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Evaluating retch wire screen as a new tool for faeces collection in digestibility assessment in fish: the impact of nutrient leaching on apparent digestibility of fishmeal, soybean meal and rapeseed meal diets in rainbow trout (*Oncorhynchus mykiss*)

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Master of Science in Feed Manufacturing Technology

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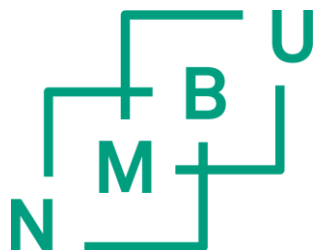
Master of Science Thesis (30 credits)

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Ås (2017)

Acknowledgements

I am grateful to God for the successful completion of this Master thesis, one of many stages in my career.

Ås, May 2017

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Abstract

The study was carried out to evaluate the retch wire screen as a potential tool for collecting feces from water, by comparing its apparent digestibility estimates with that of the conventional stripping method. The retch wire screen is designed as a wire mesh for collecting or trapping feces that is removed from the tank along with the outlet water. The slots between the wire screens allow the passage of the outlet water while the trapped feces are collected from the wire screens for analysis. Three different diets with fishmeal, soybean meal and rapeseed meal were produced by extrusion. The diets were fed once a day to triplicate groups of rainbow trout with an average weight of 120 grams, reared in 14ºC freshwater. The experiment lasted for 22 days. Feces were obtained by careful stripping from the distal abdomen and collected from the wire screen 15, 30, 60, 120 and 240 minutes past feeding. Faecal dry matter, organic matter, carbon, nitrogen, sulphur and yttrium oxide (Y_2O_3) were analysed in diets and freeze dried feces samples, and apparent digestibilities (AD) were calculated.

AD estimates of organic matter, nitrogen and sulphur obtained by collecting feces on the wire mesh collector were higher than AD estimates obtained by the stripping method, except for some values obtained for AD of carbon. This shows that leaching of nutrients from the feces was immediate. Compared to this initial leaching of nutrients, the leaching loss caused by prolonged collection times from 15 to 240 minutes was small. The percentage difference observed in AD estimate between stripping and feces collected with the retch wire screen were lower than values reported in previous methods of feces collection from water media. The relative ranking of the apparent digestibility among the 3 diets in the ANOVA analysis showed the same statistical ranking, and this may facilitate use of the tool, eventually by the employment of a correction factor.

There were significant differences observed in the faecal dry matter among the diets for both methods of faecal collection. Soybean meal showed the lowest faecal dry matter among the diets. AD of nitrogen was significantly higher for the soybean meal diet than for the fishmeal diet when feces were obtained by stripping. Soybean meal also gave the highest estimates of nitrogen AD for feces collection at all the time intervals while rapeseed meal recorded the lowest Nitrogen AD. Rapeseed meal also showed the poorest digestibility in both methods of faecal collection for organic matter, carbon and sulphur. Sulphur AD obtained with the

rapeseed meal diet by the stripping method was particularly low (54.7%), compared to the fishmeal and soybean meal diets that had 72.4 and 70.1% respectively. The likely reason for this was attributed to low digestibility of sulphur containing amino acids in the rapeseed, possibly due to antinutrient factors.

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1   Introduction

Growth in the aquaculture sector has resulted in the limited availability and increased cost of fishmeal. Plant ingredients such as soybean meal and rapeseed meal are readily available and moderately expensive alternative protein sources, but contain a significant portion of antinutrients (Francis et al. 2001), that limit their digestibility and reduce performance in carnivorous fishes. The evaluation of their nutritional value, in which digestibility assessment is often a first step, is therefore important to facilitate use in feeds for aquatic species.

Determination of digestibility in fish is important for defining the proportion of nutrients absorbed from a given diet or feed ingredients. This gives an indication of the utilization of nutrients from feed ingredients and helps in optimizing fish diets. It is also useful in the development of least-cost feed formulations and minimization of nutrient run-offs leading to water pollution (Vandenberg & De La Noüe 2001; Vielma et al. 1998). Digestibility measurement involves the estimation of the ratio of nutrients in feed intake to that in the feces. Merely comparing these ratios does not give an exact representation of the apparent digestibility and could lead to inaccuracies such as underestimation and overestimation of digestibility estimates.

Underestimation of apparent digestibility could be due to the presence of enzymes, cellular materials eroded from the intestinal lining and other materials secreted into the gut, not reabsorbed by the body and estimated as undigested feed (McDonald et al. 2011). The possibility of contamination of feces with uneaten feed is also a cause for underestimation. Overestimation on the other hand is a possibility when estimating digestibility in fishes. This is due to their aquatic environment where possible leaching of nutrients would have occurred before faecal samples are pooled (collected) for analysis. Therefore, a certain degree of overestimation may have to be factored into the digestibility estimates when collecting feces from water media. Quantitative or direct method of faecal collection is difficult in fishes and hardly used. Digestibility estimates are therefore reliant on collection of representative samples (Halver & Hardy 2002). To avoid or limit inaccuracy in the estimation of digestibility, indirect methods, requiring the use of indigestible markers are added to test diets (Austreng 1978; Edin 1918; Nose 1961). This allows for the determination of the apparent digestibility coefficient by calculating the ratio of the marker in diet to the feces excreted (Halver & Hardy 2002).

1.1 Digestive physiology in Rainbow trout

Nutrients are released and absorbed from feed in the gastro-intestinal tract which has a total length of 0.6 to 0.8 times the body length in salmonids (Smith 1989). The sites responsible for the digestive processes in the gastro-intestinal tract of rainbow trout are the posterior oesophagus, stomach, intestine (upper and lower region) and the pyloric caeca (Kryvi & Totland 1997) while other supportive organs such as the pancreas, gall bladder and liver aid the digestion process (Kryvi & Totland 1997; Rust 2002). The digestive tract may also consist of some tissues like the mucosa, submucosa, muscularis and serosa with specific functions in aiding the process of digestion (Rust 2002). The mucosa consists a range of columnar epithelium cells bearing large or undulated folds (Weinreb & Bilstad 1955). Submucosa is made up of a thick layer of connective tissues that support the mucosa, the muscularis has two muscle layers responsible for the movement of lumen contents while the serosa is a group of mesothelial cells found on loose connective tissues (Rust 2002).

1.1.1 Digestion in the stomach

The stomach consists of all the previously mentioned tissues. Digestion of feed starts from the stomach, the rainbow trout has a U or J-shaped stomach with an enlarged lumen that is divided into three regions: cardiac (anterior region), intermediate or transitional and fundic or pyloric (posterior region) (Rust 2002; Smith 1980; Weinreb & Bilstad 1955). The mucosa, in the stomach consist of epithelial layers such as gastric glands that secrete the enzyme, pepsin and hydrochloric acid from the oxynticopeptic cells (Rust 2002). These gastric juices along with water taken in with the feed hydrates the feed while further metabolism by hydrochloric acid which activates pepsinogen to pepsin liberates soluble nutrients from the ingested food (Krogdahl 2001).

1.1.2 Digestion in the intestine and pyloric caeca

The intestine of carnivore fishes has been reported to be shorter than that in herbivores species (Smith 1989). It is divided into two parts, the anterior and posterior region. Digesta from the stomach is transferred or moved into the upper intestine or midgut through the contraction and relaxation of the pylorus (Krogdahl 2001). This process of contraction and relaxation determine the rate of gastric emptying (Rust 2002) but is also influenced by factors such as the physical properties and chemical composition of the feed (Krogdahl 2001). Regulation of the amount of acidic content entering the intestine and pyloric caeca from the

stomach is also achieved through the previously mentioned contraction and relaxation process (Ostrander 2000) while secretions in the posterior pylorus such as sodium bicarbonate produced from the enterocytes contribute in regulating the acidic pH of the digesta coming from the stomach (Bakke et al. 2010). pH in the anterior and posterior pylorus are reported to be within the ranges of 2-5 and 7-8 (Rust 2002).

Posterior to the pylorus are blind appendages known as the pylorus caeca. It is surrounded by pancreatic tissues and gall bladder responsible for supplying pancreatic juices and bile through their respective ducts into the digestive tract (Bakke et al. 2010; Rust 2002). The upper intestine and the pyloric caeca have similar functions of nutrient absorption and enzyme secretion in the digestive tract (Weinreb & Bilstad 1955). The number of the pyloric caeca or total intestinal absorptive surface is reported to be higher in rainbow trout compared to the Atlantic salmon (Refstie et al. 2000) and may be an important factor to consider when comparing nutrient digestibility in both species. The secretion of enzymes and mucous is done through the mucous cells while hormones that regulate digestion are secreted by endocrine cells in the columnar epithelium of the mucosa (Krogdahl 2001; Rust 2002; Takashima & Hibiya 1995). The mucosa which is in contact or borders the lumen consists of a layer enterocytes surrounded by endocrine and mucous cells (Hartviksen 2015). These enterocytes consists of apical membrane with a large number of microvilli called the brush border which is covered with mucus (Krogdahl 2001) and reducing in length towards the posterior intestine (Khojasteh et al. 2009; Weinreb & Bilstad 1955). The brush border gives the enterocyte the large surface area that makes it suitable for digestion through the release of enzymes and absorption of released nutrients (Krogdahl 2001; Kuz'Mina & Gelman 1997; Rust 2002).

The posterior intestine has a larger diameter and thicker wall compared to the upper intestine but otherwise built up similarly (Krogdahl 2001; Kryvi & Totland 1997). Enzyme secretion and nutrient absorption is reported to be decreased in this section (Rust 2002) but the possibility of nutrient absorption and transportation is shown through the presence of numerous and large supranuclear vacuoles which has been observed to be few or absent in fishes that are fasted (Krogdahl 2001).

1.2 Faecal collection methods

The faces collection methods used in digestibility measurements have been shown to have significant effect on the results (Glencross et al. 2005; Glencross et al. 2007;

Storebakken et al. 1998a; Vandenberg & De La Noüe 2001). The common methods used for faecal collection include direct collection from the intestine such as anal suction (Spyridakis et al. 1989), stripping of feces 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 feces from the water medium (Cho & Slinger 1979; Choubert et al. 1982; Spyridakis et al. 1989; Windell et al. 1978). Both methods are subject to criticisms in terms of digestibility measurements. For example, direct collection from the intestine have been criticised for removal of feces before completion of natural retention time, thereby limiting digestion and nutrient absorption capacity (Possompes 1973; Vens-Cappell 1985) while collection from water medium are criticized for overestimation due to leaching (Choubert et al. 1979; Choubert et al. 1982; Glencross et al. 2007; Spyridakis et al. 1989; Vandenberg & De La Noüe 2001; Windell et al. 1978).

