

Faecal waste production, characteristics and recovery in European sea bass (*Dicentrarchus labrax*) is affected by dietary ingredient composition

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Abstract

The quantitative and qualitative characteristics of faecal waste was studied in European sea bass (*Dicentrarchus labrax*) fed diets with high inclusion of different feed ingredients (field peas, PEA; feather meal, FeM; sunflower cake meal, SFM); wheat dried distillers grain with soluble, WDG; corn gluten meal, CGM and soya protein concentrate, SPC). Each of the test ingredient was partially replaced with the basal mixture used in the control diet (CON). The ingredients were chosen for their varying levels of starch, protein, soluble and insoluble non-starch polysaccharide contents. Fish having an initial body weight of 120g were used (20 fish/tank in triplicate groups) in both trials and were fed at 2% of their body weight for 40 days. Apparent digestibility coefficients (ADC) of dry matter, nutrients (protein, fat, carbohydrate, ash and phosphorus) of the test diets were significantly altered between groups in trial I, with SFM showing least ADC for DM, carbohydrate and phosphorus; starch AC was the least in PEAbut only DM digestibility and phosphorus availability were different in trial II. The quantity, recovery percentage, physical characteristics, appearance and chemical composition of the feaces were affected by the test ingredients. Carbohydrate fraction of the diet was the most influential in affecting the quantity and chemical composition of faeces produced. Increased inclusion of NSP rich ingredients (WDG, soluble or SFM, insoluble) resulted in higher faecal recovery percentage, despite higher feaces load. Overall, high inclusion of alternate ingredients affected quantitative and qualitative characters of the faecal waste in European sea bass, which has implications for environmental sustainability of European sea bass aquaculture.

Key words: European sea bass; alternate ingredients; non-starch polysaccharides; nutrient digestibility; faecal recovery.

1. Introduction

To sustain the growth of aquaculture sector with the limited availability of marine based ingredients such as fishmeal (FM) and fish oil (FO), alternative ingredients must be included into aqua feeds (FAO, 2020; Naylor et al., 2009; Tacon and Metian, 2015; Tacon et al., 2010). Alternate ingredients used to replace FM in aqua feeds are protein sources of plant or animal origin. Plant origin ingredients include oilseed meals, glutens, protein concentrates etc. (Glencross, 2020; Glencross et al., 2007; Gomes et al., 1995; Kaushik et al., 1995); whereas, protein sources of animal origin include rendered animal products mainly of avian or porcine origin such as well as novel products such as insect meals (Bureau et al., 1999; Henry et al., 2015). Knowledge on the impact of feedstuffs on nutrient digestibility, production performance and health of the fish has increased over the years (Naylor et al., 2021). However, the impact of alternate ingredients on the faecal waste produced needs better understanding.

Concerns as regards the management of aquaculture wastes such as suspended solids, soluble nitrogenous excreta, phosphorus among others and the possible nutritional strategies have been put forward since long (Cowey, 1995) and have also been practically implemented at least as regards the salmonids (Cho and Bureau, 2001). Change in feed composition alters the type of nitrogen (N), phosphorus (P) and carbon (C) in the feed (Cho and Bureau, 2001). These changes affect nutrient retention in fish and thus nutrients released as waste in the rearing system (Schneider et al., 2004). Compared to fishmeal, feed ingredients of plant origin can contain higher levels of indigestible carbohydrates such as non-starch polysaccharides (NSPs) and oligosaccharides, which decrease the digestibility of the feed and increase faecal load to the water medium (Amirkolaie et al., 2005b; Prabhu et al., 2019). Phytic acid and other anti-nutritional factors can lead to increased excretion of phosphate and nutrients by the fish (Francis et al., 2001; Kokou and Fountoulaki, 2018). The increased faecal and nutrient load will vary depending on the rearing systems and eventually affect the environmental sustainability of aquaculture (Waite et al., 2014).

