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Evaluation of wedge wire screen as a new tool for faeces collection in digestibility assessment in fish: The impact of nutrient leaching on apparent digestibility of nitrogen, carbon and sulphur from fishmeal, soybean meal and rapeseed meal-based diets in rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

The study was carried out to evaluate wedge wire screen as a potential tool for collecting fish faeces from the tank outlet water. Apparent digestibility (AD) estimates of carbon (AD_C), nitrogen (AD_N), sulphur (AD_S), organic material (AD_{ORG}) and individual amino acids obtained by the wire screen were compared with stripping. Three diets, with fishmeal, soybean meal (SBM) or rapeseed meal (RSM) as main protein source were extruded and fed to triplicate groups of 120-g rainbow trout (*Oncorhynchus mykiss*). Faeces were obtained by gentle stripping from the distal abdomen, and by collection from a wedge wire screen 15, 30, 60, 120 and 240 min post feeding. AD_{ORG}, AD_N and AD_S obtained by collection on the screen were significantly ($P < .05$) higher than those obtained by stripping, except for some values for AD_C. Differences in AD between stripping and faeces collected with the wire screen were lower than reported for other methods of collection from water media. Most of the increase in AD of nutrients for wedge wire screen occurred during the first 15 min after defecation. The AD_{ORG}, AD_C, AD_N and AD_S were lowest in the RSM diet. The AD of amino acids followed the same pattern as AD_N. AD_S in the rapeseed diet was particularly low (54.7%), compared to the fishmeal (72.4%) and SBM diet (70.1%). In addition to AD of cysteine, low digestibility of glucosinolates, its degradation products and other sulfonated components are likely reasons for the low AD_S. Faeces from the RSM based diet had higher dry matter (DM) concentration than the SBM based diet. Faecal DM recovery for SBM was lowest among the diets for both collection methods. The low DM recovery for the SBM faeces did not affect AD estimates of faeces at the different time intervals, possibly due to simultaneous leakage of nutrients and indigestible marker. In conclusion, the use of wedge wire screen for collection of faeces represents an interesting supplement to stripping. The AD estimates had low random variation and nutrient leaching during faeces collection were dependent on dietary composition.

1. Introduction

Determination of apparent digestibility (AD) is an important first step in nutrient optimization of fish feed. Digestibility measurement in fish relies on collection of faecal samples and use of inert markers added to the feed (Edin, 1918). The methods that have been used for faecal collection in fish include direct collection from the intestine through anal suction (Spyridakis et al., 1989; Windell et al., 1978), stripping of faeces from the posterior intestine (Austreng, 1978), dissection and removal of faecal material from the posterior intestine (Austreng, 1978; Percival et al., 2001) and collection of faeces from the water medium (Cho and Slinger, 1979; Choubert et al., 1982; Spyridakis et al., 1989; Windell et al., 1978).

Stripping of faeces has been reported to give reliable and highly correlated AD estimates to the dissection method (Austreng, 1978). However, this method and other direct collection methods from the posterior intestine are criticized for the risk of removal of faeces before natural retention time is completed, and thereby limiting digestion and nutrient absorption capacity (Possompes, 1973; Vens-Cappell, 1985). Faeces collected from water media have a natural retention time in the gut but are subject to leaching which will result in overestimation of AD (Choubert et al., 1979; Choubert et al., 1982; Glencross et al., 2007;

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Spyridakis et al., 1989; Vandenberg and De La Noüe, 2001; Windell et al., 1978). The rate of nutrient leaching from faeces increases with time exposed to water (Windell et al., 1978), but is reported to be rapid within the first 5 min in water (Possompes, 1973). Rapid collection of faeces from water is therefore necessary to reduce leaching. The rapid faeces collection techniques, such as the continuous filtration method and immediate pipetting of faeces from water showed the lowest rate of leaching compared to the decantation method in the study by Spyridakis et al. (1989).

Diet composition affects faecal consistency and is a key factor that can determine the rate of nutrient leaching from faeces. The presence of non-starch polysaccharides (NSP) from plant ingredients in salmonid feed has been reported to cause increased water content in faeces (Storebakken, 1985). Soybean meal (SBM) in salmonid diets is also associated with increased faecal water content caused by osmotically active short oligosaccharides that reduce water absorption in the distal intestine (Kraugerud et al., 2007; Olli et al., 1994; Refstie et al., 1997; Refstie et al., 1999). Rapeseed meal (RSM) have often low protein digestibility in salmonids and contains antinutritive components such as glucosinolates, lignins, phytic acid, tannins, protease inhibitors, indigestible oligosaccharides and NSPs (Francis et al., 2001; Knudsen, 1997; Mwachireya et al., 1999).

The wedge wire screen is a stainless-steel wire mesh placed at an inclined position in the outlet water column of the tank. It is used for the collection of uneaten feed and faeces that are removed from the tank along with the outlet water. The design of the wire screen ensures efficient drainage, such that uneaten feed and faeces trapped on the screen have minimal contact with water. This may provide an advantage of reduced nutrient leaching from faeces required for the AD estimation of nutrients.

