient (ADC) of nutrients and the faecal removal efficiency, faeces were collected by settling during final week of the experiment (Meriac et al., 2014; Prakash et al., 2024). Collection time was kept at 48 h for rainbow trout and 120 h for Arctic charr and Atlantic salmon. Feed intake was lower in case of Atlantic salmon and Arctic charr, and thus to have adequate quantity of faecal sample collected for analytical purposes, a longer faecal collection period was required. To avoid bacterial degradation of faeces, bottles attached to the swirl separators were submerged in ice slurry. Faecal samples were pooled per tank and stored at −20 °C until further analysis. For determining faecal particle size distribution (PSD) under both a stressed and non-stressed scenario, samples were collected in bottles attached to swirl separators during the final week of the experiment. Sample collection began after ensuring the left over pellets in the fish tank or swirl separator were removed and was done for 3 h between 10:30 h and 13:30 h. The collected faeces were kept on ice till further analysis. A 100 g feed subsample was pooled for each diet weekly and used for feed composition analysis.

2.4. Analytical methods

The analytical methods used in this trial were similar to that described by Prakash et al. (2023). To assess digestibility and faecal removal efficiency, faecal samples were dried to a constant weight in an oven at 70 °C. The dried faecal samples were ground (Retsch ZM, 200; Retsch GmbH, Haan, Germany) before the analysis. The feed and faeces were analysed for chemical composition as per standard methods. Dry matter was estimated by drying the samples to constant mass at 103 °C (ISO 6496, 1999). Ash was determined gravimetrically by incinerating the samples in a muffle furnace for 4 h at 550 °C (ISO 5984, 1978). The ash fraction was dissolved in concentrated sulphuric acid by autoclaving (121 °C, 20 min) to determine phosphorus, calcium, magnesium and yttrium by inductively coupled plasma optical emission spectrometry, following Dutch analytical standards (NEN 15510:2017). The crude protein was determined based on nitrogen content of samples by Kjeldahl-method (ISO 5983, 1997). Crude fat was determined gravimetrically using acid hydrolysis followed by extraction with petroleum-ether (Soxhlet method; ISO 6492, 1999). Gross energy was measured by bomb calorimetry, by direct combustion (C7000; IKA®-Werke GmbH & Co. KG). Starch, including free sugar fraction in feed and faeces were determined enzymatically using amyloglucosidase without ethanol extraction for removing free sugars (Goelema et al., 1998).

The faecal PSD was measured using different mesh size sieves (1600 μm, 850 μm, 300 μm, 100 μm and 40 μm) following the protocol described in Prakash et al. (2023). PSD was determined for undisturbed faecal waste (hereafter termed non-stressed faeces) and faeces exposed to mechanical stress (hereafter termed stressed faeces). To expose faeces to mechanical stress, faeces collected in the bottle attached to the swirl separator were poured thrice through a 1-m-long PVC pipe held at a 40-degree slope. Change in faecal PSD following exposure to mechanical stress provided an estimate of faecal stability/consistency. To determine the mass of collected organic matter (OM) fractions on filters, filters were dried and incinerated as described for feed and faeces samples. Faecal PSD data was expressed on mass % basis (on organic matter basis) for each fraction for non-stressed and stressed faecal waste.

2.5. Calculations

Absolute weight gain (WG, g/fish) was estimated as the difference between the mean individual final (W_f) and initial (W_i) body weight per fish. Feed intake per fish per day (FI, g/fish/d) was calculated using the formula:

$$FI = \frac{\text{Total DM feed offered each day} - \text{Uneaten DM feed each day}}{\text{Fish number}}$$

FI was summed for the whole period to obtain feed intake per fish over the entire experimental period (FI_{tot}).

Specific growth rate (SGR; %BW/d) was calculated as:

$$SGR = \frac{[ln(W_f) - ln(W_i)]*100}{t}$$

where t is the duration of trial in days.

The feed conversion ratio (FCR) on dry matter basis was calculated as:

$$FCR = \frac{FI_{tot}\ (g\ DM/fish)}{WG\ (g/fish)}$$

The dry matter ADC of diets was calculated as follows:

$$ADC_{DM}(\%) = 100*[1 - (Y_{diet}/Y_{faeces})]$$

where Y_{diet} and Y_{faeces} is the concentration of yttrium in diet and faeces respectively expressed on DM basis.

The ADC of macronutrients and macro-minerals of diets were calculated according to the formula described by Cheng and Hardy (2002):

$$ADC\ (\%) = 100*[1 - (Y_{diet}*N_{faeces})/(Y_{faeces}*N_{diet})]$$

Where N_{diet} and N_{faeces} represent the nutrient percentage (g/kg DM or kJ/g DM gross energy) of the diet and faeces respectively.

Carbohydrate content in feed and faeces was determined by the difference as [1000 – (crude protein + fat + ash)]. NSP level is calculated as the difference between the carbohydrate and starch content.

Faecal waste production (g DM/kg DM FI) was calculated on dry matter basis as the amount of non-digested feed per kilogram feed intake as:

$$\text{Faecal waste production}\ (g\ DM/Kg\ DM\ FI) = (100\% - ADC_{DM})*1000$$

Faecal removal efficiency (FR, %) was estimated as the percentage of total faeces collected by settling in proportion to total faecal waste produced (Prakash et al., 2024). In detail, this was calculated based on yttrium collected in settled faeces ($Y_{removed}$, g) in relation to the amount of yttrium supplied by diet (Y_{diet}, g) as:

$$FR\ (\%) = (Y_{removed}/Y_{diet})*100\%$$

Non-removed faeces (g DM/kg DM FI) was calculated as the difference between the total amount of faeces produced and faeces removed as:

$$\text{Non} - \text{removed faeces} = [(100\% - ADC_{DM})*(100\% - FR)]*1000$$

2.6. Statistical analysis

Tanks were used as the experimental unit ($n = 12$) for analysing the data separately for each species. Normality of data and equality of variance was assumed. A two-way ANOVA was used to investigate the effect of starch level, NSP level and their interaction. In the case of a significant interaction effect ($p < 0.05$), a Tukey HSD test (honest significant difference; 95% significance) was performed to compare treatment means. Statistical analyses were performed by using the statistical program SPSS Statistics 27 (IBM, New York, United States of America). Since, standard deviation for most parameters in Arctic charr was high due to high variability in replicates in comparison to the other two species, this might distort the result of statistical analysis if considering species as a factor. Hence, in the main paper we are only presenting the effect within species. For completeness, the results of statistical analysis with species as a factor are presented in the supplementary material (Supplementary Table S3 to S5) .