1.2.1 Anal suction

The method involves the insertion of a glass cannula into the anus of anaesthetized fish and slight suction with using a rectal bulb to collect digesta from the posterior intestine (Spyridakis et al. 1989; Windell et al. 1978). Digestibility measurements with the use of this method have showed similar results with the dissection method (Spyridakis et al. 1989). The advantage of this method is reported to be the reduction of contamination with endogenous materials and that fishes do not need to be killed (Tytler & Calow 2012). The method however involves handling which imposes significant level of stress on the fish that can cause sudden defaecation (Spyridakis et al. 1989; Tytler & Calow 2012) and decrease in appetite. Sufficient time intervals between collection periods is thus essential when using this method to ensure that fishes return to the same appetite level.

1.2.2 Stripping method

The stripping method of faecal collection has been shown to give reliable estimates of apparent digestibility coefficient (Storebakken et al. 1998a). It is done by applying gentle abdominal pressure to squeeze out feces from the posterior intestine of fishes when anaesthetized (Austreng 1978). Austreng (1978) recommended the collection of feces from the posterior intestine to ensure complete nutrient absorption and eliminate the possibility of collecting undigested feed. This was due to significant increases in digestibility throughout the gastrointestinal tract except between the proximal and distal intestine in the study. Other

probable causes of underestimation include endogenous materials such as urine, blood and semen collected along with the feces. Studies have shown that digestibility estimates gotten by the stripping method are higher than that of dissection method with reasons attributed to contributions from endogenous materials (Austreng 1978; Spyridakis et al. 1989; Vandenberg & De La Noüe 2001). Stripping of feces can be difficult in small fishes due to the small amount of feces obtained as a result of stripping from the posterior intestine (Spyridakis et al. 1989; Storebakken et al. 1998a). This is a limiting factor that can necessitate repeated collections leading to stress on the fishes or possible injuries to the intestinal mucosal membrane (Vandenberg & De La Noüe 2001). The handling stress can affect feed intake, growth performance (Storebakken et al. 1998a) and cause reduced apparent digestibility coefficient of nutrients (Hajen et al. 1993; Stone et al. 2008).

1.2.3 Dissection method

Feces is collected from the posterior intestine as with the other direct collection methods from the intestine. The difference with the dissection method is that it involves killing the fish, cutting open along the belly, dissecting out the intestine and squeezing out digesta from the posterior intestine (Austreng 1978). Collection of feces from the posterior intestine is based on the same reasons as previously explained in the stripping method. Digestibility estimates obtained by the dissection method have been shown to be lower than the other methods of faecal collection (Austreng 1978; Spyridakis et al. 1989; Storebakken et al. 1998a). The dissection method of AD estimates are thus criticized for underestimation due to contamination with intestinal tissue or mucus (Storebakken et al. 1998a).

1.2.4 Feces collection from water medium

Collection of feces from the water medium has been done with different methods, ranging from manual collection by netting (Windell et al. 1978) and immediate pipetting (Spyridakis et al. 1989). Other collection methods are the settling column method (Cho & Slinger 1979; Cho et al. 1982) and by continuous filtration of the outlet water (Choubert et al. 1979; Choubert et al. 1982; Storebakken et al. 1998a). They have all been reported to give higher digestibility estimates when compared to the stripping and dissection method and allowance must be given for overestimation of results (Belal 2005; Glencross et al. 2005; Glencross et al. 2007; Hajen et al. 1993; Vandenberg & De La Noüe 2001). Overestimation of digestibility in these methods is due to the leaching of nutrients from faecal samples but

the rate of leaching has been shown to vary between the different methods used and time of faecal collection (Hajen et al. 1993; Spyridakis et al. 1989). Leaching of nitrogen from fish feces has been reported to be fast during the first 5 minutes in water and stabilizes after one hour (Possompes 1973). The rate of leaching may then be reduced when feces are quickly collected and have minimal contact with the surrounding water (Choubert et al. 1982). This may however be impossible or unavoidable in some cases like in Tilapia fishes where the threadlike feces hang on the anus until when it is finally emitted. In such situation, sufficient leaching would have occurred before feces are collected and may make faecal collection from water medium inappropriate. The advantage of faecal collection from water medium is the elimination of handling stress in relation to stripping (Storebakken et al. 1998a), killing associated with dissection method, and it allows for faecal collection in species where abdominal pressure is not possible (Choubert et al. 1982).

1.2.4.1 Manual feces collection from the water medium

The manual collection methods are simple methods of removing defecated feces from water medium or tanks. They require no automation, but are however time consuming and laborious as continuous observation is required to immediately remove feces from the tanks after defecation (Ostrander 2000). The feces can be collected either by netting (Windell et al. 1978) or immediate pipetting (Spyridakis et al. 1989). Collection of feces which had settled in the tanks at intervals of 1, 4, 8, and 16 hours by netting showed high digestibility values which may exceed 15% when compared to the dissection method (Windell et al. 1978). Immediate pipetting on the other hand is more accurate and the procedure involves the use of a cannula connected to a rectal bulb to suck feces from the tank once expelled (Spyridakis et al. 1989). The comparison of feces collection methods by Spyridakis et al. (1989) showed similar results between immediate pipetting and the continuous filtration collection method when feces were removed within 30 seconds from the water.

1.2.4.2 Settling column collection method

The settling column method also known as the Guelph system was developed by Cho and Slinger (1979) for digestibility studies in rainbow trout. The system was modified by Cho et al. (1982) to include slopping or slanted bottoms that led to a central drainage pipe where feces and the effluent water are removed from the respective tanks. Another modification of this system with conical bottom and reduced cross-sectional area of the drainage pipe was

developed by Hajen et al. (1993). Reduction of the cross-sectional area of the drainage pipe was done to increase the water velocity and prevent faeces from being stuck or settling in the drainage pipe. This was a problem in the earlier methods by Cho and Slinger (1979) and Cho et al. (1982), and was reported to be the reason for leaching and overestimation of digestibility in the settling column system (Hajen et al. 1993; Spyridakis et al. 1989). The settling column systems operate in the same manner with the slanted or conical fish tanks placed over a central drainage pipe that conveys feces and effluent water to the settling column. The system requires water flow rate to be adjusted between 2 – 8 l/min (Hajen et al. 1993; Ostrander 2000; Satoh et al. 1992) to ensure that feces removed from the tanks pass through the drainage pipe intact and undisturbed inside the settling column. The feces practically remain in water for about 8 – 16 hours in this method until collected for analysis (Satoh et al. 1992). Some degree of leaching can occur during this period but it is reported to be minimal if faecal pellets are undisturbed in the water and thus should have a low effect on ADC (Cho et al. 1982; Hajen et al. 1993). Spyridakis et al. (1989) reported no significant difference between in nitrogen and chromium oxide leaching when water in the settling column was removed on not. Satoh et al. (1992) also reported that there were no significant differences in the digestibility estimates obtained when the effect of timing on feces retrieval was compared between the settling column system and the TUF column feces collection system for two reference diets.
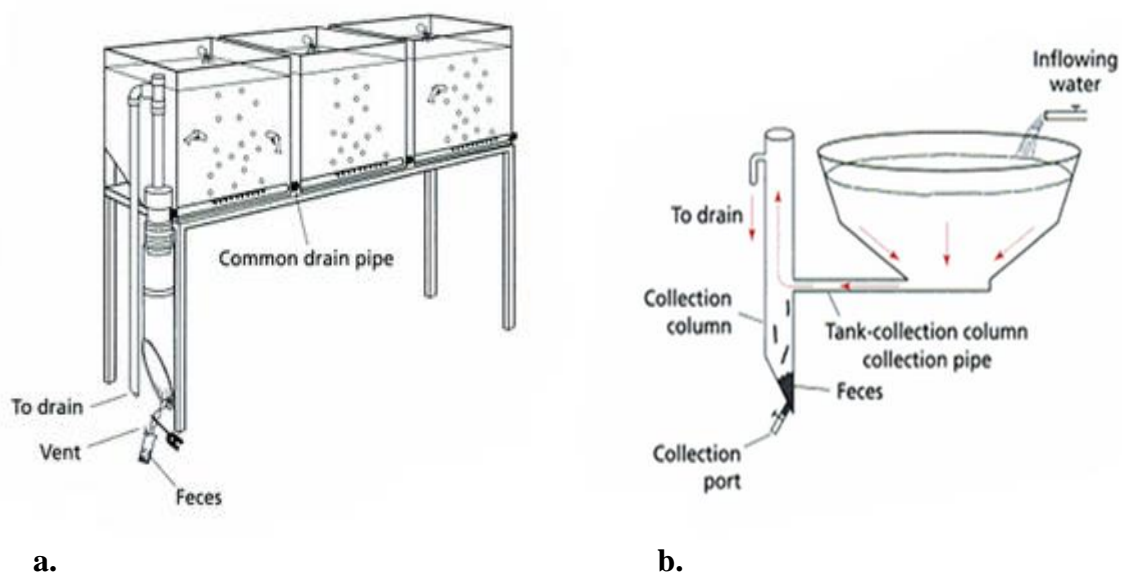
Figure 1. Modifications of the Guelph system (Satoh et al. 1992): a. (Cho et al. 1982), b. (Hajen et al. 1993)

1.2.4.3 Continuous filtration of the outlet water

Choubert et al. (1979) developed the device for the continuous automatic collection of feces from the drainage water in fish tanks. The collection device was later modified by (Choubert et al. 1982) to improve the quantitative collection of feces and collect intact faecal pellets. Cho et al. (1982) reported that leaching was reduced when faecal pellets were intact and undisturbed in the water medium. The procedure of faecal collection involves quick removal of feces within 6-15 seconds after being voided from the fish to ensure as minimal as possible contact with water. The feces are removed by the revolving hemispheric metallic screens through filtration of the drainage water. The filtered feces are then quickly propelled into refrigeration pans to prevent leaching of nutrients. The new device was reported to give a recovery rate of more than 99% for chromium oxide and also mentioned to have the advantage of minimal contact with water compared to the settling column or Guelph system developed by (Cho & Slinger 1979). Vandenberg and De La Noue. (2001), however reported no significant differences when they compared the method by (Cho & Slinger) and (Choubert et al.) in their digestibility assessment of different protein sources when fed to rainbow trout.
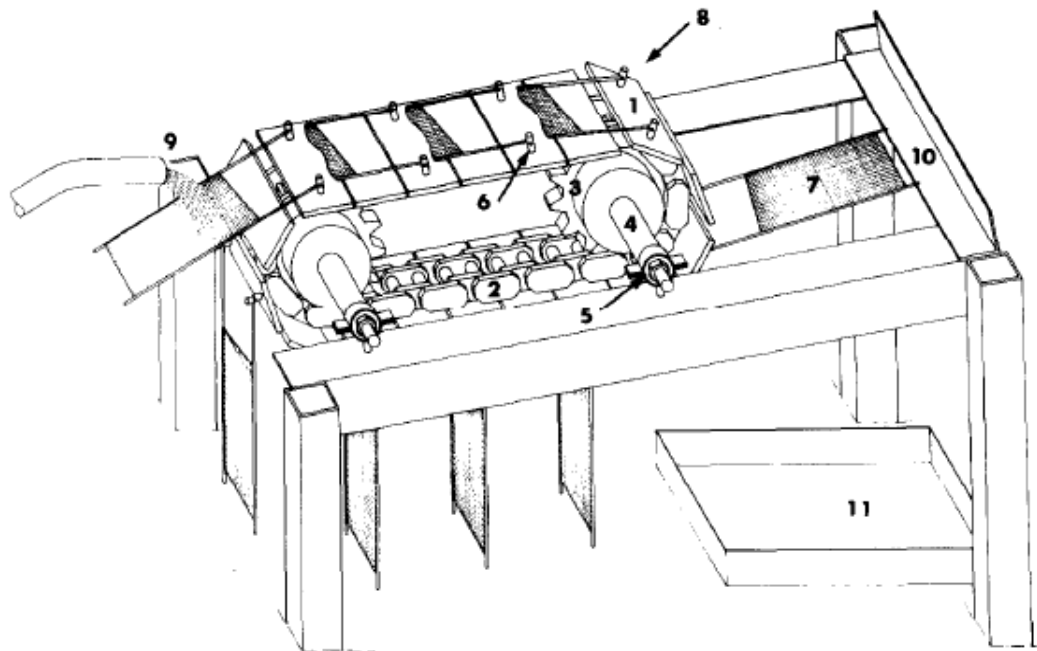
Figure 2. Continuous filtration collection of feces device by (Choubert et al. 1982)