The aim of this experiment was to evaluate the wedge wire screen as a tool for faeces collection in AD assessment in fish. The rate of nutrient leaching on the wire screen collector was also investigated with time of faeces collection, and AD estimates were compared with the stripping method. Three different diets containing fishmeal (FM), SBM and RSM differing in their contents of NSP and indigestible sugars, as main protein sources were used in the digestibility assessment. In addition, the total amount of faecal nutrient and DM, over a given period, were compared with the digestibility values and the relative amount of DM collected by the stripping method. This was done to investigate the leakage of nutrients to the recirculating aquaculture system (RAS) depending on the dietary composition of the diet given.

2. Materials and methods

2.1. Design of the wedge wire screen

The wedge wire screen, Sb-12, Pro-SLOT (Progress Industry Group, Sp. z o. o. s. k, Poland) is made with acid-proof stainless steel and used to filter or trap uneaten feed or faeces removed from the tank along with the outlet water (Fig. 1). The simple design consisting of working profile/screen, slots and the support profiles built underneath the entire length of screen (Fig. 2). Its inclined position (45°) in the outlet water column and the slots ensures efficient drainage. The support profiles further improve drainage by reducing the back flow of the outlet water onto the screen surface, leaving faeces and feed on the screen with minimal contact with water.

2.2. Formulation and production of experimental diets

Three different diets based on FM, SBM and RSM respectively, as main protein sources were produced at the NMBU Centre for Feed Technology (Fôrtek), Ås, Norway. The RSM used in the present experiment was a fine fraction from air classified RSM containing 37.8% protein (Hansen et al., 2017). Diets were formulated to have a similar ratio between protein, fat and starch. Formulation and chemical

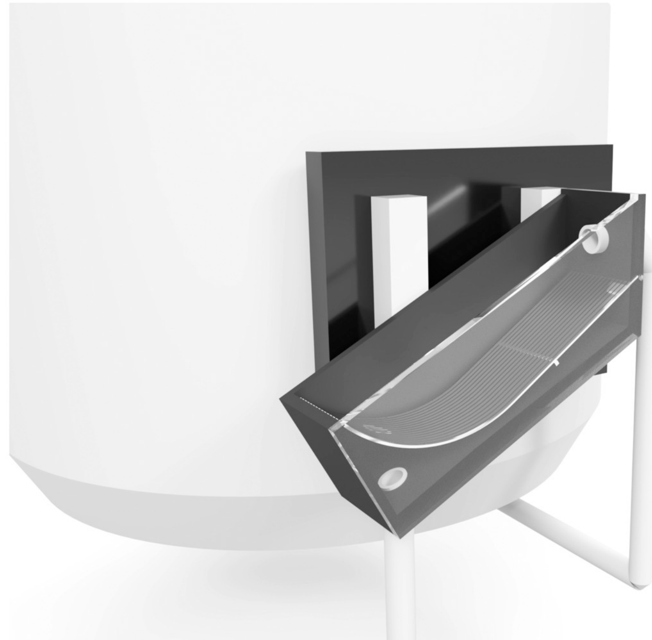

Fig. 1. Description of the feed and faecal collection system.

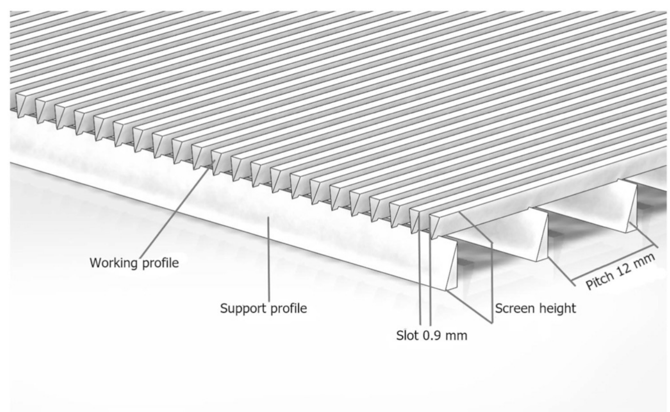

Fig. 2. Description of the wedge wire screen used for faecal collection.

composition of the diets are presented in Tables 1 and 2. The diets were supplemented with yttrium oxide (Y_2O_3) as inert marker to estimate digestibility (Austreng et al., 2000). All ingredients were mixed in a 40 l twin shaft experimental mixer, ground with a hammer mill (Alpine Upz 160, NO:13580, Augsburg, Germany) to 0.6 mm. The diets were extruded in a five-section Bühler twin-screw extruder (BCTG 62/20 D, Uzwil, Switzerland), fitted with four 3 mm die holes, bypassing the conditioner. The bulk densities were measured and recorded after extrusion by collecting pellets into a 1 l beaker. The pellets were dried after extrusion with small experimental driers for 90 min and cooled at ambient temperature. Vacuum coating with fat was done on the following day in a 6 l F-6 RVC vacuum coater (Forberg AS, Larvik, Norway).