3. Results

3.1. Rainbow trout

Survival was 100% and fish weight more than doubled during the experimental period. No interaction effect between starch and NSP content of the diet was observed for the performance parameters (Table 3; $P > 0.05$). Both high starch and high NSP content in the diet reduced growth ($P < 0.05$) and increased FCR ($P < 0.001$). The DM, crude protein and crude fat ADC were influenced by the interaction between starch and NSP content of diet ($P < 0.05$; Table 4). The inclusion of starch and NSP at high levels in the diet reduced the crude protein and crude fat digestibility. At a low starch level, the high NSP content of the diet lowered the crude protein and crude fat digestibility by 0.8% and 1.4%, respectively on an absolute basis. In contrast, at the high starch level, the impact of the NSP content of the diet in reducing crude protein and crude fat digestibility nearly doubled to 1.5% and 2.5%, respectively. Concerning starch digestibility, increasing the starch and NSP content of the diet had the opposite effect. High starch levels in the diet increased the starch digestibility by almost 8%, while high NSP content of the diet reduced the starch digestibility by 8%.

The amount of faecal waste produced was influenced by the interaction effect of starch and NSP supplementation ($P < 0.001$; Fig. 1a). At low NSP levels, starch inclusion did not alter faecal waste production. Inclusion of NSP increased waste production, being the highest at the high starch high-NSP diet ($P < 0.001$). For most other faecal waste characteristics (faecal removal efficiency; PSD of non-stressed faeces; amount of non-removed faeces) the interaction effect was not significant. Increasing dietary starch content, reduced faecal removal efficiency by 11.3% ($P < 0.001$, Fig. 2a). NSP inclusion in the diets increased the removal efficiency by 4.4% ($P < 0.05$, Fig. 2a), which fully compensated for the increased faecal waste produced. The amount of non-removed faeces was unaffected by NSP inclusion ($P > 0.05$, Fig. 3a), whereas dietary starch inclusion doubled the non-removed faeces ($P < 0.001$, Fig. 3a). Both starch and NSP inclusion significantly altered PSD of non-stressed faeces ($P < 0.05$, Fig. 5a, Supplementary Table S1). Starch inclusion reduced the size of faecal particles, while NSP inclusion increased it (Plate 1a, Supplementary Table S1). Exposing faeces to mechanical stress reduced the particle size, but this reduction was affected by an interaction effect between starch and NSP inclusion (Figs. 4a, 5a and Supplementary Table S1). After stress exposure, the amount of large faecal particles (>1600 μm) was lower at the HS-diets, but the difference between LS and HS-diets was largest in the diets without NSP inclusion (Fig. 4a).

3.2. Atlantic salmon

Survival was 100% and fish weight more than doubled during the experimental period. The interaction effect between dietary starch and NSP content influenced the weight gain and FCR of Atlantic salmon ($P < 0.01$; Table 3). NSP content of diet increased FCR depending on the starch content of the diet, with the effect being two-fold larger in the high starch diet than in the low starch diet (Table 3). No interaction effect between starch and NSP content of diet was observed for crude protein and crude fat digestibility ($P > 0.05$; Table 4). Both starch and NSP content of diet reduced the crude protein and crude fat digestibility ($P < 0.001$; Table 4), however the impact of NSP content of diet was two-

Table 3

Effect of experimental diets on growth performance of rainbow trout (RT), Atlantic salmon (AS) and Arctic charr (AC) fed restrictively during the experimental period (42 days for RT and AS, 49 days for AC).

	Low starch		High starch		SEM	Starch	NSP	Starch × NSP
	Low NSP	High NSP	Low NSP	High NSP				
Rainbow trout								
Survival (%)	100	100	100	100				
Initial body weight (g/fish)	82	81	82	80	1.9	ns	ns	ns
Final body weight (g/fish)	212	202	204	194	3.0	*	*	ns
weight gain (g/fish)	130	121	122	114	1.4	***	***	ns
feed intake (g DM/fish)	87	87	88	87	0.0			
SGR (%/d)	2.26	2.17	2.18	2.11	0.03	*	*	ns
FCR (DM basis)	0.67	0.72	0.72	0.77	0.008	***	***	ns
Atlantic salmon								
Survival (%)	100	100	100	100				
Initial body weight (g/fish)	39	39	39	39	0.9	ns	ns	ns
Final body weight (g/fish)	88	86	84	79	1.0	***	**	ns
weight gain (g/fish)	50^{d}	47^{c}	45^{b}	41^{a}	0.2	***	***	**
feed intake (g DM/fish)	34	34	34	34	0.0			
SGR (%/d)	1.97	1.90	1.83	1.72	0.03	***	***	ns
FCR (DM basis)	0.68^{a}	0.72^{b}	0.75^{c}	0.83^{d}	0.003	***	***	***
Arctic charr								
Survival (%)	100	100	100	100				
Initial body weight (g/fish)	70	71	70	70	1.2	ns	ns	ns
Final body weight (g/fish)	134	139	121	120	4.2	**	ns	ns
weight gain (g/fish)	63	68	51	50	3.5	**	ns	ns
feed intake (g DM/fish)	49	55	43	45	2.6	*	ns	ns
SGR (%/d)	1.30	1.38	1.11	1.09	0.053	**	ns	ns
FCR (DM basis)	0.78	0.81	0.86	0.90	0.014	***	***	ns

DM- dry matter; SGR- specific growth rate; FCR- feed conversion ratio (on DM basis); NSP- non-starch polysaccharides. Values are means ($n = 3$) and standard error of the means (SEM). ns- not significant, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; means along a row lacking a common superscript letter differ significantly, $P < 0.05$.