1.3   Design of the retch wire screen

The retch wire screen is a flat-welded stainless made with acid-proof steel. It was designed to fit at an inclined position in the external column of the tank where the outlet water passes through for recirculation. The wire mesh collector was designed like a sieve and had spaces or slots between the profiles. These slots allow the passage of the outlet water for recirculation, while uneaten pellets and feces that are trapped on the wire screen can be easily collected. The passage of the outlet water through the screens of the wire mesh collector reduces the amount of water gliding along the surface of the screens and ensures that feces or uneaten pellets have minimal contact with water. The inclined positon of the wire mesh collector also aids the reduction of water on the wire screen surface. The amount of water penetrating the surface of the screen is also further reduced by the inbuilt support profiles lying underneath and across the entire length of the screen. This is due to the breaking/stopping effect which the support profiles have on the outlet water passing through and running underneath the screen.
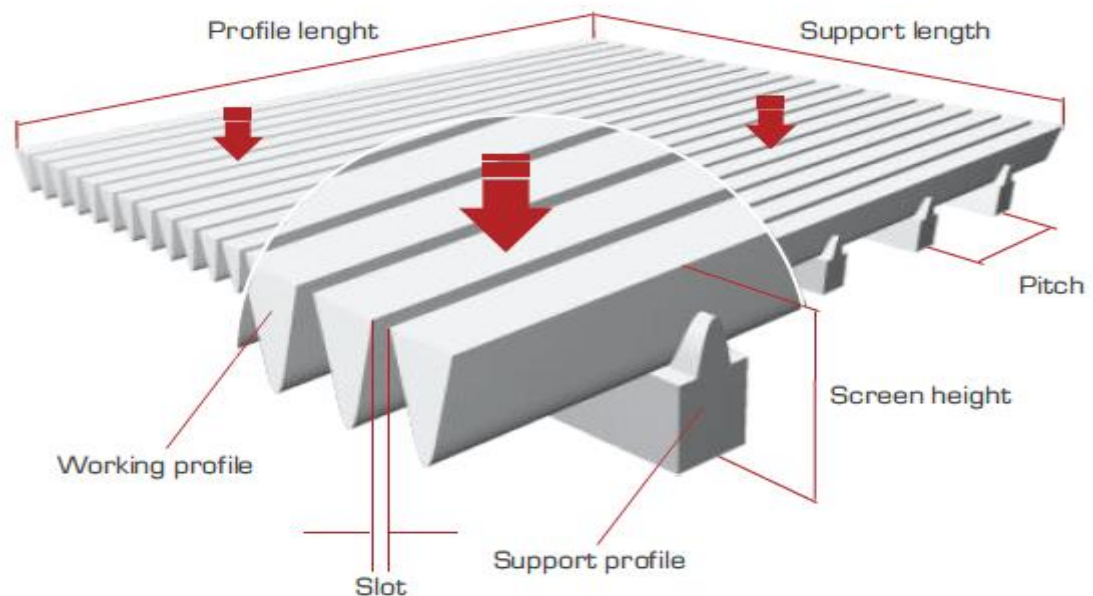
Figure 3. Wire mesh collector (Retch wire screen) - www.progress-screens.com

1.4   Utilization of the different ingredients in Rainbow trout

Fishmeal is an excellent protein source in the diets of salmonid species. The quality of fishmeal can vary due to the type of raw materials used, and processing conditions applied (Miles & Chapman 2006). Over drying of fishmeal during processing can cause reduction in

quality and nutritional value (Miles & Chapman 2006), as degradation of amino acids like cysteine and methionine can occur (Opstvedt et al. 1984). High quality fishmeal, however, has a balanced amino acid profile similar to that of the fish carcass (Mambrini & Kaushik 1995). This has led to its effective utilization as a reference diet in studies involving nutrient utilization of other potential ingredients in relation to fish performance. Results from digestibility studies have shown positive or higher values for nutrient digestibility, growth, nitrogen and energy retention when fishmeal was used either as the main protein source or reference diet (Gomes et al. 1995; Hansen & Storebakken 2007; Kraugerud et al. 2007; Refstie et al. 1997). Fishmeal is however expensive (Rumsey et al. 1993) and continued expansion of the aquaculture industry poses the need for other alternative protein sources like soybean and rapeseed meal.

1.4.1 Utilization of soybean meal

Soybean meal remains one of the most important plant protein alternative to fishmeal due to its high protein content and balanced amino acid profile (Storebakken et al. 2000). The use of soybean in salmonid diets is limited to low inclusion levels due to the presence of antinutrients (Øverland et al. 2009; Rumsey et al. 1993). The antinutrients in SBM include trypsin inhibitor, antigens, lectins, saponins (Dersjant-Li 2002) and non-starch polysaccharides such as arabinans, arabinogalactans and acidic polysaccharides (Refstie et al. 1999). The presence of indigestible carbohydrates and other antinutrients however vary with different soybean products with regards to the type of processing or degree of treatment used during production. These antinutrients are associated with low palatability and reduced feed intake (Gomes et al. 1995), reduced nutrient digestibility (Romarheim et al. 2006; Storebakken et al. 2000), low feed conversion and soybean induced enteritis (Krogdahl et al. 2003; Van den Ingh et al. 1991). The utilization of SBM in salmonid species is dependent on the degree or type of processing. The utilization of a range of SBM products as a partial substitute for fishmeal meal has been highlighted by (Rumsey et al. 1993). Their study showed 47% inclusion level of a specially processed SBM could produce similar growth rates to the fishmeal control diet in rainbow trout. Soy protein concentrate (SPC) was also shown to produce similar growth rate with a 100% fishmeal diet in rainbow trout (Kaushik et al. 1995). There is however limited success with conventional SBM products like full fat or defatted SBM. In the study by (Kaushik et al. 1995), 25% inclusion level of defatted SBM in a fishmeal diet did not significantly affect growth in rainbow trout, higher inclusion rates of

50% however resulted in growth reduction. Similar results of reduced growth with increasing SBM inclusion levels has been shown in the Atlantic salmon (Olli et al. 1994b).

Although there exists a preference for fishmeal when compared to SBM, rainbow trout can likewise adapt and effectively utilize SBM. Refstie et al. (1997) showed that rainbow trout raised in fresh water could adapt within a month to a diet consisting of 67% crude protein from SBM. Their results showed that growth rate, although lower to that of fishmeal after adaptation to the SBM diet was not significantly different. A similar study showing higher feed intake and mean weight in rainbow trout after adaptation to SBM was conducted by Romarheim et al. (2006). Growth was although significantly lower compared to the fishmeal diet, this was reported to be due to lower feed intake of the SBM diet, for which there were no obvious reasons. The starch content of the SBM diet in this study was lower compared to the fishmeal diet and may have influenced the growth due to the digestible protein to energy ratio. These are therefore indications that rainbow trout may perform well given sufficient time to adapt to SBM diet.

The presence of indigestible carbohydrates and other antinutrients in SBM have been suggested to have a negative effect on the performance of salmonids. There are however specie differences in terms of response to these antinutrients (Rumsey et al. 1993). For example, rainbow trout has been shown to tolerate higher inclusion levels of SBM compared to the Atlantic salmon (Refstie et al. 2000). In their study, Refstie et al. (2000) reported that rainbow trout were less affected by the antinutrients in SBM, digested nutrients more efficiently but had lower utilization of absorbed nutrients compared to the Atlantic salmon. They further stated that the lower nutrient utilization for rainbow trout in their study may have been due to the low ratio of digestible protein to digestible energy in the trout diet. Low digestible energy in SBM could mean that amino acids are used for energy purpose through oxidation to glucose or fat. This can however be compensated for through increased intake of the SBM diet. SBM have a balanced amino acid profile but is low in methionine (Storebakken et al. 2000). This is another factor that can limit nutrient utilization of SBM but can be compensated for through amino acid supplementation. Glencross et al. (2004) reported that there was no significant difference in protein digestibility when NSP was reduced with resultant increase in protein in rainbow trout compared to Atlantic salmon which showed better protein utilization with reduced NSP. Indigestible carbohydrates in SBM is reported to have an effect on the reduced utilization of nutrients especially lipids (Kaushik et al. 1995). This is to a higher extent in Atlantic salmon than rainbow trout (Glencross et al. 2004) and it is caused by increased gut water content resulting from the presence of osmotically active

short oligosaccharides (Refstie et al. 2000). Another cause of reduced lipid digestibility in salmonids is the reduction in absorptive capacity of the distal intestine due to soybean antinutrients (Storebakken et al. 2000). Reduced faecal dry matter in salmonids resulting from the consumption of SBM (Kraugerud et al. 2007; Olli et al. 1994b; Refstie et al. 1997; Refstie et al. 1999) has also been implicated as the effect of the increased gut water content and osmotically active short oligosaccharides (Kraugerud et al. 2007; Olli et al. 1994b; Refstie et al. 1999). Some other antinutrients in SBM that have significant effect on the utilization of SBM are protease inhibitors and phytic acid.

Protease inhibitor activity has shown to be high in rainbow trout and related to increasing energy concentration in the diet (Krogdahl et al. 1994). The inhibition activity of soybean proteases is achieved through the blocking or reduction of trypsin and chymotrypsin molecules by either the Kunitz trypsin inhibitor and Bowman–Birk protease inhibitor, which leads to increased and possible suppression of these proteolytic enzymes by the pancreas (Krogdahl & Bakke 2015; Olli et al. 1994a). Fishes can however tolerate lower than 3 mg/g trypsin inhibitor activity in the diet through increased production of trypsin (Storebakken et al. 2000) and optimal heat treatment has been shown to reduce the trypsin inhibitor activity in SBM diets. The degree of heat treatment of SBM varies among different fish species (Storebakken et al. 2000) and careful consideration should be given to the potential loss of amino acids during heat treatment (Francis et al. 2001).