2.3. Biological experiment and fish rearing facilities

The fish experiment was conducted at the NMBU Fish Laboratory. The experimental procedures were performed in accordance to the national guidelines for the care and use of animals (The Norwegian Animal Welfare Act and the Norwegian Regulation and Animal Experimentation). 360 rainbow trout (*Oncorhynchus mykiss*) with an

Table 1
Formulation of the experimental diets.

	FM	SBM	RSM
Ingredients (g/kg)			
Fishmeal LT[a]	380	134	128
Soybean meal[b]		278	
Rapeseed meal[c]			308
Corn gluten meal[d]	154	154	154
Vital wheat gluten[e]	72	72	72
Wheat[f]	164	136	126
Fish oil[g]	80	80	70
Rapeseed oil[h]	100	94	89
Choline chloride[i]	0.3	0.3	0.3
Monocalcium phosphate[j]	18.2	18.2	18.2
Limestone[k]	4.1	4.1	4.1
Stay C 35%[l]	1.0	1.0	1.0
L-Lys[m]	11.3	13.3	13.3
DL Met[n]	3.1	7.2	7.2
L-Trp[o]	0.6		
L-Arg[p]	5.1	2.0	2.0
L-Thr[q]	1.8	2.0	2.0
Y^2O^3[r]	0.1	0.1	0.1
Vitamin/mineral premix[s]	5.1	5.1	5.1

[a] LT fishmeal, Norsildmel, Egersund, Norway.
[b] Soybean meal, hexane extracted and toasted, Non-GMO, Denofa AS, Fredrikstad, Norway.
[c] Rapeseed meal, fine fraction after air classified solvent extracted RSM, Bunge, Poland.
[d] Heinz & Co. AG, Zurich, Switzerland.
[e] Vital wheat gluten, Amilina AB, Panevezys, Lithuania.
[f] Wheat, Regal, Lantmännen Cerealia, Stockholm.
[g] NorSalmOil, Norsildmel, Egersund, Norway.
[h] Rapeseed oil, AAK, Karlshamn, Sweeden.
[i] Choline chloride, 70% Vegetable, Indukern s.a., Spain.
[j] Monocalcium phosphate, Bolifor® MCP-F, Oslo, Norway Yara.
[k] Limestone, Agri ForVK, Fransefoss, Norway.
[l] STAY-C Stabilized Vitamin C, Dry Mixture, L-Ascorbyl-2-Polyphosphate (AsPP), 35% ascorbic acid activity, Argent Aquaculture, Washington
[m] L-Lysine CJ Biotech CO., Shenyang, China.
[n] Rhodimet NP99, Adisseo ASA, Antony, France.
[o] L-tryptophan minimum 98%, PT Cheiljedang, China
[p] L-Argenine, CJ Biotech CO., Shenyang, China.
[q] L-Threonine, CJ Biotech CO., Shenyang, China.
[r] Yttrium, Metal Rare Earth Limited, Shenzhen, China.
[s] Premix fish, Norsk Mineralnæring AS, Hønefoss, Norway. Per kg feed; Retinol 3150.0 IU, Cholecalciferol 1890.0 IU, α-tocopherol SD 250 mg, Menadione 12.6 mg, Thiamin 18.9 mg, Riboflavin 31.5 mg, d-Ca-Pantothenate 37.8 mg, Niacin 94.5 mg, Biotin 0.315 mg, Cyanocobalamin 0.025 mg, Folic acid 6.3 mg, Pyridoxine 37.8 mg, Ascorbate monophosphate 157.5 g, Cu: CuSulfate $5H_2O$ 6.3 mg, Zn: ZnSulfate 151.2 mg, Mn: Mn(II)Sulfate 18.9 mg, I: K-Iodide 3.78 mg, Ca 1.4 g.

average weight of 120 g were distributed into 9 tanks with a conical base (diameter 77 cm; water depth 50 cm). The tanks were randomly divided into three replicate dietary groups and contained 40 fishes each. The fishes were subjected to 24 h light regime and supplied 14 °C fresh water from a RAS with an average recirculation of 97.2%. The water flow rates of the tanks were standardized to about 11 l min^{-1} and the oxygen content of the outlet water was kept within 7.0–8.0 mg l^{-1}. Feeding was done once a day for 120 min, using automatic belt feeders for 21 days. The uneaten feed were readily removed from the tanks by the flow of the outlet water and deposited onto the wedge wire screen for collection. The uneaten feed were collected daily from the wire screens, weighed and stored at −20 °C.

2.4. Collection of faeces with wedge wire screen

The collection of faeces from the wedge wire screen started on feeding day 12 and ended on day 16 of the experiment. Faeces

Table 2
Chemical composition of diets.