Dietary strategies are central to the good industry practices and suggestions of Water Framework Directive (WFD) and the Marine Strategy Framework Directive (MSFD) in relation ~~to~~ for mitigation against the impacts of organic enrichment through aquaculture (EC, 2016). Proper management of the faecal waste in aquaculture and consequently the suspended matter release into the environment requires control over not only the quantity but also the quality of the faeces produced. The amount of faecal waste produced is determined by the dry matter (DM) digestibility of the feed. For example, a decrease in DM digestibility from 90% to 80% implies a doubling of the solid waste excreted per kg of feed (Prabhu et al., 2019). In open aquaculture systems, the physical and chemical characteristics of the faeces respectively determines the nature (particulate or dissolved) and eutrophication potential of the waste (Amirkolaie, 2011). In open flow through systems, the biophysical characteristics of faecal waste (i.e., stability, density, settling velocity etc.) have significant implications for the deposition and dispersal of solid waste and environmental impact on receiving water bodies (Reid et al., 2009). In recirculation aquaculture systems, the characteristics of the faecal waste affects the operational management of the system such as solid removal, bio-filtration, water refreshment rate etc. Previous studies have shown that physical characteristics of the faeces can be affected by diet composition in tilapia, carp and trout and thereby alter the effectiveness of water quality management measures (Amirkolaie et al., 2005b; Brinker et al., 2005; Prabhu et al., 2019). Possible changes in the consistency and properties of fish faeces can also change their dispersion and affect the distance over which the effects of aquaculture on sediment are apparent (Ballester-Moltó et al., 2017; Cripps, 1995; Kelly et al., 1997). For example, dietary inclusion of guar gum in Nile tilapia, African catfish and rainbow trout has shown to alter physical properties of faeces thereby affecting the efficiency of solid waste removal

(Amirkolaie et al., 2005a; Brinker, 2007; Leenhouwers et al., 2007). It is known that specific dietary components (bulk agents), can significantly affect feed transit time and digestibility in European seabass (Dias et al., 1998). Research on FM and FO replacement in fish feeds and particularly in European seabass feeds has focused much on growth performance, product quality and health aspects (Glencross, 2020; Kaushik et al., 2004; NRC, 2011; Torrecillas et al., 2017). Our understanding on the dietary factors that affect the biophysical properties of fish faeces is limited, and little is known of the consequences of alternative feed ingredients on waste characteristics, especially for marine fish. Given the importance of inclusion of increasing levels of cost-effective feed ingredients of plant or animal origins to replace FM, knowledge on the possible environmental impacts of such changes on waste outputs is warranted. . The aim of the present study was to evaluate the effect of high inclusion levels of seven different ingredients in feeds for European sea bass (*Dicentrarchus labrax*) on nutrient digestibility, waste production and faeces properties. The ingredients were chosen based on their nutrient/antinutrient composition and most of them are widely used in aqua feeds.

2. Material and methods

2.1. Test ingredients and experimental diets

Six raw materials differing in their nutrient/antinutrient properties were evaluated in two separate feeding trials for their effects on nutrient digestibility, faecal properties and waste production. The ingredients studied were feather meal, high in indigestible protein; peas, high in starch; sunflower meal and wheat distilled grain from soluble, high in insoluble and soluble NSP respectively; corn gluten meal and soy protein concentrate high in protein content but with no starch and NSP. The proximate composition of the ingredients used for feed production is presented in Table 1. The formulation of the experimental diets used in trials I and II are presented in Table 2. A fish meal-based diet was formulated and used as a control in both feeding trials (CON diet). All the test diets contained 70% of a basal ingredient from the control diet and 30% or 25% of the test ingredient in trials I and II, respectively. In trial I, four raw materials were selected as follows: field peas, having a high starch, but low NSP content (PEA diet), hydrolysed feather meal, having a high indigestible protein content (FeM diet), sunflower meal, having a low in starch, but a high insoluble NSP content (SFM diet), wheat dried distilled grain with soluble, having a low starch, but a high soluble NSP content (WDG diet). In the second feeding trial, two ingredients were selected according to their high protein content, low or zero content of starch and NSP. The ingredients evaluated in trial II were corn gluten meal (CGM diet) and soya protein concentrate (SPC diet), included at 25% to a basal diet. All diets contained 0.05% yttrium oxide as an inert marker for digestibility evaluation. The experimental diets were produced by SPAROS LDA (Portugal). In brief, all ingredients were finely ground, mixed and extruded by means of pilot-scale twin-screw extruder (model BC45, CLEXTRAL, France) with a screw diameter of 55.5 mm and temperature ranging 109-113°C. Upon extrusion, feeds were dried in a vibrating fluid bed dryer (model DR100, TGC Extrusion, France). Following drying, pellets were allowed to cool at room temperature and subsequently, the oil fraction was applied to the extruded pellets by coating under vacuum conditions (PG-10VCLAB, DINNISEN, The Netherlands). Analysed proximate composition of the diets are given in Table 3.