Phytate phosphorus is not readily available to fish due to lack of phytase activity in the intestine of salmonids (Vielma et al. 1998) to hydrolyse the phytic acid. The amount or level of phytic acid in soy products are also dependent on the type of processing (Storebakken et al. 2000). High phytic acid levels in fish diets have negative effects on absorption of nutrients and minerals (Francis et al. 2001) and cause subsequent water pollution (Vielma et al. 1998). Reduction in nutrient utilization, for example, protein absorption is observed through the formation of phytic acid and protein complexes (Krogdahl & Bakke 2015). Whereas, the reduction in the bioavailability of minerals occurs through the formation insoluble chelates with divalent and trivalent ions such as magnesium, iron, zinc, copper and calcium (Akande et al. 2010; Lönnerdal 2002; Spinelli et al. 1983). For efficient utilization of phosphorus in soy products for salmonids, heat treatment or hydrolyzation with the enzyme phytase is used to treat phytic acid. The challenge with the above methods is denaturation of phytase on exposure to high temperatures and low temperature of cold water fishes which limits phytase activity (Storebakken et al. 2000). These challenges were however eliminated with pre-incubation of soy protein concentrate at low at low temperatures 40 to 45ºC (Denstadli et al.

2007; Storebakken et al. 1998b). Other studies by (Sajjadi & Carter 2004) and (Vielma et al. 1998) have also demonstrated the positive effect of improved phosphorus level through phytase supplementation on growth in the Atlantic salmon and rainbow trout.

1.4.2 Utilization of rapeseed/canola meal

Rapeseed obtained after oil extraction contains about 32 - 45% dry matter protein with a well-balanced amino acid profile (Burel et al. 2000b) which makes it an excellent alternative to fishmeal. Canola meal is another variety of rapeseed that contains low levels of glucosinolates (30µmol g^{-1}) and 2 % erucic acid (Enami 2011). The amino acid profile of canola meal is similar to SBM but contains higher amounts of cysteine and methionine and lower lysine levels (Khajali & Slominski 2012). Acceptable feed intake and performance has been reported at 20 % inclusion of canola meal fines in juvenile rainbow trout diet (Thiessen et al. 2003). The inclusion level in salmonid diets is also limited to 20% (Newkirk 2009) due to the presence of antinutrient factors like phytic acid, tannins, protease inhibitors, sinapine, glucosinolates (Francis et al. 2001; Mwachireya et al. 1999) and a significant portion of fibre, 30-40% (Burel et al. 2000b). The presence or quantity of these antinutrients in rapeseed/canola products as well as their nutrient composition varies with the degree or type of processing to which they are subjected (Mwachireya et al. 1999). Rainbow trout and the Atlantic salmon have shown to effectively utilize rapeseed meal when included in fish diets (Aslaksen et al. 2007; Mwachireya et al. 1999). The proportion of nutrient utilization is although lower than in fishmeal (Aslaksen et al. 2007) and accompanied with reduced growth. Mwachireya et al. (1999) reported in their study reported low dry matter digestibility of canola meal. This was said to be majorly the effect of high indigestible carbohydrate content and phytic acid, with glucosinolates and phenolic compounds affecting nutrient digestibility to a lesser extent. Majority of rapeseed or canola products used in studies have been subjected to various treatments or processing like dehulling, air classification, solvent extraction, enzyme and thermal treatment resulting in reduction or elimination of antinutrient to an acceptable level in fish diets (Aslaksen et al. 2007; Diosady et al. 1986; Mwachireya et al. 1999; Thiessen et al. 2004; Vose et al. 1976). The resultant products like rapeseed protein concentrate, canola protein concentrate (CPC), canola protein isolate are high sources of protein which have shown good potential for high inclusion in fish diets. The methods of production of these concentrates determines their nutritional composition and antinutrients levels which has a direct influence on of fish performance (Collins et al. 2012). Thiessen et

al. (2004) in their study concluded that CPC has similar nutritive value with fishmeal as there were no significant differences in the performance of juvenile rainbow trout fed a fishmeal diet with increasing levels of CPC (500 and 750 g kg^{-1} of dietary fishmeal) when compared to the fishmeal control diet. Higher growth rate was also observed in the same study when CPC replaced of 100g kg^{-1} dietary fishmeal protein in a commercial-like trout diet consisting of other protein sources. The supplementary effect of fishmeal in the study can, however, not be ignored and may have contributed to the performance of the fish fed the CPC diet.

There are considerable differences in the overall nutrient utilization between rapeseed diets with high and low fibre levels (Hajen et al. 1993). The most important influence of NSP on reduced nutrient utilization in salmonids seems to be on energy digestibility with lesser effect on protein digestibility (Aslaksen et al. 2007; Hajen et al. 1993; Mwachireya et al. 1999). This results in increased utilization of digested protein for energy purposes and eventual reduced performance. The adverse effect of fibre in rapeseed on nutrient utilization may be through the reduction of intestinal transit time by insoluble fibres or the trapping of nutrients by the high viscosity formed by soluble fibres in the intestine (Storebakken et al. 1998b). The study by Aslaksen et al. (2007) showed higher intestinal viscosity resulting from rapeseed meal diet compared to SBM in salmon. The high intestinal viscosity observed for the rapeseed meal diet was also related to the higher faecal dry matter and caused by its high NSP content.

Glucosinolates are secondary plant metabolites containing sulphur (Khajali & Slominski 2012). The levels of glucosinolates vary with different rapeseed/canola products and high levels in diets have a negative effect on palatability, feed intake, (Mwachireya et al. 1999), decreased thyroid function and hormone levels (Burel et al. 2000a) which reduces iodine uptake and causes goitre (Krogdahl & Bakke 2015). The toxic effect of glucosinolates is mediated through the hydrolysis of its toxic metabolites like thiocyanates, isothiocyanates, goitrin, nitriles, 5-vinyl-2-oxazolidinethione, and 5-vinyl-1,3-oxyzolodine-2-thione (VOT) by thioglucosidases/myrosinase present in the rapeseed/canola meal or in the intestinal microflora (Khajali & Slominski 2012; Krogdahl & Bakke 2015; Tripathi & Mishra 2007). This hydrolysis and release of toxic metabolites occurs due to breakage or rupture of the seed upon processing which results in disruption of the barrier which separates thioglucosidases from their substrate (Burel et al. 2000a; Khajali & Slominski 2012). Specific effects of the glucosinolates metabolites ranges from reduced iodine availability triggered by thiocynates, morphological and physiological changes of the thyroid caused by VOT while the nitriles are affect liver and kidney functions (Krogdahl & Bakke 2015). The efficient utilization of

rapeseed meal in salmonid diets may also depend on the elimination of glucosinolate and myrosinase activity during treatments such as solvent extraction or thermal treatment. Burel et al. (2000a) reported that low feed utilization and growth was observed at the lowest glucosinolate level of 1.4 mol/kg DM in rainbow trout fed rapeseed meal diet while even more severe reductions were observed with increasing glucosinolate levels up to 19.3 mol/kg DM in the diet. It was however not concluded that reduction in growth was only due to the increasing glucosinolate levels. The effect of other antinutrient factors in rapeseed meal, contributing to lower protein digestibility was not eliminated and could be a possibility. Other changes such as increased volume of the thyroid tissue and reduced plasma thyroxin levels that are indications of suppressed iodine uptake were also observed at low dietary glucosinolate levels

1.5   Objectives of the study

The current study was attempted to evaluate the retch wire screen as a new tool for feces collection in the assessment of apparent digestibility in fish.

The main objectives of the research work are:

➤ investigate the possibility of collecting feces from small rainbow trout by filtering the outlet water, using a new type of retch-wire screen.

➤ compare the apparent digestibility estimates of fishmeal, soybean meal and rapeseed meal from feces obtained by the retch wire screen and the stripping method.

➤ investigate the rate of nutrient leaching from feces with time on the retch wire screen.

2   Materials and methods

2.1   Experimental design

The experiment was carried out to evaluate the retch wire screen as a new tool for feces collection in the assessment of apparent digestibility in fish. The impact of nutrient leaching on apparent digestibility of fishmeal, soybean meal and rapeseed meal diets in rainbow trout (*Oncorhynchus mykiss*) was determined by comparing feces obtained by the retch wire screen with the stripping method. A total of 9 tanks with 3 replicates for each diet were used in the experiment. The tanks contained 40 fishes each and the 3 experimental diets were randomly assigned among the replicate groups. The experiment lasted for 22 days.

2.2   Formulation and production of experimental diets

Three different diets with fish meal, soybean meal and rapeseed meal as main protein sources were produced at the NMBU Centre for Feed Technology (Fôrtek), Ås, Norway. Formulation of the diets is presented in Table 1. The diets were supplemented with yttrium oxide (Y_2O_3) as inert marker to estimate digestibility (Austreng 1978). All ingredients were mixed in a 40 l twin shaft experimental mixer and ground with a roller mill (Alpine Upz 160, NO:13580, Augsburg, Germany) to 0.6 mm to ensure homogeneity. The diets were extruded in a five-section Bühler twin-screw extruder (BCTG 62/20 D, Uzwil, Switzerland) where the feed mash was directly added in the first section without conditioning. The extruder was fitted with four 3mm die holes and a throughput of 5 kg h^{-1}, 4.8 kg h^{-1} and 5.5 kg h^{-1} for fishmeal, soybean meal and rapeseed meal diets, respectively.

Final bulk densities of the pellets were achieved by applying varying amount of pressure through changing the RPM and addition of water to the extruder barrel. The extrusion parameters are described in Table 2. Bulk densities were measured and recorded after extrusion by collecting pellets into a 1 cm^3 beaker, and were 570g, 565 and 575g, respectively, for the fishmeal, soybean meal and rapeseed meal. The pellets were dried after extrusion with small experimental driers for 90 minutes and cooled at ambient temperature. Vacuum coating with fat was done on the following day in a Forberg 6^{-1} mini vacuum coater (Larvik, Norway). The fishmeal and soybean meal diets absorbed the added oil well, while the rapeseed diet had lower oil absorption due to lower expansion of the pellets. The digestible protein to energy ratio for the fishmeal, soybean meal and rapeseed meal are 1.16, 1.17 and 1.16 respectively. Extrusion parameters are shown in Table 2.

Table 1.