	FM	SBM	RSM
Dry matter, g/kg feed	896	904	899
Crude protein (N × 6.25), g	458.0	413.9	382.3
Crude fat, g	162.1	154.5	169.2
Starch, g	154.7	136.7	122.2
Ash, g	77.0	60.0	64.0
Amino acids, g/kg			
Total amino acids[a]	448.0	418.3	384.1
Methionine	13.0	13.7	14.4
Lysine	31.4	28.0	25.9
Threonine	19.7	17.6	17.2
Valine	21.1	18.7	18.0
Isoleucine	18.0	17.0	15.1
Leucine	41.3	38.6	35.7
Phenylalanine	20.0	20.1	17.5
Histidine	8.64	8.58	8.10
Arginine	28.1	23.2	19.8
Cysteine	5.07	5.34	5.95
Asparagine	33.2	33.1	25.3
Serine	22.3	21.6	19.1
Glutamine	89.2	89.2	82.2
Proline	32.1	30.2	29.9
Glycine	23.6	17.5	17.3
Alanine	28.4	22.8	21.6
Tyrosine	12.9	12.9	11.4

[a] Tryptophan not included.

collection was done after the removal of uneaten pellets from the wire screens to prevent contamination of the faeces. Collection of faeces from the wire screens was done at the following intervals 15, 30, 60, 120, and 240 min after feeding, over a total time of 480 min for all intervals. The faeces were collected from the wire screens with plastic spatulas and immediately stored at −20 °C.

2.5. Collection of faeces by the stripping method

Stripping of faeces was done twice during the experiment, on day 17 and day 22. Faeces were carefully stripped from all 40 fishes in each tank from the posterior intestine as described by Austreng (1978). Prior to stripping, the fishes were anaesthetized with Trikainmesilat (Finquel®, Scan Aqua, Årnes, Norway) 60 mg l^{-1} in small aerated tanks. The stripped faeces were immediately weighed and stored at −20 °C prior to freeze drying.

2.6. Chemical and physical analysis

The diet and faeces samples were ground with a pestle and mortar and analysed for carbon (C), nitrogen (N) and sulphur (S) (Vario El Cube elemental analyzer system GmbH, Hanau, Germany). Ash content was determined by combustion at 550 °C. Yttrium (Y) in feed and faeces was dissolved by microwaving (Milestone UltraClave III; Milestone, Sorisole, Italy) in HNO_3 prior to assessing Y concentration by inductively coupled plasma mass spectroscopy (ICP-MS; Agilent 8800 Triple Quadrupole; Agilent Technologies, Santa Clara, CA, United States). Amino acids in diet and faecal samples were analysed on a Biochrom 30 amino acid analyzer (Biochrom Ltd., Cambridge, UK). Physical quality parameters of pellets were measured after oil coating. Expansion of pellets was measured with an electric Vernier calliper as the mean value of the diameter of 10 randomly picked pellets per feed. Sinking velocity was assessed as the mean value of the time required for 10 randomly picked pellets to sink in a 1 m tube filled with 23 °C tap water. Pellet durability analysis was done in triplicates for each of the diets and measured in a LignoTester (Serial N° LT 110, Borregaard Lignotech, Sarpsborg, Norway). Fifty gram of pre-sieved pellets were weighed out for the diets and subjected to mechanical stress for 30 s. The durability was estimated as the percentage of the remaining pellets after sieving through a 3 mm sieve. Water stability was assessed according to Baeverfjord et al. (2006).

2.7. Calculations and statistical analysis

Apparent nutrient digestibilities were calculated as described by Edin (1918). Feed conversion ratio (FCR) was calculated as feed intake (final weight – initial weight)$^{-1}$. The average values of the 3 replicates were obtained for pellet expansion, sinking speed and pellet durability. Fish performance, faecal dry matter, AD estimates of organic matter, C, N and S were analysed by one-way analysis of variance (ANOVA) and regression. Linear and 2nd degree polynomial regressions were used to observe the effect of time on rate of nutrient leaching in the diets. The best models were selected based on R^2, residual plot and significance level. Significant difference ($P < .05$) among the means were ranked by Tukey's multiple range test and are indicated by different subscript letters in the tables. Digestibility of amino acids were analysed by two-way ANOVA with the effects of diet and faecal collection method were the independent variables. Significant differences among means of amino acid digestibility were identified by comparing least square means. All statistical analyses were conducted by the General Linear Models procedure in SAS software package (SAS/STAT Version 9.4. SAS Institute, Cary, NC, USA).

3. Results

3.1. Feed production and pellet quality

The specific mechanical energy (SME) employed during feed production was 770, 681 and 874 Wh kg^{-1} for the FM, SBM and RSM diets. The bulk densities of the diets followed the same trends as the SME results with 570, 565 and 575 g l^{-1}, respectively. The diet with rapeseed meal had the numerically lowest expansion and fastest sinking velocity. Pellet durability ranged from 99.1 to 99.5% among diets. The water stability test gave lower dry matter retention at all time intervals for the SBM and RSM diets compared to the FM diet. Feed production parameters are shown in Table 3.