2.2. Experimental conditions, feeding and faecal collection

In both trials, European sea bass (*Dicentrarchus labrax*) juveniles of 120g initial body weight were used. Triplicate groups of 20 fish were assigned to 15 x 250 L cylindroconical fibreglass tanks in an open flow through water system kept at 5 L min^{-1} so that the faeces after being egested were quickly transferred to the traps (time needed less than 20 sec) in order to avoid leaching and disintegration of the faeces pellets. Photoperiod was kept at 12:12 h, light: dark regime and all water physicochemical parameters (dissolved oxygen, temperature etc.) were checked daily. The conditions regarding the experimental system used were identical in both experiments, except for water temperature. Water temperature during trial I ranged from 20-22°C, whereas in trial II it was 25-27°C. Salinity in both trials was 34-35 ppt.

Each diet was hand-fed to triplicate groups of fish at 2% of their body weight in two meals per day, the 1^{st} at 9:00 and the 2^{nd} at 16:00, for 40 days. Faeces collection started after 30 days of rearing and was performed every day over the last 10 days. Faeces were collected in faecal traps submerged in ice to prevent bacterial decay of the collected faeces, using a modification of the Guelph method (Cho et al., 1982). The fish were fed at 09.00 h and faeces collection commenced 2 hours after feeding until late in the afternoon in 100 ml containers. The faeces collected in the container each day were pooled per tank into an aluminium tray and kept frozen in -20^{0}C until they were freeze-dried and analysed. Further, freshly ejected faecal pellets were sieved for 20 min in circular 2 mm mesh placed in the faecal traps and carefully collected in petri dish for the estimation of dry matter, density, sinking velocity, osmolality, viscosity and faeces appearance.

2.3. Physical characteristics of the faeces

Dry matter was measured by drying a pre-weighed pooled sample (n=10-20) of freshly collected faeces pellets at 105^{0}C for 24 hours to constant weight. Density of faecal pellets was determined by immersing 10-20 pre-weighted faecal pellets in graduated cylinder (0.05 ml) containing sea water. The water level change was recorded and values were expressed in g/ml. Sinking velocity was measured using a graduated cylinder (height, 100 cm and diameter, 7.5 cm) filled with sea water. Briefly, freshly collected faecal pellets (n=10) from each experimental tank were placed on petri dishes and dropped individually into the measuring cylinder. The time needed for each individual feacal pellet to descend a determined distance (cm) per second (s) was recorded as the sinking velocity (cm/sec). Viscosity and osmolality were determined in supernatant of centrifuged stripped faeces (12,000 g for 10min) as described elsewhere (Leenhouwers et al., 2007).

Osmolality was determined in 0.05ml of supernatant collected after centrifugation (12.000g for 10min) from a pooled sample of freshly collected faeces pellets (n=10-20). The measurement was performed by a cryoscopic osmometer (OSMOMAT 030 Gonotec GmbH Berlin) and expressed as osmol/kg. Viscosity measurements were performed in feed and faeces (Leenhouwers et al., 2007). Briefly, 1g of feed sample and 3 ml of distilled water was added and left to incubate in a centrifuge tube for 30 min at 38^{0}C. Later, the tube was subjected to centrifugation at 10000 rpm for 10 min at room temperature (RT), and the supernatant was used for viscosity measurements. In the faeces, a sample of freshly collected faeces was centrifuged at 12000 rpm for 10 min at RT and the supernatant was used for viscosity measurements. Subsequently, the viscosity was measured in the collected supernatant immediately using a Brookfield LVDV-I+ cone/plate viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, USA). All measurements were done at 25^{0}C at a shear rate of 2.25-750 s^{-1} Absolute viscosity was expressed in centipoise (cP) at a shear rate of 750 s^{-1}.~~2.4.~~