Table 4
Apparent digestibility coefficient (ADC, %) of nutrients in rainbow trout (RT), Atlantic salmon (AS) and Arctic charr (AC) fed experimental diets during the experimental period (42 days for RT and AS, 49 days for AC).

	Low starch		High starch					
	Low NSP	High NSP	Low NSP	High NSP	SEM	Starch	NSP	Starch × NSP
Rainbow trout								
Dry matter	84.7^{c}	79.5^{b}	85.1^{c}	78.1^{a}	0.15	*	***	***
Organic matter	88.2^{c}	81.8^{b}	87.6^{c}	79.5^{a}	0.16	***	***	***
Crude protein	96.7^{c}	95.9^{b}	95.5^{b}	94.0^{a}	0.10	***	***	*
Crude fat	97.0^{c}	95.6^{b}	95.9^{b}	93.4^{a}	0.09	***	***	***
Ash	48.7	47.8	50.9	49.3	0.35	***	**	ns
Carbohydrate	37.8^{b}	29.8^{a}	72.7^{d}	59.1^{c}	0.72	***	***	**
Starch + sugar	86.1^{b}	74.7^{a}	91.5^{c}	85.7^{b}	0.37	***	***	***
Energy	92.3^{d}	87.4^{b}	90.9^{c}	84.2^{a}	0.10	***	***	***
Phosphorus	55.7	56.8	58.4	59.7	0.26	***	**	ns
Calcium	10.4	11.2	15.5	16.6	0.77	***	ns	ns
Magnesium	29.4	29.9	36.1	38.9	0.71	***	*	ns
Atlantic salmon								
Dry matter	85.3^{d}	79.5^{b}	83.8^{c}	77.3^{a}	0.10	***	***	**
Organic matter	89.3^{d}	82.8^{b}	87.0^{c}	79.8^{a}	0.12	***	***	*
Crude protein	96.8	95.6	96.2	94.8	0.11	***	***	ns
Crude fat	97.6	95.7	95.6	93.5	0.31	***	***	ns
Ash	53.4	52.2	51.7	50.0	0.27	***	***	ns
Carbohydrate	36.5^{b}	28.2^{a}	66.8^{d}	55.5^{c}	0.25	***	***	***
Starch + sugar	76.4^{b}	67.3^{a}	83.5^{d}	78.3^{bc}	1.46	***	**	ns
Energy	92.7^{d}	87.5^{b}	90.3^{c}	83.5^{a}	0.20	***	***	**
Phosphorus	56.2^{a}	57.0^{ab}	58.9^{c}	58.0^{bc}	0.24	***	ns	**
Calcium	11.4	10.0	9.7	7.9	0.68	*	*	ns
Magnesium	39.2	40.6	37.7	36.7	0.43	ns	ns	ns
Arctic charr								
Dry matter	80.1	76.7	81.2	74.7	1.23	ns	**	ns
Organic matter	84.5	80.5	84.8	77.9	1.32	ns	**	ns
Crude protein	94.4	93.7	92.1	92.5	0.69	*	ns	ns
Crude fat	94.7	93.3	92.5	91.2	0.67	*	ns	ns
Ash	45.6	45.0	44.3	40.2	0.90	**	*	ns
Carbohydrate	15.7	24.3	68.4	54.4	6.36	***	ns	ns
Starch + sugar	80.3	80.6	89.7	84.5	3.83	ns	ns	ns
Energy	88.0	84.4	87.2	81.5	1.12	ns	**	ns
Phosphorus	50.9	53.0	53.6	54.6	0.62	**	*	ns
Calcium	11.2^{ab}	11.8^{ab}	12.4^{b}	7.7^{a}	0.98	ns	*	*
Magnesium	9.8	15.3	13.3	15.3	2.29	ns	ns	ns

NSP- non-starch polysaccharides; values are means (n = 3) and standard error of the means (SEM); ns- not significant, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; means along a row lacking a common superscript letter differ significantly, $P < 0.05$.

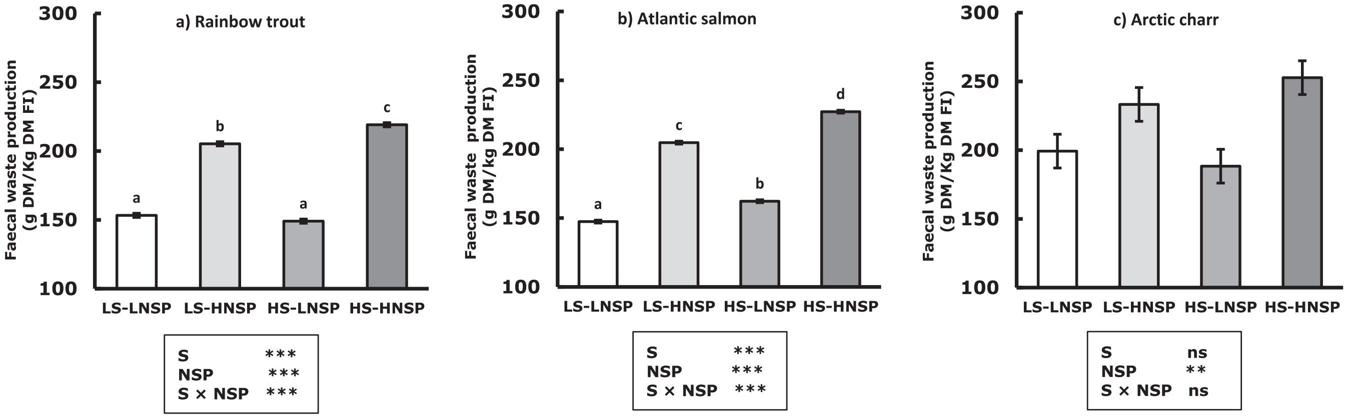


Fig. 1. Effect of inclusion of dietary starch and NSP on faecal waste production (g DM/kg DM FI) in (a) rainbow trout, (b) Atlantic salmon and (c) Arctic charr fed restrictively during the experimental period. S- starch; NSP- non-starch polysaccharides; DM- dry matter; FI- feed intake; LS-LNSP- low starch and low NSP diet; LS-HNSP- low starch and high NSP diet; HS-LNSP- high starch and low NSP diet; HS-HNSP- high starch and high NSP diet; different superscripts labelled above the bars indicate dietary differences for faecal waste production when an interaction effect was observed between starch and NSP sources in the diet; error bars indicate standard error of the means; box below each graph indicates the dietary differences for starch, NSP levels and their interaction; ns- not significant, $P > 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