3.2. Growth performance

The rainbow trout fed the FM diet had the highest weight gain of 85 g fish^{-1} (Table 4). Feed intake for the groups fed the FM diet was similar to the RSM group but significantly different from the SBM group which had the lowest feed intake of 50 g fish^{-1}. The FM group also showed the lowest feed conversion ration (FCR) with 0.66 g intake (g gain)$^{-1}$ compared to the SBM (0.76) and RSM diets (0.82).

Table 3
Feed production and pellet quality parameters.

	FM	SBM	RSM
Extrusion parameters			
Temperature in section 3, °C	118	119	121
Temperature in section 5, °C	113	118	120
SME[a], Wh kg^{-1}	770	681	876
Bulk density	570	565	575
Physical pellet quality			
Sinking speed, cm s^{-1}	8.3	8.3	8.0
Durability, %	99.5	99.5	99.1
Diametric expansion, %	8.0	12.7	5.6
Water stability (% DM[b] retained)			
30 min in water	90.8	80.6	82.5
60 min in water	88.3	80.9	78
120 min in water	89.1	79.3	77

[a] SME: Specific mechanical energy.
[b] DM: Dry matter.

Table 4
Growth and feed conversion ratio (FCR) for rainbow trout fed fishmeal (FM), soybean meal (SBM), and rapeseed meal (RSM) diets during 21 days of feeding.

Diet	FM	SBM	RSM	s.e.m.[1]	*P*-value
Start weight, g fish^{-1}	119.7	119.2	120	1.76	0.83
Final weight, g fish^{-1}	204.6^a	185.3^b	189.3^b	4.51	0.0045
Weight gain, g fish^{-1}	84.9^a	66.2^b	69.3^b	4.97	0.01
Feed intake, g fish^{-1}	56.2^a	50.4^b	56.7^a	2.33	0.08
FCR, g intake (g gain)$^{-1}$	0.66^b	0.76^a	0.82^a	0.02	0.0007

a,b Indicate significant (P ≤ .05) differences among diets within a row.
[1] Pooled standard error of mean.

Table 5
Faecal dry matter in percentage and faecal dry matter (g) collected by stripping and wedge wire screen method over a total collection period of 480 min from rainbow trout fed *fishmeal (FM), soybean meal (SBM), and rapeseed meal (RSM) diets.*

	FM	SBM	RSM	s.e.m.[1]	P-value
Faecal dry matter, %					
Stripping	14.4^a	11.4^b	14.7^a	1.26	0.033
Screen collection intervals					
15 min	11.9^a	8.4^b	11.9^a	0.62	0.0005
30 min	11.7^a	8.3^c	11.1^b	0.23	< 0.0001
60 min	11.7^a	8.6^c	10.9^b	0.37	0.0001
120 min	11.2^a	8.4^b	11.0^a	0.19	< 0.0001
240 min	11.9^a	8.4^b	11.1^a	0.61	0.0005
Faecal dry matter, g					
Stripping	3.05	2.79	3.70	0.40	0.074
Screen collection intervals					
15 min	3.02 ^{a}	1.52 ^{b}	4.16 ^{a}	0.56	0.0036
30 min	3.06 ^{a}	1.37 ^{b}	3.46 ^{a}	0.31	0.0004
60 min	2.69 ^{a}	1.22 ^{b}	2.93 ^{a}	0.43	0.0055
120 min	2.90 ^{a}	1.09 ^{b}	3.37 ^{a}	0.25	< 0.0001
240 min	3.60 ^{a}	1.57 ^{b}	3.47 ^{a}	0.66	0.0173

abc Indicate significant (P ≤ .05) differences among diets within a row.
[1] Pooled standard error of mean.

3.3. Faecal dry matter and apparent digestibility

Fish fed the FM and RSM diets had higher faecal DM content with both methods of faecal collection compared to fish given the SBM diet (Table 5). The faecal DM percentage remained constant over time of faeces collection, whereas the total content (g) of DM collected over an 8-h period followed a 2nd degree polynomial pattern, with a minimum DM content obtained between 60 and 120 min.

AD of organic matter followed the same pattern for both methods of faeces collection with the FM diet fed fish having higher digestibility than the SBM diet, while the RSM diet showed the lowest AD of organic material (Table 6). The differences between the stripping method and first collection interval were 1.2, 6.6 and 9.7 percentage points for FM, SBM and RSM diets, respectively.

The AD of C for the three dietary treatments showed the same statistical ranking as the AD of organic matter, except an increased linear manner with increasing time on the wire screen for the fish fed the FM diet (Table 6). There was no difference observed between the first collection interval (15 min) and the stripping method for AD of C in the FM diet, whereas 4.7 and 6.7 percentage differences were observed in the SBM and RSM diets, respectively.