2.4. Biochemical analysis

Ingredients and feed samples were ground using a 1 mm screen before analysis. Freeze-dried faeces from each tank were ground and thoroughly homogenised to obtain representative sub-samples. All chemical analyses were performed in triplicate. Proximate composition of feeds and freeze-dried faeces was determined according to standard laboratory methods (AOAC, 2005) as follows: Dry matter after drying at 105^{0}C until a constant weight was obtained. Ash content by incineration in a muffle furnace for 12 h at 550^{0}C (AOAC, 2005. Crude protein (Nx6.25) according to the Kjeldahl method (Kjeltec Auto Tecator, Foss Tecator). Total fat in extruded feeds was determined by first hydrolysing the sample with HCl followed by ether extraction in a Soxhlet apparatus (SOXTEC SYSTEM HT, 1043 Extraction unit Foss Tecator). Total lipid in faeces by the phosphovanillin method (Nengas et al. 1995). Starch by an enzymatic method (Megazyme Total Starch Assay kit (AA/AMG), Megazyme International, Ireland) (McCleary et al., 1994), using thermostable α-amylase and amyloglycosidase. For the raw materials, a slight modification of the dimethylsulphoxide methodology was followed in order to achieve better solubilisation of starch. This included incubation of the pea samples for 15 min in dimethylsulphoxide in a boiling water bath under continuous stirring. Total non-starch polysaccharides (NSP) were determined spectrophotometrically with a modification in the calculation of the total NSP content (Englyst et al., 1994). The content was calculated using a standard curve derived from a standard sugar solution, consisting of 4.01 g/l of arabinose, 9.92 g/l of glucose and 3.56 g/l of galacturonic acid. The modified standard solution was based on the actual sugar proportions found in raw materials from literature (Knudsen, 1997). Carbohydrate content of faeces and diets was also calculated by difference [DM-(crude protein+crude fat+ash)]. Phosphorus in both feeds and faeces was determined using the vanado-molybdate method after sample combustion at 550^{0}C and digestion with acid (Aspila et al., 1976). Yttrium was determined by ICP-MS method. Briefly, samples were homogenized, and microwave digested with nitric acid in a closed vessel (microwave digester; CEM Marsxpress). The resulting digestate was then diluted to volume with ultrapure water and analyzed for yttrium. Volatile fatty acids (VFA) were measured in the digesta as an indicator of the occurrence of fermentation in the intestine of the fish. Fresh collected faeces (0.5 g) were added to 1 ml distilled water and 50 μl of 85% phosphoric acid mix and stored at -20$^{\mathbf{0}}$C until analysis. The analysis was based on the method described by García-Villalba et al. (2012), by GC-MS on a Hewllet-Packard 6890 GC-MSD system. A DB-WAXetr (30mX0.25mm, 0.25μm film thickness) analytical column was used and the oven temperature program was: initially 90 ^{o}C, then increased to 150 ^{o}C at 15 ^{o}C/min, to 170 ^{o}C at 5 ^{o}C /min and finally to 250 ^{o}C at 20 ^{o}C/min and kept at this temperature for 2 min.

2.5. Calculations and statistical analysis

2.5.1. Digestibility

The apparent digestibility coefficient (ADC) of dry matter, crude protein, crude fat, starch, carbohydrates and phosphorus was estimated by an indirect method using yttrium oxide as the inert marker by the equation (Cho and Kaushik, 1985):

ADC% = 100 * (1-(Fnutr * Dy) / (Dnutr * Fy));

where, Fnutr= nutrient concentration in faeces, Dnutr= nutrient concentration in diet, Dy = yttrium concentration in diet and Fy =yttrium concentration in faeces.

2.5.2. Faeces recovery measurements

During the last 10 days of the experiment, fish were fed their daily ration of feed (g) and then faeces were quantitatively collected dried and weighted. The percentage of faeces recovery was calculated using the total amount of yttrium in excreted faeces and the total amount of yttrium in the consumed feed (Amirkolaie et al., 2005a), where the total amount of yttrium in the excreted faeces is the amount in DM of collected faeces multiplied by the yttrium concentration in the faeces. The total amount of yttrium oxide of consumed feed is the total amount of consumed feed in DM multiplied by the yttrium concentration in the feed.