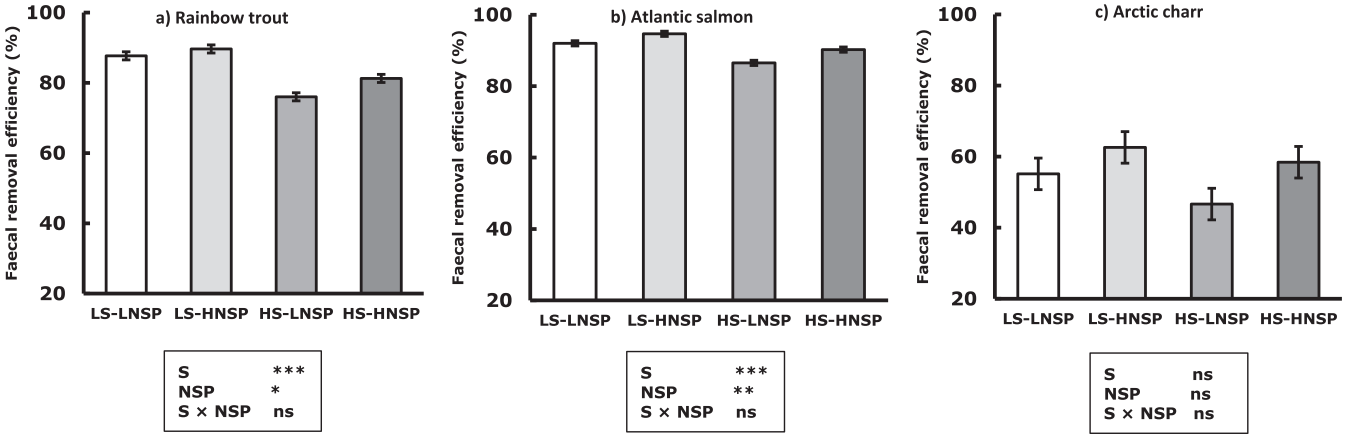


Fig. 2. Effect of inclusion of dietary starch and NSP on faecal removal efficiency (%) in (a) rainbow trout, (b) Atlantic salmon and (c) Arctic charr fed restrictively during the experimental period; S- starch; NSP- non-starch polysaccharides; DM- dry matter; LS-LNSP- low starch and low NSP diet; LS-HNSP- low starch and high NSP diet; HS-LNSP- high starch and low NSP diet; HS-HNSP-high starch and high NSP diet; error bars indicate standard error of the means (SEM); box below each graph indicates the dietary differences for starch, NSP levels and their interaction. ns- not significant, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

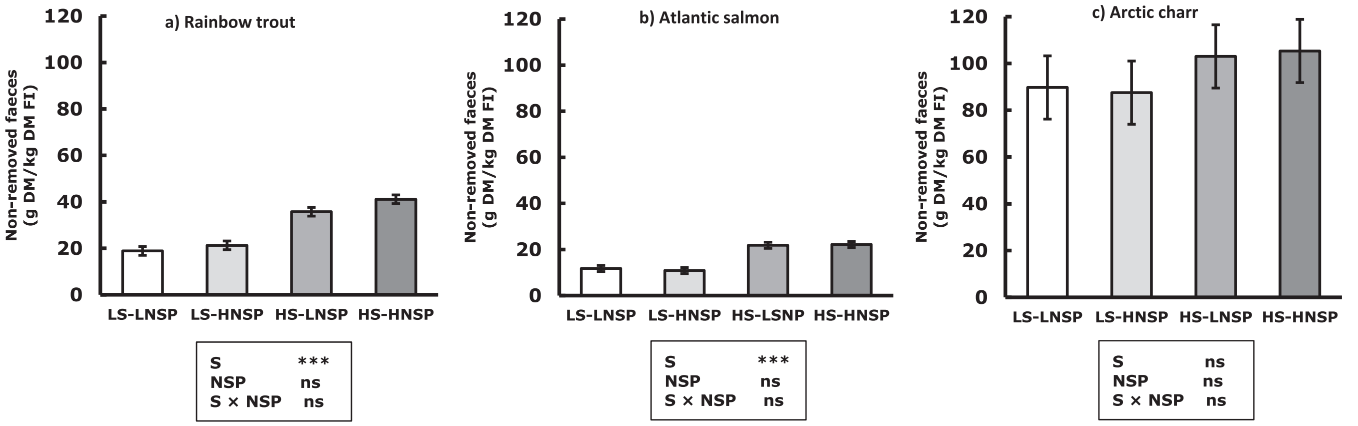


Fig. 3. Effect of dietary inclusion of starch and NSP on non-removed faeces (g DM/kg DM FI) in (a) rainbow trout, (b) Atlantic salmon and (c) Arctic charr fed restrictively during the experimental period; S- starch; NSP- non-starch polysaccharides; DM- dry matter; FI- feed intake; LS-LNSP-low starch and low NSP diet; LS-HNSP- low starch and high NSP diet; HS-LNSP- high starch and low NSP diet; HS-HNSP- high starch and high NSP diet; error bars indicate standard error of the means (SEM); box below each graph indicates the dietary differences for starch, NSP levels and their interaction; ns- not significant, $P > 0.05$; ***, $P < 0.001$.

fold larger than that of starch content of diet.