Using the stripping method, the SBM diet had slightly higher AD of N than the FM diet, while the RSM diet resulted in the lowest AD of N. Regression analysis showed increased AD estimates for N with increasing time of faecal collection for FM and RSM diets. No significant effects of exposure time were observed for the SBM diet. The percentage differences in AD of N between the first collection interval (15 min) and the stripping method was significant for all treatments and the percentage difference was 2.9, 4.3 and 7.4 for the FM, SBM and RSM diets,

Table 6
Apparent digestibility of nutrients obtained by the stripping and wedge wire screen method in rainbow trout fed fishmeal (FM), soybean meal (SBM), and rapeseed meal (RSM) diets.

		Screen collection interval, min								
	Stripping	15	30	60	120	240	s.e.m.[1]	P-value[2]	R^2	Regression model
AD Organic matter, %										
Fishmeal diet	88.4^{xB}	89.5^{xAB}	90.1^{xAB}	90.0^{xAB}	90.6^{xA}	91.2^{xAB}	0.80	0.07		
Soybean meal diet	80.7^{yB}	86.4^{yAB}	87.0^{yA}	86.3^{yAB}	86.6^{yA}	87.5^{yAB}	2.13	0.03		
Rapeseed meal diet	71.6^{zB}	79.3^{zA}	79.2^{zA}	78.7^{zAB}	79.6^{zA}	80.4^{zA}	2.73	0.004		
AD Carbon, %										
Fishmeal diet	89.3^{xB}	89.3^{xYB}	89.9^{xB}	89.6^{xB}	90.2^{xAB}	91.2^{xA}	0.43	0.0009	0.71***	$89.3 + 0.008\,T$
Soybean meal diet	82.8^{yB}	86.9^{yA}	87.6^{yA}	86.7^{yA}	87.0^{yA}	87.9^{yA}	0.91	0.0002		
Rapeseed meal diet	73.9^{zB}	79.2^{zA}	78.5^{zA}	77.8^{zA}	78.2^{zA}	79.6^{zA}	0.96	0.0001		
AD Nitrogen, %										
Fishmeal diet	90.7^{yC}	93.4^{yB}	93.4^{yB}	93.8^{yAB}	94.3^{yA}	93.8^{yAB}	0.28	< 0.0001	0.72***	$93 + 0.02\,T - 5.9 \times 10^{-5}T^2$
Soybean meal diet	91.8^{xB}	95.9^{xA}	96.0^{xA}	96.2^{xA}	96.2^{xA}	96.1^{xA}	0.43	< 0.0001		
Rapeseed meal diet	85.1^{zB}	91.9^{zA}	91.9^{zA}	92.5^{zA}	92.8^{zA}	92.4^{yA}	0.43	< 0.0001	0.51*	$91.6 + 0.02\,T - 6 \times 10^{-5}T^2$
AD Sulphur, %										
Fishmeal diet	72.4^{xB}	85.7^{yA}	87.2^{yA}	89.2^{A}	90.1^{yA}	89.1^{A}	2.45	< 0.0001		
Soybean meal diet	70.1^{xB}	91.9^{xA}	93.4^{xA}	91.8^{A}	95.6^{xA}	96.0^{A}	1.95	< 0.0001	0.37*	$92.1–0.018\,T$
Rapeseed meal diet	54.7^{yC}	82.5^{yB}	87.4^{yAB}	89.5^{A}	92.5^{xyA}	91.3^{A}	2.12	< 0.0001	0.78***	$81.8–0.15\,T - 4.6 \times 10^{-4}T^2$

xyz Indicate significant ($P < .05$) differences among diets within a column.
ABC Indicate significant differences among collection interval for individual diets within a row.
AD: Apparent digestibility.
[1] Pooled standard error of mean.
[2] P-value given for the ANOVA. R^2 is given for the regression analysis were; * $P < .05$, ** $P > .01$, ***$P > .001$.

respectively.

Fish fed the FM and SBM diet had higher AD of S compared to the RSM diet, using the stripping method. The regression analysis showed a trend for the FM diet to reach a peak in AD of S after approximately 150 min on the wire screen while the AD of S increased with time for the SBM and RSM treatment. Additionally, the difference in AD of S between the stripping and the screen collection method was higher for all dietary treatments compared to the other nutrients.

There were significant differences in the amino acid digestibility among the diets for both methods of faeces collection (Table 7). The RSM showed the lowest digestibility for all the amino acids compared to FM and SBM and followed same pattern as N digestibility. The difference in AD of AA was significant between the stripping method and collection at 15 min on the wire screen.

4. Discussion

The experimental diets were formulated to have same ratio of protein and lipid. However, the vacuum coating of oil led to a higher lipid level in the RSM diet than planned. The expansion of the pellet was

Table 7
Apparent digestibility of amino acids from the stripping method and faecal collection at 15 min post feeding in rainbow trout fed fishmeal (FM), soybean meal (SBM), and rapeseed meal (RSM) diets.