2.5.3. Particle size distribution of faeces

The faeces were collected as previously described by sieving through a 2 mm mesh. Samples were measured in length (mm) and weighed individually to 1 mg accuracy. The purpose of these measurements was to determine if the different raw materials used in the diets affected the appearance of faecal pellets and to check if there was a correlation between faeces length and faeces weight. The appearance of the faeces was performed by an Image analysis software (Digital Image Systems, Athens, Greece).

2.5.4. Amount of waste produced

The total faecal waste produced consists of the recovered faeces plus the non-recovered faeces. The total amount of faeces produced was calculated based on the dry matter digestibility. The amount of non-recovered faeces is the difference between the faeces recovered from the settling tanks and the calculated amount of total faeces produced.

2.5.5. Statistical analysis

All results were introduced in a data-base (MS-Excel®) and mean and standard deviations of each treatment were calculated. All data were checked for normal distribution using the One-Sample Kolmogorov-Smirnov test and for homogeneity of variances using Levene's test. Differences between diets were determined by a one-way ANOVA using SPSS 16.0® at 0.05 significant levels. The figures were made in GraphPad Prism v8.

3. Results

3.1. Digestibility

Apparent digestibility coefficient (ADC) values from experiments I and II are provided in Table 4 and 5, respectively. In trial I, the results showed that ADCs of nutrients were significantly affected by the raw materials added in the diet ($p<0.001$). Control diet (CON diet) exhibited the highest ADC values for dry matter, protein and fat. ADC values for protein and fat were high for all diets (>91%), except for the FeM diet that gave the lowest values (91.5%, 91% respectively) significantly different from all other diets. PEA diet showed the lowest ADC value for starch (78.7%) significantly different from all other diets while the SFM diet showed the lowest dry matter digestibility (64.1%) which was significantly different from all other diets. Phosphorus digestibility in the SFM diet (57.1%) was significantly lower than all other diets. FeM diet showed the higher value (72.5%) significantly different from all other diets except WDG diet (67.4%) while values for the CON and the PEA were intermediate (66.9 and 66.3% respectively). In trial II, dry matter digestibility was significantly higher in the CON and CGM diet compared to the SPC diet ($p<0.05$). Protein, fat and starch ADC's were high for all diets and no significant differences were found. Phosphorus digestibility was significantly higher in CON and CGM diet, 40.0% and 41% respectively ($P<0.01$), while SPC diet gave the lowest value (26.8%). Comparison of ADC values for the CON diet between experiments I and II showed that values for all nutrients, except fat, were significantly lower in the II trial, reflecting the impact of temperature (20-22 versus 25-27°C) on nutrient digestibility ($p < 0.01$). The highest difference was observed on phosphorous ADC (66.9% vs. 40%, $p<0.000$).

3.2. Faecal composition

The proximate composition of the faecal waste in both feeding trials is summarised in Tables 6 and 7. In trial I, the proximate composition of the faecal waste was strongly affected by diet fed to sea bass ($p<0.001$). The ash fraction represented the largest fraction of the faecal waste. However, it should be noticed that this is related to the drying of the wet faeces which includes sea water. The second largest fraction was the carbohydrate fraction. PEA diet fed group, contained the highest starch (16%), while carbohydrate content other than starch was the highest in the SFM diet (39.2%). Faeces from the FeM diet had the highest crude protein and fat content and the lowest carbohydrate content. Faecal phosphorus content was significantly higher in the CON group compared to all others. In trial II, the largest fraction in the faecal waste (on DM basis) was that of carbohydrate same for all diet groups. The next largest fraction was ash, which was significantly higher in both the SPC and CON diets (32.5 and 31.4% respectively) compared to the CGM diet. Starch content in faeces differed between diets ($p<0.01$) being higher in the CON and CGM group (22.2% and 20.2% respectively). No differences were apparent in protein, fat and phosphorus content of the faeces. Faecal waste composition from fish fed the CON diet between the experiments I and II showed minor differences for dry matter, crude protein, fat and phosphorous. Starch and ash content in the faeces showed significant differences between the two trials. Faecal starch content was significantly higher (22.2% vs 2.8%, $p <0.001$ trial II and I respectively) and ash content was lower (31.4% vs 46.3%, $p <0.000$ trial II and I respectively). The analysis of total volatile fatty acids (VFA) in faecal waste in the present study indicate the occurrence of microbial fermentation in the intestine (Fig. 3). The only VFA produced was acetic acid and significant differences were evident between the CON and the SFM diet while no difference occurred with the rest of diets. The highest value was given by SFM diet (4.54μmol/g) and the lowest by the CON diet (2.6 μmol/g).