The amount of faecal waste produced was influenced by the starch and NSP content of the diet and their interaction ($P < 0.01$; Fig. 1b). Both high starch and high NSP content of diet increased the faecal waste production (Fig. 1b). The high NSP diet had four-fold higher waste production compared to high starch diet. Averaged over all 4 diets, the faecal removal efficiency in Atlantic salmon was 91% (Fig. 2b). NSP inclusion in the diet increased the faecal removal efficiency by 3%, compensating for the increased faecal waste production. Accordingly, the amount of non-removed faeces was unaffected by NSP inclusion ($P > 0.05$; Fig. 3b), while dietary starch inclusion, resulted in a doubling of the non-removed faeces ($P < 0.01$; Fig. 3b). No interaction effect was observed between the starch and NSP content of the diet for faecal particle size classes ($P > 0.05$, Figs. 4b, 5b and Supplementary Table S1). From visual observation of faeces in bottle, HS-low NSP diet appeared to have largest share of faecal fines (Plate 1b). Dietary starch inclusion increased the proportion of small-size faecal particles (<40 μm) and reduced large size faecal particles (>1600 μm) under both scenarios i.e. without and with exposure to mechanical stress ($P < 0.01$; Fig. 5b). In contrast, NSP inclusion reduced the proportion of smaller-size (< 40 μm) faecal particles ($P < 0.05$; Fig. 5b) under both scenarios.

3.3. *Arctic charr*

Survival was 100% and the average body weight almost doubled during the experimental period. No interaction effect between starch and NSP content of the diet was observed for growth, FCR and nutrient digestibility (except for Ca) ($P > 0.05$, Tables 3, 4). In general, nutrient digestibility values in Arctic charr were lower than in Atlantic salmon and rainbow trout (Table 4). Highest (94.4%) and lowest (92.1%) crude protein ADC were observed for low starch low-NSP and high starch low-NSP diets respectively. Low starch low-NSP diet had highest crude fat ADC (94.7%), whereas the lowest crude fat ADC (91.2%) occurred in high starch high-NSP diet. High starch inclusion in the diet reduced crude protein and crude fat digestibility by 1.8% and 2.2%, respectively, whereas high NSP inclusion had no significant effect (Table 4).

The amount of faecal waste produced, removal efficiency and non-removed faeces were unaffected by the interaction between starch and NSP supplementation ($P > 0.05$, Figs. 1c, 2c, 3c). High NSP inclusion increased faecal waste production. Averaged over all the diets, faecal removal efficiency was estimated to be 56%. The amount of non-removed faeces in Arctic charr was 3 to 6 times larger than in rainbow trout and Atlantic salmon, respectively. Dietary composition had no impact on amount of non-removed faeces but this was due to a much higher variability between replicates compared to rainbow trout and

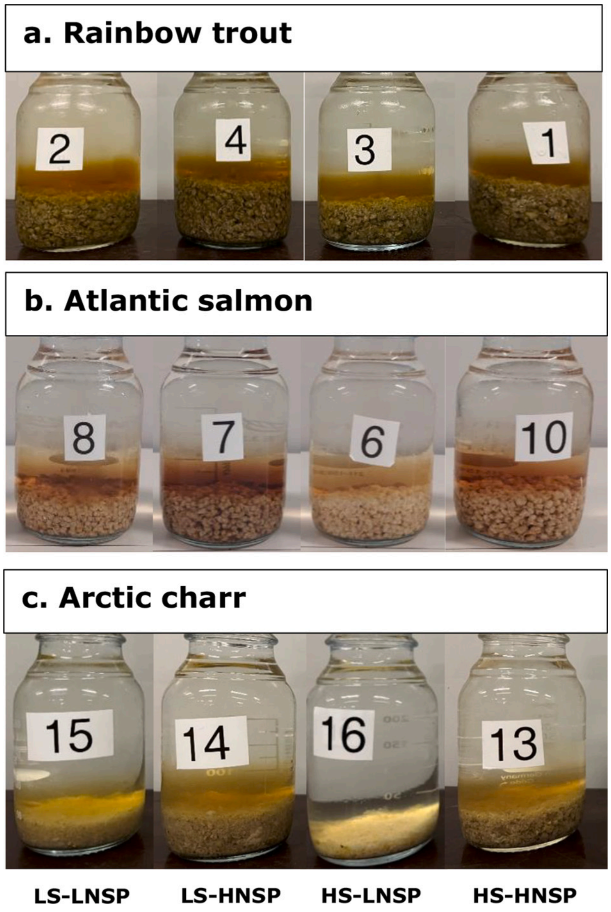


Plate 1. Overnight collected faeces of rainbow trout (a), Atlantic salmon (b) and Arctic charr (c) fed restrictively during the experimental period. Bottles from left to right for each species represent faeces collected from fish fed with low starch and low NSP diet (LS-LNSP), low starch and high NSP diet (LS-HNSP), high starch and low NSP diet (HS-LNSP), and high starch and high-NSP diet (HS-HNSP), respectively.