	Stripping			Faecal collection, 15 min				
	FM	SBM	RSM	FM	SBM	RSM	s.e.m[1]	P-value
Essential amino acids								
Methionine	93.2^{b}	95.2^{cd}	92.3^{a}	94.7^{c}	97.5^{e}	95.7^{d}	0.20	< 0.001
Lysine	94.8^{b}	95.0^{b}	90.9^{a}	96.2^{c}	97.8^{d}	95.4^{bc}	0.21	< 0.001
Threonine	91.6^{b}	90.7^{b}	84.1^{a}	93.7^{c}	95.4^{d}	90.7^{b}	0.27	< 0.001
Valine	92.3^{c}	92.0^{bc}	84.2^{a}	94.0^{cd}	95.6^{d}	90.4^{bc}	0.40	< 0.001
Isoleucine	92.1^{c}	92.5^{c}	84.1^{a}	94.0^{d}	96.1^{e}	90.1^{b}	0.32	< 0.001
Leucine	94.2^{c}	93.9^{c}	87.6^{a}	95.4^{d}	96.5^{e}	92.1^{b}	0.22	< 0.001
Phenylalanine	92.9^{bc}	94.1^{c}	88.5^{a}	94.1^{c}	96.4^{d}	92.5^{b}	0.29	< 0.001
Histidine	90.9^{b}	91.7^{bc}	86.8^{a}	93.0^{c}	96.0^{d}	92.6^{c}	0.28	< 0.001
Arginine	95.4^{c}	95.9^{cd}	91.3^{a}	96.2^{d}	97.6^{e}	94.1^{b}	0.14	< 0.001
Non-essential amino acids								
Cysteine	84.0^{b}	84.8^{b}	77.0^{a}	88.2^{c}	93.6^{d}	88.7^{c}	0.48	< 0.001
Asparagine	83.8^{b}	86.5^{c}	78.6^{a}	89.2^{d}	95.3^{e}	89.9^{d}	0.39	< 0.001
Serine	91.2^{bc}	92.0^{c}	84.8^{a}	93.3^{d}	95.8^{e}	90.8^{b}	0.20	< 0.001
Glutamine	95.4^{b}	95.6^{b}	91.1^{a}	96.8^{c}	98.0^{d}	95.1^{b}	0.21	< 0.001
Proline	94.5^{b}	94.0^{b}	87.8^{a}	96.2^{c}	97.2^{c}	93.2^{b}	0.30	< 0.001
Glycine	88.7^{b}	88.2^{b}	81.7^{a}	93.0^{d}	95.1^{e}	90.6^{c}	0.31	< 0.001
Alanine	93.3^{b}	93.0^{b}	87.2^{a}	95.1^{c}	96.2^{c}	92.4^{b}	0.28	< 0.001
Tyrosine	92.8^{b}	94.1^{c}	89.1^{a}	93.8^{bc}	96.3^{d}	92.7^{b}	0.25	< 0.001

abcde Indicate significant differences among collection interval for individual diets within a row.
P-values for both the effect of diet, time and interaction was significant $P < .001$.
Interaction was similar for all digestibility estimates $P < .001$.
[1] Pooled standard error of mean.

generally low and had a negative correlation with SME, showing that the RSM diet obtained numerically lowest expansion despite highest SME and temperature during extrusion. Since the RSM diet have the highest level of non-soluble polysaccharides (NSP), this is in line with Hansen and Storebakken (2007), describing decreased pellet expansion with increased non-soluble NSP (cellulose) in the feed mash despite higher torque and SME during extrusion.

The higher growth rate observed in the FM diet fed fish compared to the plant-based diets is consistent with previous studies in rainbow trout (Burel et al., 2000; Collins et al., 2012; Rumsey et al., 1993; Zhang et al., 2012). The lower growth rate observed in the SBM diet fed fish was mainly due to reduced feed intake. The feed conversion ratio for the SBM diet was, however, lower than that for the RSM diet, resulting in similar growth.

In general, the AD estimates for the wedge wire screen method was higher than the values obtained by stripping, in keeping with previous studies showing higher AD estimates for faeces collected from the water medium compared to other faecal collection methods (Spyridakis et al., 1989; Vandenberg and De La Noue, 2008; Hajen et al., 1993). This is also in agreement with observation by Possompes (1973) describing rapid nitrogen leaching within the first 5 min. In the present study, lower differences were observed between the AD of N with the stripping and wedge wire screen method for all the diets compared to Spyridakis et al. (1989), which described a continuous filtration as optimal method for faeces collection from the water medium. The differences observed between stripping and sieve collection with freeze drying for AD of N in the study by Storebakken et al. (1998) was smaller than observed in this study for FM and SBM diets.