3.3. Characteristics of faecal pellets

The results on faeces characteristics from the measurements performed on the faecal pellets from trial I and II are respectively given in Table 8 and 9. In trial I, faeces density was similar for all the diets, ranging from 1.15 to 1.27g/ml. Dry matter of faecal pellets was significantly affected by the test ingredients ($p<0.001$). Faecal pellets from fish fed the CON and WDG diets had the highest and lowest values respectively DM (19.8 vs. 15.5%, $p<0.001$), while the other groups had intermediate values and did not differ significantly between them. Sinking velocity of the faecal pellets was similar for all diets ($P>0.05$). Osmolality showed significant differences among diets. CON and WDG groups had the highest and lowest values (1.26 and 1.15 Osmol/kg respectively ($P<0.05$)). Viscosity measurements in faeces ranged from 2.42 - 3.62cP and faeces pellets from fish fed the WDG diet was significantly more viscous than all other groups ($P<0.001$). In trial II, dry matter of the faecal pellets was significantly affected by the dietary treatments being lower for the SPC fed group (13.3%) compared to the CON and CGM (15.8 and 16.1% respectively). Density, osmolality, viscosity and sinking velocity of the faecal pellets did not differ between the three experimental groups.

Concerning the measurements of the faecal pellets in length and weight, a high variability was exhibited, making difficult to draw reliable data. Faeces appearance was evaluated by an image analysis and therefore, representative images were taken (Plate 1). The large variability in faeces appearance hampered the classifications of the faeces into a large and small fraction (expressed in %). Qualitative observations suggested that faecal pellets from the CON diet tended to be longer and firmer, being followed by faecal pellets from the WDG diet and SFM diet. On the contrary faecal pellets of the fish fed the PEA and FeM diets were shorter in length and appeared to be disintegrated. Faeces appearance was evaluated but as in trial I, no reliable data could be extracted. In general, faeces pellets were smaller and appeared to be disintegrated in all diet groups. When comparing the faecal pellets of the CON diet between trial I and II, those in trial II appeared to be more fragile and disintegrated easier.

3.4. Faeces recovery

Total amount of faces produced, recovered and non-recovered in absolute quantities and their proportion from trial I and II are presented in Figure 1 and 2, respectively. In trial I, faeces produced per kg of feed (on DM basis) was significantly high in SFM fed fish, WDG and PEA groups were intermediate, while CON and FeM fed fish had the lowest (Fig. 1A, $p<0.001$). The amount of recovered faeces in g kg^{-1} feed (on DM basis) was the highest in SFM followed by WDG group whereas the lowest was observed in FeM (Fig 1A, $P<0.001$). The quantity of non-recovered faeces was significantly high in PEA and FeM groups, followed by SFM and the lowest in CON and WDG group ($P<0.001$). The highest recovery percentage as shown in Figure 1B, was observed in CON, SFM and WDG diets fed fish (88%, 76% and 85%, respectively), FeM and PEA fed fish exhibited significantly lower recovery percentage of 60 and 53% respectively. In trial II, the total amount of faeces produced was significantly higher in CGM fed fish diet compared to CON and SPC (Fig. 2A). The amount of recovered faeces did not differ significantly between groups; however, the amount of non-recovered faeces was significantly higher in the CGM diet ($P<0.01$). Consequently, percentage faeces recovery was the lowest in CGM diet fed group (52.2%, $p<0.05$) while the highest was found in SPC (72%), followed by CON (60%) (Fig. 2B). Differences were significant only between the SPC and CGM diets. Total faeces production in CON fed fish diet was significantly lower in trial I than in trial II (208 vs. 281g DM/kg feed DM), as faeces recovery percentage was significantly lower (60% vs 87.5) in trial II compared to trial I, thus resulting in increased non-recovered faeces at higher temperature.