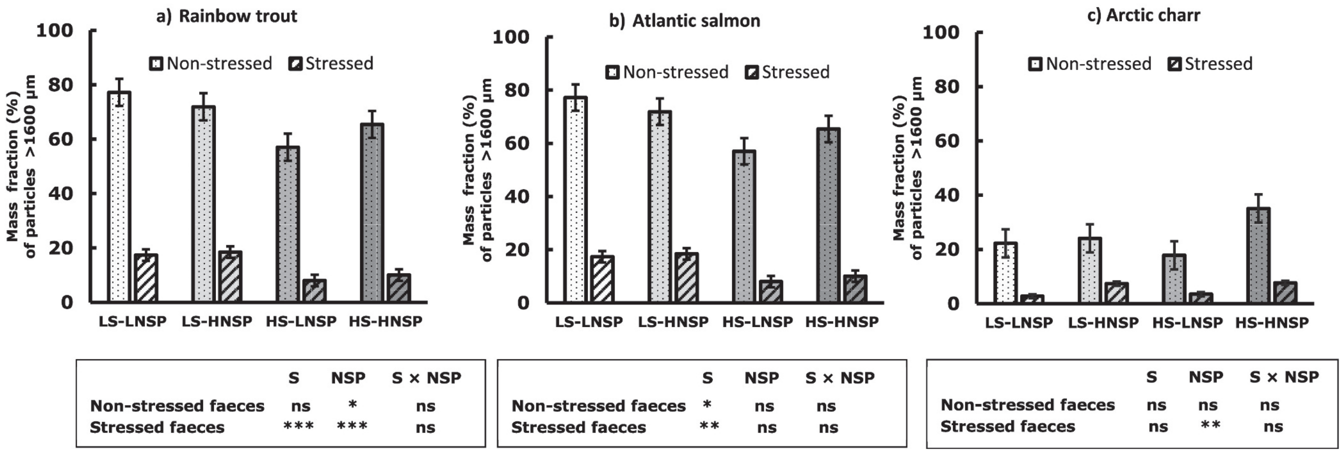
Atlantic salmon. Visual observation of faeces collected in bottles indicated that the majority of faeces consisted of fine faecal particles (Plate 1c). No faecal pellets or strings were observed in contrast to rainbow trout and Atlantic salmon. The fraction of faecal particles <40 μm under both stressed and non-stressed scenarios increased at low NSP levels when starch level in the diet was high, whereas at high NSP levels, this effect was not evident. This resulted in an observed interaction effect between starch and NSP content of the diet for faecal particles <40 μm ($P < 0.05$, Fig. 5c). Arctic charr had the highest faecal waste production, the lowest faecal removal efficiency and the largest amount of non-removed faeces among the 3 salmonid species investigated in this study (Figs. 1, 2, & 3).

4. Discussion

Comparative evaluation of faecal characteristics across fish species is challenging due to confounding variables such as diet composition, feed production parameters, tank hydrodynamics, water velocity and faecal removal technique used in different studies. Tank hydrodynamics, in particular, can affect the residence time of faecal matter in tanks, potentially impacting measured variables such as faecal size, stability and removal efficiency. The current study is a unique attempt to assess the interspecies variability in faecal characteristics of 3 different salmonid species (rainbow trout, Atlantic salmon, and Arctic charr) when feeding identical diets produced in a single batch and assessed using an identical set-up with similar tank shapes and hydrodynamic conditions.

In this study, our initial aim was to keep feed intake (FI) similar across the species, to avoid its impact on faecal characteristics. However, FI varied between the fish species, as quantity of feed consumed at 90% of expected satiation corresponded to varying values for different species (Table 3). In the case of Arctic charr, FI was much lower, and animals took longer than two weeks to adapt to the experimental diets. Visual observation during feeding indicated the existence of a social hierarchy, as reported earlier for this species (Bailey et al., 2000; Jobling et al., 1993), probably influencing the FI. Irrespective of the interspecies variability, the FI and growth, as observed in this study, are comparable to the previously reported values for rainbow trout (Prakash et al., 2023) Atlantic salmon (Handeland et al., 2008; Hemre et al., 2016; Sissener et al., 2021) and Arctic charr (Jobling et al., 1993; Langeland et al., 2016) of equivalent size.

The amount of faecal waste produced has consequences for the waste management measures to be implemented for species reared in open systems such as raceways and cages as well as in closed systems such as RAS (Dalsgaard et al., 2013; Pedersen et al., 2017). Waste production follows dry matter digestibility, a function of the proportion of nutrients

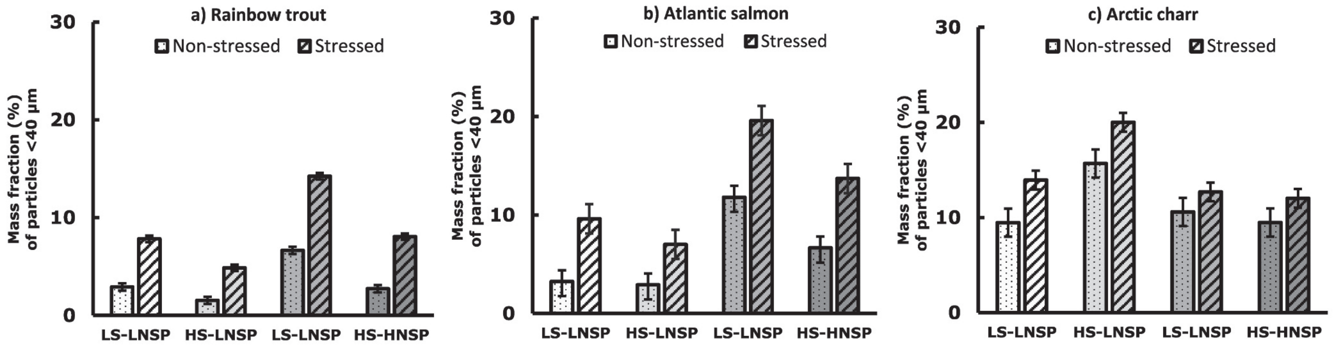


	S	NSP	S × NSP
Non-stressed faeces	ns	*	ns
Stressed faeces	***	***	ns

	S	NSP	S × NSP
Non-stressed faeces	*	ns	ns
Stressed faeces	**	ns	ns

	S	NSP	S × NSP
Non-stressed faeces	ns	ns	ns
Stressed faeces	ns	**	ns

Fig. 4. Effect of inclusion of starch and NSP on mass fraction (%) of particles >1600 μm in non-stressed and stressed faeces of rainbow trout (a), Atlantic Salmon (b), and Arctic charr (c) fed restrictively during the experimental period; LS-LNSP-low starch and low NSP diet; LS-HNSP- low starch and high NSP diet; HS-LNSP- high starch and low NSP diet; HS-HNSP- high starch and high NSP diet; error bars are standard error of the means (SEM); box below each graph indicates the dietary differences for starch, NSP levels and their interaction. ns, not significant $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.