Formulation and composition of experimental diets

	Fishmeal diet	Soybean meal diet	Rapeseed meal diet
Ingredients (g/kg)			
Fishmeal, LT [a]	370	131	125
Soybean meal [b]	0	271	0
Rapeseed meal [c]	0	0	300
Corn gluten meal	150	150	150
Vital wheat gluten [d]	70	70	70
Wheat [e]	160	132	123
Fish oil [f]	90	90	80
Rapeseed oil	110	104	99
Choline chloride [g]	0.3	0.3	0.3
Monocalcium phosphate [h]	18.2	18.2	18.2
Limestone	4	4	4
Lysine [i]	11	13	13
Methionine [j]	3	7	7
Tryptophan [k]	0.6	0	0
Arginine	5	2	2
Threonine [l]	1.8	2	2
Stay C 35% [m]	1	1	1
Y_2O_3 [n]	0.1	0.1	0.1
Premix [o]	5	5	5
Dry matter (DM), g kg^{-1} feed	933	929	928
Chemical composition, kg $^{-1}$ DM			
Crude protein (N X 6.25), g	441.6	401.8	386.6
Crude fat, g	241.7	218.9	213.2
Starch	137.6	124.2	119.6
Ash, g	73.7	65.9	59.5
Protein: Starch	3.21	3.23	3.23
Protein: Fat	1.83	1.84	1.81

[a] LT fishmeal, Norsildmel, Egersund, Norway. [b] Soy bean 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] Vital wheat gluten, Amilina AB, Panevezys, Lithuania.[e] Wheat, Regal, Lantmännen Cerealia, Stockholm. [f] NorSalmOil, Norsildmel, Egersund, Norway. [h] Monocalcium phosphate, Bolifor® MCP-F, Oslo, Norway Yara. [g] Choline chloride, 70 % Vegetable, Indukern s.a., Spain. [h] Monocalsium phosphate, Bolifor® MCP-F, Oslo, Norway Yara. [i] L-Lysine CJ Biotech CO., Shenyang, China. [j] Rhodimet NP99, Adisseo ASA, Antony, France. [k] L-tryptophan minimum 98%, PT Cheiljedang, China. [l] L-Threonine, CJ Biotech CO., Shenyang, China. [m] STAY-C Stabilized Vitamin C, Dry Mixture, L-Ascorbyl-2-Polyphosphate (AsPP), 35% ascorbic acid activity, Argent Aquaculture, Washington. [n] Yttrium, Metal Rare Earth Limited, Shenzhen, China. [o] 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.

Table 2.
Feed production parameters for the experimental diets

Diet	Fishmeal	Soybean meal	Rapeseed meal
Die size	3	3	3
Number of die holes	4	4	4
Calibration (kg/h)	50	50	50
Feeder (kg/h) HZ	5	4.8	5.5
Extruder temperature (^{o}C)			
Section 1	28	25	24.4
Section 2	62	47.6	52.1
Section 3	118	118.9	121
Section 4	116.4	117.9	118
Section 5	113.3	117.6	120
Die temperature	106	116	118
Die pressure (bar).	7	15	12
Pressure, section 4	0.74	0.8	1
SME (Wh/kg)	770	681	874
Torque (Nm)	188	217	200
Torque (Relativ, %)	43	50	45
Drive power (kW)	6.7	6.5	8
Screw speed (rpm)	300	290	380
Extr. water (% - kg/h)	8.2	9.5	9.2
Knife speed (rpm)	1550	1550	1600
Bulk density, 1st	590	567	580
Bulk density, 2nd	565	570	570
Bulk density, 3rd	570	565	575
Diameter (mm)	3.24	3.38	3.17

2.3 Fish rearing facilities and conditions

The experiment was conducted at the Norwegian University of Life Sciences (NMBU) Fish Laboratory. Rainbow trout (*Oncorhynchus mykiss*) with an average weight of 119.6 g obtained from the same fish laboratory were used in this study. The experiment was carried out in tanks with a conical base (diameter 77cm; water depth 50cm). The fishes were subjected to 24h light regime and supplied fresh water from a Recirculating Aquaculture System (RAS) with an average recirculation of 97.2 %. The water flow rates of the tanks were standardized to about 11 l/min and the oxygen content of the outlet water was kept within 7.0 mg l^{-1} - 8.0 mg l^{-1}. Other parameters such as pH 7.2 and temperature 14°C were

also constant throughout the experiment. There were no health issues or mortalities during this experiment.

2.4   Feed intake assessment

The fishes were allowed to adapt for 11 days before the commencement of faecal sampling. During this period, feed intake of the experimental diets was regulated to satiety level. The fishes were not fed the day before and after transfer from the rearing tanks into experimental tanks. Feeding was done once a day between 9:30am and 11:30am with an automatic belt feeder which ensured that feeding was done at the same rate, started and ended at the same time. Feeding started on Day 2 of the experiment with each tank receiving 120 grams of feed according to body weight but feed intake was expectedly low due to stress. The feed weighed out to each tank was then reduced to 80 grams between Day 3 to Day 5 of the experiment in an effort to gradually introduce the fishes to the diets. Feed intake started to improve on the third day of adaptation and approached normal levels of feeding except for tanks 3 and 8 that were fed soybean meal diet which showed low feed intake. Low feed intake in tank 3 was due to adaptation to the new diet and improved to a certain level as the experiment went on, while tank 8 was due to a drop in the oxygen level to 6.4 mg l^{-1} which was adjusted to 8.0 mg l^{-1} when water flow rate was increased. Feed intake then improved to normal levels as with other tanks receiving the soybean meal diet on the fourth day of the experiment. The increase in feed intake continued among the tanks and the weighed-out feed to the tanks were also increased to 100 grams on Day 6 and 7. There was further increase in feed intake on Day 8 with the tanks receiving 110 grams while satiety level was reached with 120 grams of feed on Day 9 of the experiment. Feeding was set at this satiety level except on the evenings before stripping and mornings before stripping where half of the satiety level was fed for all the diets. The collection of uneaten feed was aided with the self-cleaning nature of the tanks as uneaten feeds were easily removed from the water medium through the flow of the outlet water and deposited onto the retch wire screen. The uneaten feeds were then collected from the screens, weighed and stored at -20ºC. Cleaning of the outlet tubes was done twice during the period of the experiment.

2.5 Feces collection

The two faecal collection methods used are collection with the retch wire screen and stripping method.

2.5.1 Collection of feces with retch wire screen

The collection of feces from the retch wire screen started on DAY 12 and ended on Day 16 of the experiment. The collection of feces was done after the removal of uneaten pellets from the screens to prevent contamination and overestimation of nutrient in feces. Feces were collected from the screens with plastic spatulas into small plastic containers for eight hours daily and were immediately stored in the freezer at -20°C after each collection. Specific collection intervals (15 minutes on DAY 16, 30 minutes on Day 15, 60 minutes on Day 12, 120 minutes on DAY 14 and 240 minutes on Day 13) were used on each day of collection to compare the rate of nutrient run-off from feces on the screens.

Figure 4. Feces collection on the retch wire screen.

2.5.2 Stripping of feces

Stripping of feces was done twice during the experiment on DAY 17 and DAY 22. Feces were stripped from all 40 fishes in each of the 9 tanks from the posterior intestine according to the method reported by (Austreng 1978). The stripping was carefully done by applying gentle abdominal pressure to prevent contamination of the faecal samples with mucous and urine. Stripping of feces on DAY 17 started from tank 1 and ended with tank 9. Prior to stripping, the fishes were netted in batches from their respective experimental tanks after feeding and anaesthetized with tricanine methanesulfonate (MS 222) 60 mg l^{-1} in small aerated tanks. The stripped feces from the respective tanks were collected in small plastic containers and immediately weighed and stored at -20°C. After stripping was completed, the fishes were returned into respective experimental tanks and were not fed on DAY 18 due to the handling stress of stripping. Feeding resumed on DAY 19 and feed intake was as normal before stripping.

The procedure for the second stripping on DAY 22 was the same as DAY 17 except that stripping started from tank 9 and ended with tank 1. The feces collected by stripping on DAY 22 were added to the previous samples from DAY 17 and immediately stored at -20°C prior to freeze-drying and chemical analysis. After final stripping, the fishes were killed in a tricanine methanesulfonate (MS 222) 120 mg l^{-1} bath and final fish weight for the respective tanks were recorded.

Figure 5. Stripping method of feces collection.

2.6 Chemical and physical analysis

Faecal samples were freeze dried and analysed for dry matter content. The diets and faecal samples were then grounded with a pestle and mortar and analysed for carbon, nitrogen and sulphur respectively (Vario El Cube elemental analyzer system GmbH, Hanau, Germany). Ash content was determined by combustion at $550^{\circ}C$. Yttrium (Y_2O_3) analysis in diet and feces was done using ICP spectrometry. Physical quality parameters of pellets such as expansion, sinking velocity, pellet durability and water stability were measured after coating with fat. Expansion of pellets were measured with an electric vernier caliper as the mean value of the diameter of 10 randomly picked pellets. Sinking velocity was measured as the mean value of the time required for 10 randomly picked pellets to sink in a 1 metre tube filled with $23^{\circ}C$ tap water. Pellet durability was done in triplicates for each of the diets and measured in a Ligno tester (LignoTester Serial N^{o} LT 110, BORREGAARD LIGNOTECH, Sarpsborg, Norway). 50 g of pre-sieved pellets were weighed out for the diets and tested for 30 seconds respectively. The durability was then estimated as the percentage of the remaining pellets after sieving through a 3mm sieve. Water stability test was done according to the method described by (Baeverfjord et al. 2006). The test was done in triplicates at 120 shakings per minute in a water bath filled with distilled water. 10g samples of each diet were weighed into pre-weighed conical shaped wire net baskets with 3 mm mesh size and a diameter of cm. The bottom of the baskets used were flat and situated 2cm inward such that the basket could only stand with the surrounding edge. The shape of the bottom allowed particles lost from the pellets in the baskets to settle at the bottom of the beaker while shaking in the water bath. The baskets containing the respective feed samples were then placed in 600ml beakers filled with 300ml tap water and incubated at $23^{\circ}C$ for 30, 60 and 120 minutes. At the end of each incubation, the baskets were removed from the beakers and placed on paper tissues for the water to drain out. The baskets were then weighed and dried overnight in a heating cabinet at $101^{\circ}C$. After drying, the baskets were weighed again to determine the dry matter content of the pellets.

2.7 Calculations and statistical analysis

Apparent nutrient digestibility was calculated as described by (Maynard & Loosli 1969). Weight gain was calculated as the Final weight – Initial weight. Feed conversion ratio (FCR) was calculated as Feed intake/(Final weight – Initial weight). The average values of the 3 replicates for pellet expansion, sinking speed and pellet durability were used in the

results table. The other results were statistically analysed with the General Linear Models procedure in SAS software package (SAS/STAT Version 9.4. SAS Institute, Cary, NC, USA). Fish performance, ADC estimates and faecal dry matter content were analysed by one way analysis of variance (ANOVA). Significant differences ($P \leq 0.05$) for the effect of diet and time were ranked by Tukey's multiple range test and indicated in the result tables as different superscripts. Linear and 2nd degree polynomial regressions were used to observe the effect of time on rate of nutrient leaching in the diets.