The AD values of nutrients from the different dietary groups obtained by stripping were all within expected values. The AD of organic matter and carbon followed the same pattern with decreased digestibility with increased level of indigestible NSP in the diet and is in line with Dalsgaard et al. (2012). AD of N was higher using the wire screen than the stripping method for all the diets. The similar AD of N observed for FM and SBM diet with the stripping method agrees with previous study in rainbow trout by Refstie et al. (2000). The lower digestibility of RSM compared to FM and SBM in the present study is in line with Dalsgaard et al. (2012).

The AD of S observed by the stripping method was lower in the RSM fed fish compared to the FM or SBM fed fish. The AD estimates of the S containing amino acids, methionine and cysteine in the RSM diet provided an indication that additional, non-amino acid sulfonated components have contributed to the low digestibility of S. A digestibility estimate of S not arising from methionine and cysteine was calculated for the stripping method and obtained a digestibility of −1.2%, indicating that this non-amino acid S were not digested by the RSM fed fish. In addition, the same AD calculation was done for faeces from the 15 min collection showing 63% digestibility of non-amino acid S by the RSM fed fish. Further, approximately 50% reduction in S concentration, g/kg in faecal DM from stripping compared to 15 min collection was observed, which again demonstrate a high leakage of undigested and soluble S compared to the other investigated nutrients. RSM contains glucosinolates and there is little information regarding digestibility of glucosinolates in fish. However, in pigs, glucosinolates is partly degraded in the stomach and intestine, or absorbed as intact molecules in either blood or urine (Sørensen et al., 2016). Nitriles and isotiacyanids are volatile degradation products of glucosinolates produced in the intestine that may explain the high leakage of indigestible S in the RSM fed fish.

The difference in AD of organic matter and C between stripping and the 15 min collection was low in fish fed FM diet compared to the SBM and RSM diets. Low NSP content in the FM diet compared to the SBM and RSM diets gives a generally higher AD of C by the stripping method and may, therefore, result in a reduced difference between striping and 15 min collection.

The elevated water content in faeces from SBM fed fish is in line with previous findings (Olli and Krogdahl, 1994; Refstie et al., 1997; Refstie et al., 1999; Kraugerud et al., 2007). Considering the low AD of organic matter and C observed for the SBM fed fish compared to the FM control, it is natural to believe that this is the reason for the difference in AD between the two sampling methods for the SBM fed fish. However, the difference in AD of organic matter and C for the RSM fed fish between the two methods was elevated compared to the FM control, despite similar DM content in faeces. The high DM content in RSM faeces can be due to high water absorption capasity of RSM compared to SBM (Dev and Mukherjee, 1986; Khattab and Arntfield, 2009). The elevated difference between methods for the RSM fed fish, despite high DM content, can be due to the small particle size (average 25 μm) of the air classified RSM used in the present experiment.

The differences observed between the ADs by using the stripping method and an alternative faecal collection method from water media is often related to a result of leaching of nutrients (Windell et al., 1978). The lack of change in faecal DM percentage over time on the screen in the present experiment, independent of dietary treatment, demonstrated a low ability of the faeces to change DM percentage by absorbing water. This may indicate a low rate of nutrient leaching over time on the wire screen. However, the total amount of faecal DM (g) collected on the screen were highly dependent on diet and were significantly decreased when fish were fed SBM. With the given setup for this trial, it was not possible to have a total budget of nutrients fed and nutrients collected by the screen system. But for the fish given the FM and RSM diet, the amount of faeces (DM) collected by the stripping method and after 15 min on the screen for a total period of 8 h were approximately the same, 3 and 4 g, respectively. For the SBM fed fish the amounts of faeces were reduced from 2.79 g (stripping) to 1.52 g (15 min) and further decreased to 1.09 g for the 120 min sampling. Even though there is large uncertainty by comparing weights of faeces from stripping and collection by screen over time, these numbers give strong indications of high faecal nutrient leakage from SBM fed fish compared to FM and RSM, that is not detected by digestibility calculation using the indigestible marker technique. Further, this gives strong indications that both nutrients and marker are leaking from the faeces at approximately the same rate, as observed in the low variation in AD of N, independent of time on the screen and total amount of faeces collected from the fish.

This means that the present digestibility results obtained for faeces collected by the screen method should not be used as indicator for nutrient loss from faeces. It may be expected that the difference in AD between the stripping method and collection of faeces from the screen after 15 min should be a good estimate for nutrient leakage. But with a corresponding increase in AD of N with 4 percentage points between these methods and stable ADs of N over time on the screen for the SMB fed fish, it is difficult to link this up to the high loss of total DM and nutrients collected over time on the screen.

5. Conclusions

The low rate of leaching observed with time using the wedge wire screen, compared to other methods of faeces collection in water, indicates that it is an effective tool for faeces collection from the water medium. The major challenge observed with collection of faeces from water media in the present study was the immediate loss of nutrients after defecation. Digestibility results obtained for faeces collected from the water column, independent of collection method, should not be used as indicator for nutrient loss from faeces. Furthermore, feed composition and characteristics play a significant role in determining faecal properties and can to a significant extent reduce nutrient loss from faeces.

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