	S	NSP	S × NSP
Non-stressed faeces	***	***	**
Stressed faeces	***	***	***

	S	NSP	S × NSP
Non-stressed faeces	***	*	ns
Stressed faeces	***	*	ns

	S	NSP	S × NSP
Non-stressed faeces	*	*	**
Stressed faeces	*	**	**

Fig. 5. Effect of inclusion of starch and NSP sources in the diet on mass fraction (%) of particles <40 μm in non-stressed and stressed faeces of rainbow trout (a), Atlantic Salmon (b), and Arctic charr (c) fed restrictively during the experimental period; error bars are standard error of the means (SEM); LS-LNSP-low starch and low NSP diet; LS-HNSP- low starch and high NSP diet; HS-LNSP- high starch and low NSP diet; HS-HNSP- high starch and high NSP diet; box below each graph indicates the dietary differences for starch, NSP levels and their interaction. ns, not significant $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

in the diet and their corresponding nutrient digestibility values. Adding NSP sources (soya hulls and wheat bran) to the basal diet increased waste production in all three salmonids investigated in this study. NSP sources used in this study are mainly rich in insoluble fractions. Nearly all fibre in wheat bran exists as insoluble fraction (Marlett, 1990; Stevenson et al., 2012) whereas in case of soy hull the insoluble fraction constitutes about 80% of total dietary fibre fraction (Cole et al., 1999; Faryadi et al., 2023). An increase in faecal waste production with inclusion of NSP sources in the diet was expected as the NSP fraction is largely indigestible. An additional reason for the increased waste production with inclusion of an NSP source might also be related to a decline in crude protein and crude fat digestibility (Table 4). On the contrary, the inclusion of gelatinised wheat flour in the diet increased waste production in the case of Atlantic salmon and rainbow trout but not in the case of Arctic charr. It is hard to speculate the exact reason for this differential impact of starch in affecting the faecal waste production between the species, but in part this could be related to the high degree of variability between the replicates in the case of Arctic charr. The increase in faecal waste production with starch inclusion in case of rainbow trout and Atlantic salmon was not a consequence of the decline in starch digestibility (Table 4). One may find it surprising that starch digestibility did not decline following increased dietary starch levels. This may be related to the fact that starch digestibility depends not only on the inclusion level of starch but also on the form (gelatinised vs non-gelatinised) in which starch is present in the diet. In the low starch diets, the starch fraction is derived from protein sources (wheat gluten, pea protein concentrate, soya protein concentrate and krill meal) and from insoluble-NSP sources (wheat bran and soya hulls), which may be difficult to digest in comparison to starch derived from gelatinised wheat flour in the high starch diets (Table 2). The decline in crude protein and fat digestibility with high starch inclusion in the diet (Table 4) in all 3 fish species investigated in this study is in line with earlier observations in various fish species, such as rainbow trout (Prakash et al., 2024), Atlantic salmon (Glencross et al., 2004) and yellowtail king fish, *Seriola lalandi* (Horstmann et al., 2024). The insoluble-NSP inclusion in the diet are suggested to increase the gut evacuation rate in case of pigs (Wilfart et al., 2007). Increased bulk of digesta in response to insoluble dietary fibre inclusion in the gut may exert a direct physical action increasing the peristaltic action and propulsive movement of digesta in the different sections of intestine (Wenk, 2001). Faster gut evacuation would reduce the time for interaction between digestive enzymes and substrate and the time available for absorption of digested nutrients. This might be a probable reason for the observed effect of NSP inclusion in the diet reducing crude protein and crude fat digestibility. However, in a trial with European sea bass (*Dicentrarchus labrax*) inclusion of an increasing level of dietary fibre did not influence the time required to empty 90% of hindgut contents (Bonvini et al., 2018). In the current study, Arctic charr had the lowest dry matter digestibility, which corresponds to the largest amount of faecal waste produced per kg of feed fed (Table 3 and Fig. 1a). The increased faecal waste production and thus lower dry matter digestibility in Arctic charr compared to rainbow trout and Atlantic salmon was related to the lower digestibility of nutrients such as crude protein, crude fat, carbohydrates and ash (Table 3). Nevertheless, crude protein and fat digestibility, as observed for Arctic charr in this study, are much higher than the values reported in the literature for this species. Most of the earlier studies in Arctic charr were conducted at lower temperatures (Abro et al., 2014 (10 °C); Carlberg et al., 2018 (8.5 °C); Langeland et al., 2016 (10 °C); Ringø, 1991 (8 °C)) and the faecal samples for digestibility measurements were collected by stripping or dissection. These interstudy differences might account for the higher dry matter and nutrient digestibility in the current study (faeces collection by sedimentation and rearing temperature of 14 °C) than in comparison to earlier studies. Additionally, the anatomical and physiological differences, such as the number of pyloric caeca (Bergot et al., 1981; Nilsson and Filipsson, 1971) and the activity of digestive enzymes (Krogdahl et al., 2004) among the fish species may also account for the differences in digestibility between species. In the current study, the largest differences in nutrient digestibility between rainbow trout and Atlantic salmon, occurred for starch while crude protein and crude fat digestibility also differed but much less than starch (Krogdahl et al., 2004).