3   Results

3.1   Feed production and pellet quality

The SME obtained during production of the diets was 770, 681 and 874 (Wh kg^{-1}) for fishmeal, soybean meal and rapeseed meal, respectively. This resulted in differences in bulk densities among the diets with 570, 565 and 575g l^{-1}. The bulk density of the respective diets influenced their sinking velocity, expansion and fat absorption properties with rapeseed meal having the numerically lowest expansion and fat absorption and fastest sinking velocity (Table 3). High pellet durability was recorded for all the diets. The results of the water stability test showed significant differences among the diets at the different time intervals. The fishmeal diet had the highest dry matter percentage at 30, 60 and 120 minutes. The soybean meal diet showed lower dry matter percentage at 30 minutes compared to rapeseed meal while rapeseed meal had lowest dry matter percentage among the diets at 60 and 120 minutes.

Table 3.
Physical quality parameters of fishmeal, soybean meal and rapeseed meal pellets

	Fishmeal	Soybean meal	Rapeseed meal	P-value
Expansion (mm)	3.24 ± 0.05	3.38 ± 0.03	3.17 ± 0.06	
Sinking speed (secs)	8.26 ± 0.19	8.26 ± 0.06	7.96 ± 0.17	
Pellet durability (%)	99.5	99.5	99.1	
Water stability (% DM retained)				
30 min	90.8 ± 1.02^a	80.6 ± 2.92^b	82.5 ± 2.50^{ab}	0.0430
60 min	88.3 ± 0.83^a	80.9 ± 1.20^b	78 ± 1.23^b	0.0015
120 min	89.1 ± 0.15^a	79.3 ± 1.15^b	77 ± 1.37^b	0.0004

Mean ± Standard Error.
[a,b] Indicate significant ($P \leq 0.05$) differences among diets within a row.

3.2 Growth performance

There were significant differences observed in the performance characteristics of the dietary groups at the end of the experiment (Table 4). The rainbow trout fed the fish meal diet had the highest weight gain (85 g fish[-1]), while no siginificant differences were found in the weight gain of the groups fed the soybean and rapeseed meal diets. Feed intake for the groups fed the fishmeal diet was similar to the rapeseed meal group but significantly different from the soybean meal group which had the lowest feed intake (50 g fish[-1]). The fishmeal group also showed the best feed conversion ration (FCR) at 0.66 compared to the soybean and rapeseed meal group that recorded 0.76 and 0.82 g intake g gain[-1], respectively.

Table 4.
Growth and feed conversion for rainbow trout fed fishmeal, soybean meal, and rapeseed meal diets during 21 days of feeding

Diet	Fishmeal	Soybean meal	Rapeseed meal	P-value
Start weight, g fish[-1]	119.7 ± 0.96	119.2 ± 0.9	120 ± 1.17	0.8337
Final weight, g fish[-1]	204.6 ± 1.73^a	185.3 ± 4.04^b	189.3 ± 1.02^b	0.0045
Weight gain, g fish[-1]	84.9 ± 0.92^a	66.2 ± 4.58^b	69.3 ± 1.71^b	0.0077
Feed intake, g fish[-1]	56.2 ± 0.6^a	50.4 ± 1.92^b	56.7 ± 1.18^a	0.0276
FCR, g feed intake (g gain) [-1]	0.66 ± 0.01^b	0.76 ± 0.02^a	0.82 ± 0.01^a	0.0007

Mean ± Standard Error.
[a,b] Indicate significant (P≤0.05) differences among diets within a row.
FCR: Feed conversion ratio.

3.3 Nutrient digestibility

Results of nutrient digestibility are presented in Table 5. The analysis of variance showed that faecal dry matter percentage differed significantly among the three diets for both methods of feces collection. The fishmeal and rapeseed meal diets had similar faecal dry matter content with both methods of faecal collection, while the soybean meal diet resulted in the lowest faecal dry matter percentage. Faecal dry matter percentage obtained from the stripping method differed significantly P<.0001 from feces collected at all the time intervals from the wire mesh method. There were no significant differences in the faecal dry matter percentage in individual diets with respect to time of feces collection using the wire mesh collector.

Table 5.
Apparent digestibility coefficients obtained from faecal collection at different time intervals with the wire mesh collector (retch wire screen) and stripping method

Diet	Fishmeal	Soybean meal	Rapeseed meal	P-value
Faecal dry matter, %				
15 min	11.9 ± 0.1^a	8.4 ± 0.1^b	11.9 ± 0.6^a	0.0005
30 min	11.7 ± 0.1^a	8.3 ± 0.1^c	11.1 ± 0.2^b	<0.0001
60 min	11.7 ± 0.1^a	8.6 ± 0.1^c	10.9 ± 0.4^b	0.0001
120 min	11.2 ± 0.2^a	8.4 ± 0.1^b	11 ± 0.1^a	<0.0001
240 min	11.9 ± 0.1^a	8.4 ± 0.1^b	11.1 ± 0.6^a	0.0005
Stripping	14.4 ± 0.4^a	11.4 ± 0.2^b	14.7 ± 1.2^a	0.0328
AD Organic matter, %				
15 min	$89.5 \pm 0.2^{a\,Z}$	86.4 ± 0.7^b	79.3 ± 0.5^c	<0.0001
30 min	$90.1 \pm 0.2^{a\,YZ}$	87 ± 0.2^b	79.2 ± 0.5^c	<0.0001
60 min	$90 \pm 0.1^{a\,YZ}$	86.3 ± 0.5^b	78.7 ± 0.5^c	<0.0001
120 min	$90.6 \pm 0.1^{a\,XY}$	86.6 ± 0.4^b	79.6 ± 0.6^c	<0.0001
240 min	$91.2 \pm 0.1^{a\,X}$	87.5 ± 0.3^b	80.4 ± 0.2^c	<0.0001
Stripping	88.4 ± 0.1^a	80.7 ± 0.2^b	71.6 ± 0.1^c	<0.0001
AD Carbon, %				
15 min	$89.3 \pm 0.1^{a\,Y}$	86.9 ± 0.7^b	79.2 ± 0.6^c	<0.0001
30 min	$89.9 \pm 0.4^{a\,Y}$	87.6 ± 0.2^b	78.5 ± 0.5^c	<0.0001
60 min	$89.6 \pm 0.1^{a\,Y}$	86.7 ± 0.7^b	77.8 ± 0.6^c	<0.0001
120 min	$90.2 \pm 0.1^{a\,XY}$	87 ± 0.7^b	78.2 ± 0.9^c	<0.0001
240 min	$91.2 \pm 0.4^{a\,X}$	87.9 ± 0.5^b	79.6 ± 0.4^c	<0.0001
Stripping	89.3 ± 0.2^a	82.8 ± 0.2^b	73.9 ± 0.1^c	0.0001
AD Nitrogen, %				
15 min	$93.4 \pm 0.1^{b\,Y}$	95.9 ± 0.1^a	91.9 ± 0.03^c	<0.0001
30 min	$93.4 \pm 0.1^{b\,Y}$	96 ± 0.7^a	91.9 ± 0.3^c	<0.0001
60 min	$93.8 \pm 0.1^{b\,XY}$	96.2 ± 0.1^a	92.5 ± 0.2^c	<0.0001
120 min	$94.3 \pm 0.1^{b\,X}$	96.2 ± 0.2^a	92.8 ± 0.1^c	<0.0001
240 min	$93.8 \pm 0.3^{b\,XY}$	96.1 ± 0.1^a	92.4 ± 0.4^b	0.0026
Stripping	90.7 ± 0.2^b	91.8 ± 0.1^a	85.1 ± 0.3^c	<0.0001
AD Sulphur, %				
15 min	85.7 ± 0.4^b	91.9 ± 0.8^a	$82.5 \pm 1.9^{b\,Y}$	0.0035
30 min	87.2 ± 0.7^b	93.4 ± 0.3^a	$87.4 \pm 0.3^{b\,XY}$	0.0002
60 min	89.2 ± 0.5	91.8 ± 2.3	$89.5 \pm 0.3^{\,X}$	0.4035
120 min	90.1 ± 0.7^b	95.6 ± 0.5^a	$92.5 \pm 1.1^{ab\,X}$	0.0080
240 min	89.1 ± 3	96 ± 0.8	$91.3 \pm 1.2^{\,X}$	0.1063
Stripping	72.4 ± 1.3^a	70.1 ± 0.7^a	54.7 ± 1.7^b	0.0001

Mean ± Standard Error.
[abc] Indicate significant (P≤0.05) differences among diets within a row.
[XYZ] Indicate significant differences among time of collection for individual diets within a column.
AD: Apparent digestibility

3.3.1 Organic matter digestibility

Apparent digestibility of organic matter followed the same pattern for both methods of feces collection with the fishmeal diet fed fish having higher digestibility than the soybean meal diets, while the rapeseed meal diet showed the lowest AD. The differences in ADs among the diets were significant for both methods of feces collection. The percentage differences 1.1, 5.7 and 7.7 for fishmeal, soybean and rapeseed meal diets were observed between the stripping method and first collection interval. No significant effects were observed in ADs of the respective diets with increasing collection interval on the wire mesh collector as shown by the regression analysis (Figure 6). The ranking by Tukey's multiple range test showed, however, significant difference between 15 and 240-minute collection interval for the fishmeal diet.
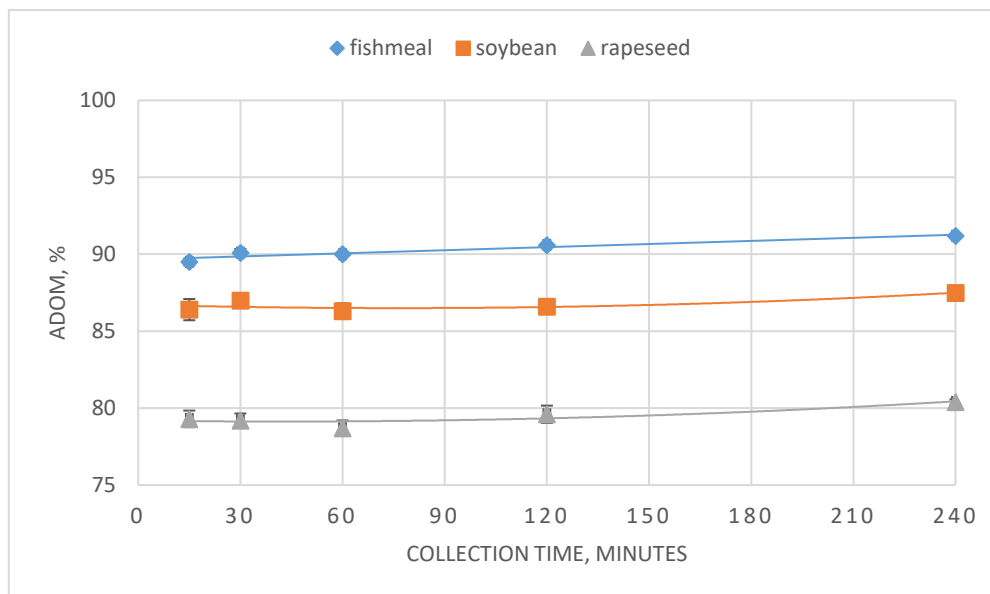

Figure 6. Apparent digestibility of organic matter (ADOM, mean ± standard error) in fishmeal, soybean meal and rapeseed meal diets at 15 – 120-minute collection intervals (T). The regression lines for the given treatments is presented as:

Fishmeal: ADOM = 89.9 + 0.002T, R^2 = 0.03, P_{model} = 0.5211
Soybean meal: ADOM = 86.4 + 0.09T – 7.2 × $10^{-5}T^2$, R^2 = 0.17, P_{model} = 0.3251
Rapeseed meal: ADOM = 77.4 - 0.07T – 2.3 × $10^{-4}T^2$, R^2 = 0.21, P_{model} = 0.2462

3.3.2 Carbon digestibility

AD for carbon showed significant differences among the diets for both methods of feces collection. The fishmeal diet showed the highest carbon digestibility using the stripping method while rapeseed had the lowest AD for carbon. The same pattern also followed in the

retch wire screen method with fishmeal diet having the highest carbon digestibility and rapeseed having the lowest AD across all the time intervals. Regression analysis showed a linear and significant increase in the AD of carbon in the fishmeal diet with increasing time interval on the wire mesh collector. No significant differences were observed in ADs of the soybean bean and rapeseed meal diet. There was no percentage difference observed for fishmeal diet between the first collection interval (15 minutes) and the stripping method for carbon AD whereas 4.1 and 5.3 percentage differences were observed for soybean and rapeseed meal diets, respectively.
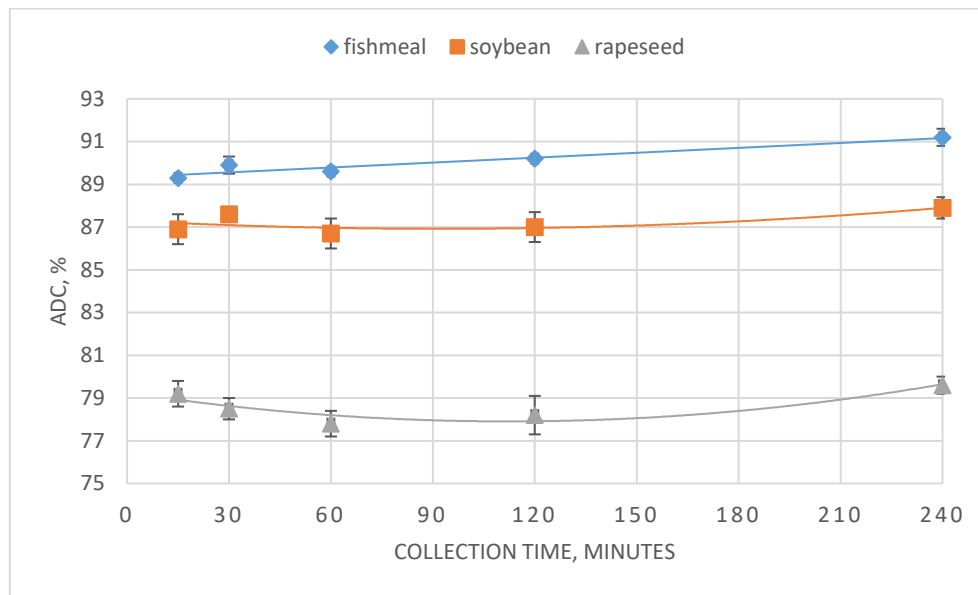

Figure 7. Apparent digestibility of carbon (ADC, mean ± standard error) in fishmeal, soybean meal and rapeseed meal diets at 15 − 120-minute collection intervals (T).
The regression lines for the given treatments is presented as:

Fishmeal: ADC = $89.3 + 0.008T$, $R^2 = 0.71$, $P_{model} < 0.0001$
Soybean meal: ADC = $87.3 - 0.009T + 4.8 \times 10^{-5}T^2$, $R^2 = 0.15$, $P_{model} = 0.3675$
Rapeseed meal: ADC = $= 79.3 - 0.02T - 1.1 \times 10^{-4}T^2$, $R^2 = 0.31$, $P_{model} = 0.1053$

3.3.3 Nitrogen digestibility

For AD of nitrogen, the diets differed significantly, and the stripping method significantly differed from the faecal collection from water at different time intervals. Using the stripping method, the soybean meal diet had slightly higher nitrogen digestibility than the fishmeal diet. The rapeseed meal diet resulted in the lowest nitrogen digestibility. The soybean meal also had the highest digestibility using the retch wire screen method among the diets for all the time intervals. The rapeseed meal diet fed fish showed the lowest digestibility among the dietary treatments. The regression analysis showed significant differences in AD

estimates for nitrogen with increasing time interval for fishmeal and rapeseed meal diets while no significant differences were observed for the soybean meal diet. The percentage differences in ADC estimate between the first collection interval (15 minutes) and the stripping method was 2.7, 4.1 and 6.8 for fishmeal, soybean meal and rapeseed meal diet respectively.
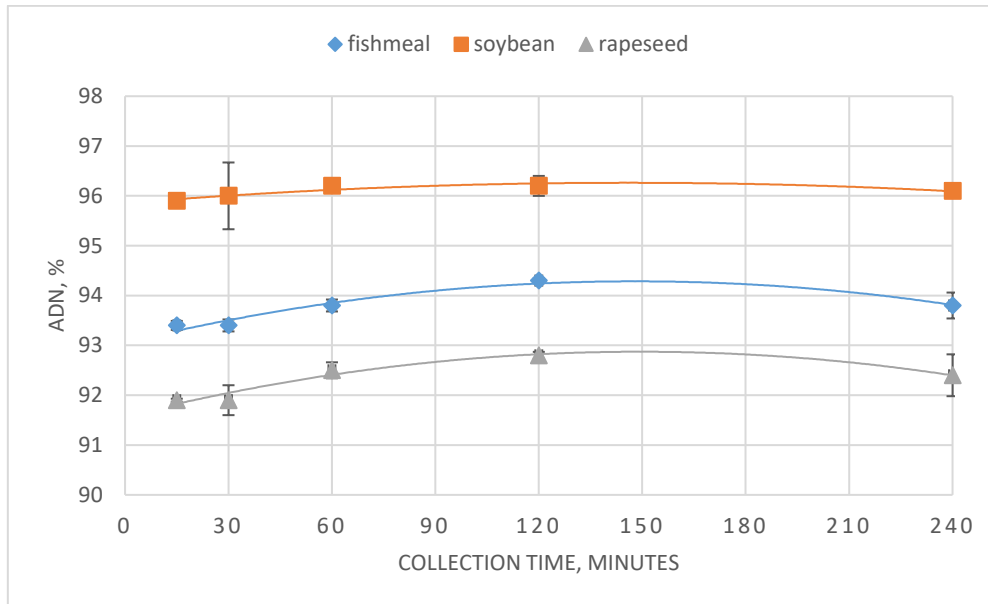

Figure 8. Apparent digestibility of nitrogen (ADN, mean ± standard error) in fishmeal, soybean meal and rapeseed meal diets at 15 – 120-minute collection intervals (T). The regression lines for the given treatments is presented as:

Fishmeal: $ADN = 93 + 0.02T – 5.9 \times 10^{-5}T^2$, $R^2 = 0.72$, $P_{model} = 0.0005$
Soybean meal: $ADN = 95 + 0.005T – 1.7 \times 10^{-5}T^2$, $R^2 = 0.06$, $P_{model} = 0.7032$
Rapeseed meal: $ADN = 91.6 + 0.02T – 6 \times 10^{-5}T^2$, $R^2 = 0.51$, $P_{model} = 0.0142$

3.3.4 Sulphur digestibility

ADC for sulphur was significantly different (P=0.0001) among the diets using the stripping method. The fishmeal diet recorded the highest digestibility and was similar to the soybean meal diet while the rapeseed meal diet showed remarkably lower digestibility than the other treatments. The soybean meal diet had higher digestibility across the time intervals in the retch wire screen method among the diets. There were significant differences observed between the diets at 15, 30 and 120 minute intervals respectively, while no significant differences were seen at 60 and 240 minute intervals. The regression analysis showed a trend for the fishmeal diet to reach a peak in ADC of sulfur after approximately 150 min on the screen. Significant effects were observed for the soybean and rapeseed meal diets with increasing time on the wire mesh collector. The rate of leaching in the faecal samples from

rapeseed meal diet was highest with a percentage difference of 27.8 between the value obtained from the stripping method and first collection interval. Percentage differences observed between the stripping method and first collection interval for fishmeal and soybean meal diets was 13.3 and 21.8%.
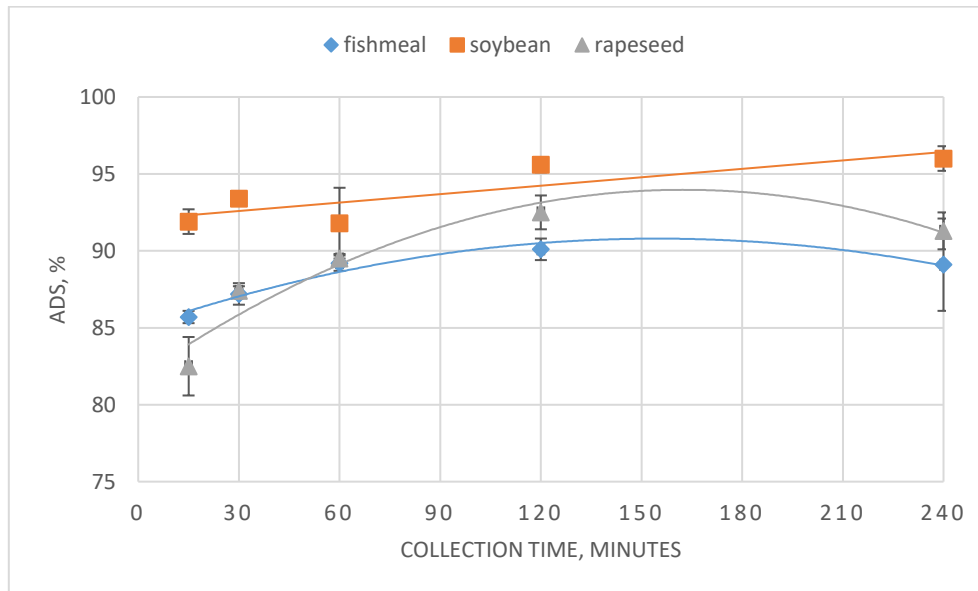
Figure 9. Apparent digestibility of sulphur (ADS, mean ± standard error) in fishmeal, soybean meal and rapeseed meal diets at 15 – 120-minute collection intervals (T).
The regression lines for the given treatments is presented as:

Fishmeal: ADS = 85.5 + 0.08T – 2.4 × 10^{-4}T^2, R^2 = 0.36, P$_{model}$ <0.0666
Soybean meal: ADS = 92.1 - 0.018T, R^2 = 0.37, P$_{model}$ = 0.0157
Rapeseed meal: ADS = 81.8 - 0.15T - 4.6 × 10^{-4}T^2, R^2 = 0.78, P$_{model}$ = 0.0002

4   Discussion

The low expansion observed in the rapeseed meal diet might be a result of the higher content of NSP compared to soybean and fishmeal diets. Hansen and Storebakken (2007) reported that NSP inhibited gelatinization by limiting access to water. The high SME observed during extrusion for the rapeseed meal diet may also be due to its higher NSP content compared to the other diets, as also reported by (Hansen & Storebakken 2007). The degree of gelatinization of the diets was not measured in this study, but the lack of conditioning during the feed production may have contributed to the lower expansion of the rapeseed meal due to incomplete or insufficient gelatinization which affects binding properties. The individual or combined effect of insufficient gelatinization and the presence of NSP may therefore be the reason for the low water stability observed in the soybean and rapeseed meal diets.