The amount of faecal waste accumulating in RAS depends on the quantity of faecal waste produced and the efficiency of removal. The faecal removal efficiency values averaged over all 4 diets ranged from 56% (Arctic charr), 84% (rainbow trout) to 91% (Atlantic salmon). This demonstrates considerable variability in the faecal characteristics between different salmonids. The species' effect on faecal removal efficiency was consistent for all 4 diets. Atlantic salmon always had the highest faecal removal efficiency values for all diets, followed by rainbow trout and Arctic charr. The underlying reasons for this variation between the salmonids are hard to speculate. One point of consideration may be that all the diets in this study were largely plant-based, and there may exist a difference between the species for their sensitivity and response mechanisms to plant-derived nutrients (Urán, 2008). For example, when fed a 1:1 mixture of natural ingredients (krill, sand eel, smelt and squid) and a commercial diet, the yellowtail kingfish, a highly carnivorous species, had an improved faecal consistency and removal efficiency than feeding a commercial diet alone (Horstmann et al., 2023a). There are few previous reports regarding the observation of faeces of poor consistency in arctic charr under captivity when fed with yeast based diets (Vidakovic et al., 2016) and commercial plant protein based diets (Noble et al., 2005). Accordingly, investigating if the species-specific variability in faecal characteristics exists in wild salmonid populations feeding on natural diets would be interesting. Other underlying reasons could be factors related to anatomical or morphological variability in the gut or differences in the gut evacuation rate and faecal moisture content between the species. Differences in moisture content in faeces collected from the distal intestine have been noted earlier in rainbow trout and Atlantic salmon (Refstie et al., 2000). Unfortunately, no such information is available in the literature for Arctic charr. However, in a recent study in our group with yellowtail kingfish, we failed to observe a correlation between the faecal moisture content and the faecal removal efficiency (Manuscript in preparation). Based on that finding, differences in faecal moisture content does not seem to be the likely reason for the differences in faecal removal efficiency between the species. The experiment with yellowtail kingfish was conducted in salt water at 34 ppt and other factors than the faecal moisture content might be influencing faecal removal efficiency. In sedimentation-based faecal waste removal, as used in this study, the size and density of faecal particles are the major determinants of their removal rate. Our observation of the lowest faecal removal efficiency in the case of Arctic charr relates to the visual observation of their faeces in the bottles (Plate 1) and the particle size distribution (PSD) data under both stressed and non-stressed conditions (Figs. 4c, 5c). Arctic charr had an almost 3 times greater proportion of faecal fines (faecal particles <40 μm size) in comparison to rainbow trout and Atlantic salmon (Fig. 5). A similar trend was observed for faecal particles >1600 μm. Findings of the current study and our previous report in rainbow trout (Prakash et al., 2023) affirm that faecal particle size is a good indicator of faecal removal efficiency in the case of salmonids. Our observation that nearly 60% (on a mass percentage basis) of faecal particles in non-stressed faeces of Atlantic salmon are >1600 μm demonstrates that by using innovative practices such as closed containment systems (Nilsen et al., 2020), the removal of solid waste with considerable success can be achieved in case of rainbow trout and Atlantic salmon. However, it remains to be ascertained if the Atlantic salmon and rainbow trout reared in sea water produces faeces of similar consistency as that of freshwater reared ones.

Due to the synergistic effect of increased waste production and reduced faecal removal efficiency, the amount of non-removed faeces in Arctic charr was about 3 times and 6 times larger than in rainbow trout and Atlantic salmon, respectively. This difference in faecal characteristics has implications for farming practices such as the selection of solid removal technique (sedimentation vs. drum filtration), hydraulic surface load, mesh size of screen filters, etc. Additionally, if RAS switch their production from one salmonid species to another, the variability in solid waste production and removal must be considered.

Regarding the specific impact of dietary factors on faecal removal efficiency, it was evident that starch level in the diet reduced faecal removal efficiency, while the insoluble-NSP content of the diet increased it (Fig. 2a, b) in rainbow trout and Atlantic salmon. A similar trend was observed for Arctic charr, but possibly due to large variability between the replicates, these did not reveal to be significant on statistical analysis (Fig. 2c). In line with our observations, starch content of diet reduced faecal removal efficiency in yellowtail kingfish (Horstmann et al., 2023b) and African catfish, *Clarias gariepinus* (Phan et al., 2022). Whether the negative impact of an increasing starch content in the diet on faecal removal efficiency is a consequence of increased faecal starch content (Supplementary Table S2) reducing the consistency of faeces (Prakash et al., 2024) or related to the processes taking place in the gut such as motility, water flux and the residence time of digesta in different intestinal segments is still unknown. Nevertheless, faecal PSD data (Supplementary Table 1) and the visual observation of faeces (Plate 1) for each diet in the investigated species affirms that an increase in the starch content of the diet increased the proportion of faecal fines (<40 μm) while increasing the insoluble-NSP content of diet decreased the proportion of faecal fines. The faecal PSD data under non-stressed condition explains our observation regarding improved faecal removal efficiency with insoluble-NSP supplementation and a decline in the removal efficiency with starch supplementation. Similar observations were made regarding the impact of dietary sources rich in insoluble NSP on faecal removal efficiency in rainbow trout (Meriac et al., 2014) and Nile tilapia (Amirkolaie et al., 2005). Authors attributed the potential beneficial impact of NSP to its ability to increase the firmness and, thereby, the settling rate of faeces (Meriac et al., 2014; Dias et al., 1998). In the current study, inclusion of insoluble-NSP sources in diet also enhanced faeces' stability, as evident with the proportion of faecal particles <40 μm under a stressed scenario in high NSP groups; however, the interaction between starch and insoluble-NSP content appeared to influence this outcome. Despite increased waste production with insoluble NSP inclusion in all three species, the amount of non-removed faeces per kg feed stayed the same (Figs. 1 and 3). This demonstrates that improvement in faecal particle size and stability with insoluble NSP inclusion has the potential to compensate for increased waste production by influencing faecal removal efficiency. Whereas starch inclusion negatively impacted waste production, faecal particle size, stability and faecal removal efficiency, and thus, the amount of non-removed faeces almost doubled with starch inclusion in the diet for rainbow trout and Atlantic salmon. Increased accumulation of faecal waste will affect not only the performance of system components such as biofilters, UV systems and drum filters but also enhance the cost of operation and consumptive use of water (Ebeling et al., 2006; Horstmann et al., 2024).

To conclude, considerable differences exist between rainbow trout, Atlantic salmon, and Arctic charr regarding faecal waste production and faecal characteristics. High starch levels in the diet reduced faecal stability and removal efficiency in salmonids. Increasing insoluble NSP in the salmonid diet improved the size and stability of faecal particles, but it also increased the quantity of faecal waste produced. The amount of non-removed faeces accumulating in the system is unaffected by insoluble NSP inclusion in the diet.

CRediT authorship contribution statement

Satya Prakash: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Roel M. Maas:** Writing – review & editing, Validation, Methodology, Conceptualization. **Anneke Bergersen:** Methodology, Investigation, Formal analysis. **Jeroen Kals:** Writing – review & editing, Methodology. **Fotini Kokou:** Writing – review & editing, Conceptualization. **Johan W. Schrama:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Antony J. Prabhu Philip:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2024.741506.

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