The main aim of this study was to evaluate the retch wire screen as a new tool for feces collection in the assessment of apparent digestibility in fish. However, the growth of the fish was also measured. The higher growth rate observed in the fishmeal diet fed fish compared to the plant based diets is consistent with previous studies in rainbow trout (Burel et al. 2000a; Collins et al. 2012; Rumsey et al. 1993; Zhang et al. 2012). The lower growth rate observed in the soybean meal diet fed fish was due to the low feed intake. The feed conversion ratio for the soybean meal diet was however lower than that for the rapeseed meal diet, resulting in similar growth.

Higher faecal dry matter content was observed for fish fed the fishmeal diet compared to the soybean and rapeseed meal diets using the stripping method. This is in line with previous finding by Aslaksen et al. (2007). Their study also showed lower faecal DM in soybean meal fed fish compared to rapeseed meal. Lower faecal DM in soybean meal diet compared to fishmeal diet was also reported by Storebakken et al. (1998a) in Atlantic salmon. Faecal DM values for fishmeal and soybean meal fed fish in a study by Storebakken et al. (1998a) showed similar values by using the stripping method as the present study. The differences in faecal DM values between the two studies could be due to the procedure used for collecting faecal samples for analysis as it was reported in their study that some materials may have been lost during melting of ice from the feces prior to analysis. The faecal samples in this study were freeze dried before analysis. The faecal DM values observed for rapeseed meal and fishmeal diet in this study were similar while lower value was observed for the soybean meal for both methods of faecal collection. This may be an indication of impaired

absorptive capacity in the intestine of rainbow trout caused by the soybean meal antinutrients (Refstie et al. 2000). Faecal DM values obtained with the retch wire screen method were similar for the different time intervals in individual diets. The similarities in faecal DM values and lack of significant difference with time of feces collection on the faecal collector for the diets demonstrates the low rates of leaching with time in the wire mesh collector. This lack of progressive leaching indicates lower exposure of feces to water on the wire mesh collector, which is important in reducing overestimation of apparent digestibility.

The results of nutrient digestibility by using the wire mesh collector indicate the low rate of leaching with time, considering the low numerical differences between the first collection interval and last. The differences in digestibility estimates demonstrating higher values in the retch wire screen method than stripping is in accordance with previous studies by Spyridakis et al. (1989) and Hajen et al. (1993) that showed higher AD estimates for feces collected from water medium to other faecal collection methods. The observed differences between ADs for the first collection interval (15 minutes) and the stripping method may suggest immediate leaching of nutrients after defecation. This is in agreement with the observation by Possompes (1973) showing that nitrogen leaching is rapid within the first 5 minutes. The same pattern of immediate leaching of other nutrients was also observed in this study.

The AD of organic matter as a measure of nutrient utilization/uptake shows that fish fed fishmeal had the highest apparent digestibility while the soybean meal diet was more completely digested than the rapeseed meal. The similarities observed among organic matter, carbon and nitrogen AD for the fishmeal diet fed group may be due to carbon and nitrogen being essential organic compounds in the nutrients digested by the fish. Fernández et al. (1998) have previously reported high correlation for dry matter, carbon and nitrogen digestibility in gilthead sea bream, fed fishmeal diets. They further explained that the high correlation observed in their study was due to the collective absorption of carbon and nitrogen, when proteins were hydrolysed into their amino acid components. The fishmeal diet in this study was highly digested due to its balanced amino acid composition and the lack of carbon containing fibres. This suggests that carbon, nitrogen and sulphur which are components of protein, that is more digested compared to other nutrients are also absorbed together. No similarities were however observed in the digestibility of soybean and rapeseed meal for organic matter, carbon and nitrogen using the stripping method. The difference in the plant based diets may be the effect of antinutrient factors that reduce digestibility of nutrients. For example, low digestibility of lipid caused by NSPs (Storebakken et al. 1998a)

or reduced protein digestibility in soybean meal (trypsin inhibitors, tannins) (Francis et al. 2001) and rapeseed meal (glucosinolates) (Burel et al. 2000a).

Carbon digestibility was higher in the fishmeal fed fish in both methods of fecal collection compared to the fishes fed the soybean meal and rapeseed meal diets. This is possibly due to the different levels of NSPs present in the plant based diets and the absence of antinutritional factors in the fishmeal. Lower AD of fat in soybean diet caused by the alcohol-soluble carbohydrate fraction and poor digestibility of fibre has been reported in soybean meal fed fish by Storebakken et al. (1998a). The lower digestibility of carbon observed in the rapeseed meal compared to soybean meal is probably caused by the higher content of fibre in rapeseed (Egli et al. 2002; Knudsen 2014). These effects of carbon containing fibres on carbon AD may be exerted through reduced lipid digestibility (Hajen et al. 1993; Kaushik et al. 1995). Considering that protein and carbohydrate also contribute to the carbon fraction in animal diets, the negative influence of antinutrients on either carbohydrate or protein digestibility may also reduce the carbon AD. Immediate leaching of carbon was not observed in the feces from fish fed fishmeal diets compared to feces from soybean and rapeseed meal diets. This is possibly due to the higher consistency of the fishmeal feces compared to the feces seen in the soybean and rapeseed meal diets. Leaching of carbon from the fishmeal feces was only evident at 60-minute collection interval, possibly due to continued exposure to water on the wire mesh collector.

Nitrogen ADs were higher using the wire mesh collector than the stripping method for the diets. The similar ADs observed for fishmeal and soybean meal diet with the stripping method is in agreement with previous study in rainbow trout by Refstie et al. (2000). The rapeseed meal diet showed lower AD, possibly because of the effect of rapeseed antinutrients (Burel et al. 2000a; Mwachireya et al. 1999) or heat treatment during rapeseed meal processing (Aslaksen et al. 2007). There was no substantial increase in leaching of nitrogen from feces over time. The differences observed between the ADs with the stripping method and the first collection interval (15 minutes) using the retch wire screen method are a result of immediate leaching after defecation. These differences were higher in the plant based diets compared to the fishmeal diet and could suggest the possible effect of NSP on increased water content in the feces. However, feces from the rapeseed meal fed fish had high dry matter content, comparable to that from fishmeal diet. In this study, lower differences were observed between the AD of nitrogen with the stripping and retch wire screen method for all the diets compared to the protein digestibility reported by Spyridakis et al. (1989), which described continuous filtration method as the most appropriate for feces collection from water

medium. The differences observed between stripping and sieve collection with freeze drying for nitrogen AD in the study by (Storebakken et al. 1998a) was smaller than observed in this study for fishmeal and soybean meal diet. This may be due to higher immediate leaching from the feces in this study, as low rates of leaching were observed for nitrogen on the wire mesh collector with time. Differences between the ADs obtained by the stripping method for the diets and AD obtained during the faecal collection intervals may also be lower, considering criticism of possible underestimation of the AD obtained by stripping the fishes (Vens-Cappell 1985). Stripping of feces in this study was however carefully done by applying pressure from the posterior intestine (pectoral fin) as recommended by (Austreng 1978). The decrease in nitrogen ADs observed among the diets between 120 and 240-minute collection interval suggests leaching of the indigestible marker.

The rate of leaching, both immediate and with time observed for sulphur was higher than what was observed for carbon and nitrogen. The pattern of high rate of leaching due to the lower consistency of feces resulting from consuming plant based diets was also observed for sulphur.

The AD for sulphur observed in the stripping method showed that sulphur was efficiently digested in the soybean meal fed group as there was no significant difference with the fishmeal fed group. The main sources of sulphur in both fishmeal and soybean meal were cysteine, methionine and taurine. The sulphur and nitrogen AD observed in the soybean meal diet confirms that protein was efficiently digested. The rapeseed meal diet fed group on the other hand showed the lowest AD for sulphur. This is possibly caused by reduced digestibility of sulphur containing amino acids in rapeseed meal or low digestibility of glucosinolates and other non-amino acid sulfonated components. The high feed intake observed for the rapeseed meal diet may therefore be to compensate for the low protein digestibility of the rapeseed diet. The low protein digestibility observed in the rapeseed meal fed fish is in agreement with previous findings in rainbow trout (Burel et al. 2000a; Mwachireya et al. 1999). Low feed conversion and growth observed for the rainbow trout fed rapeseed meal diet in this study was also reported by Burel et al. (2000a) at high and low glucosinolate levels in the rapeseed diet. Reduced protein digestibility is possibly an effect of glucosinolate metabolites, as previously reported with increasing levels of canola meal in juvenile hybrid tilapia (Zhou & Yue 2010). The effect of glucosinolates and its metabolites on reduced growth and feed efficiency seems to be caused through thyroid disturbances, leading to a reduction of the thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3) in the plasma (Burel et al. 2001). The effect of glucosinolates on the reduced digestibility of protein

is however not clear but the reduced sulphur digestibility observed in this study may provide insights for future investigations. Phytic acid has been reported to also influence protein digestibility through the formation of phytic acid-protein complexes (Francis et al. 2001; Mwachireya et al. 1999; Zhou & Yue 2010). The higher phytic acid level in rapeseed meal than soybean meal (Egli et al. 2002) may explain the lower protein AD for the rapeseed meal diet. The effect of fibre and phytate (tannins) on protein digestibility in rapeseed meal was reported to be more significant than that of glucosinolates by Mwachireya et al. (1999). It is however not clear if the reduced protein digestibility in this study is an individual effect of the antinutrients or a combinatory effect, as the antinutrient levels in the rapeseed meal were not measured.

5   Conclusion

The low rate of leaching observed with time using the retch wire screen and compared to other methods of feces collection in water indicates that it is an effective tool for feces collection from the water medium. The major challenge observed with collection of feces from water media in this study was the immediate leaching of nutrients after defecation. Further research is required to investigate possibilities of further reducing the leaching of nutrients on the retch wire screen by increasing the length or changing the shape of the support profiles under the screen. This would enable a more efficient drainage and reduce contact of feces with water on the wire screen.

The relative ranking of the apparent digestibility among the 3 diets in the ANOVA analysis showed the same statistical ranking, and this may facilitate use of the tool, eventually by the employment of a correction factor.

Nutrient digestibility was mostly affected in the rapeseed meal diet possibly due to antinutrient factors. An area of concern was the significantly low apparent digestibility observed for sulphur in the rapeseed meal fed fish. Further investigation could provide an understanding of the effect of rapeseed antinutrients on sulphur containing amino acids and more generally, protein digestibility.

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