4. Discussion

The management of waste discharged from fish farms is one of the major concerns for the further development and environmental sustainability of aquaculture (Naylor et al., 2021, 2009). Diet related strategies to manage aquaculture waste have predominantly focused on reducing waste production through improved feed conversion and/or nutrient digestibility (Gatlin III et al., 2007; Sales, 2009). In addition, dietary effects on the physical properties of fish faeces have gained significance as a waste management strategy, especially in closed systems (Meriac et al., 2014; Prabhu et al., 2019). Highly digestible feeds maximise fish production and minimise waste released to the environment. In this study, all diets exhibited high ADC values for protein, fat and significant differences were evident only with the CON diet. Values were similar with those reported in literature for other species, where different plant raw materials were used at high inclusion levels in common carp (Prabhu et al., 2019) and Nile tilapia (Amirkolaie et al., 2005b). In European sea bass, digestibility values for protein and fat reported using two different types of corn distillers dried grains fed diets were similar with those reported in the present study (Magalhães et al., 2015). The SFM diet containing the highest carbohydrate level (34% starch included) mostly as insoluble NSP showed the lowest dry matter (DM) digestibility compared to the rest of the diets affecting the quantity as well as the recovery % of the solid waste produced. In rainbow trout, different types of carbohydrate fractions affected DM digestibility of the diet, starch having the least effect, whereas NSPs like cellulose and pectin significantly reduced DM digestibility (Glencross et al., 2012). In Nile tilapia, dietary soluble NSPs reduced nutrient digestibility and faeces recovery, whereas insoluble NSPs decreased carbohydrate digestibility, but increased faeces recovery

(Amirkolaie et al., 2005b). Feeding an SFM diet to carp, the reduction of DM digestibility by 10% in comparison to the other diets (PEA, WDG, FEM) lead to a 50% increase in total faeces and a two-fold increase in non-recovered solids/kg feed consumed (Prabhu et al., 2019). In the SFM diet, the NSPs account for more than half of the carbohydrate content (Knudsen, 1997), consisting mainly of non-soluble fractions like cellulose (42%), pectins (24%), glucuronoxylans and uronic acids (24%), (gluco)mannans (5%) and fucoxyloglucans (4.5%) (Düsterhöft et al., 1992). In the present study, the major increase observed in total and non-removed faeces when fed SFM diet could be attributed to the non-soluble NSPs as found in carp (Prabhu et al., 2019). Phosphorus digestibility values in fish have been reported to have a wide range 8-75% depending on its source (Cheng and Hardy, 2002; Sugiura et al., 1998). In European seabass, the ADC of phosphorus of animal feedstuffs (fish protein hydrolysate, blood meal, meat meal) averaged 81% whereas that of soybean meal was 38% (Oliva-Teles, 1998). In trial I, replacement of FM with 30% SFM showed the lowest phosphorus digestibility, similar to our study in carp (Prabhu et al., 2019), where lower values were found in SFM based diet when compared to the other diets (34.5%). In both experiments of this study, it can be seen that the SFM diet exhibited also the lowest dry matter digestibility. NSP's content in SFM is high and consists mainly of insoluble fractions as described above. Non-soluble NSP have been reported to reduce not only dry matter digestibility but also phosphorus availability (Francis et al., 2001; Prabhu et al., 2019). The same pattern was observed in the second trial where the addition of SPC at levels of 30% exhibited the lowest values both in dry matter digestibility as well as phosphorus digestibility. The lower phosphorus ADC values of the SPC compared to the control and CGM diet is attributed to the higher phytic acid content in SPC (Storebakken et al., 2000, 1998). On the contrary, phosphorus ADC form CGM was lower compared to SPC in European seabass, nevertheless the authors attributed it to the overall poor digestibility of CGM due to bigger particle size (Dias et al., 2005). The comparison of ADC values for the

CON diet between trials I and II showed that ADC values for all nutrients, except fat, were significantly lower in trial II. The only difference between the two experiments was water temperature (20-22 vs 25-27°C) ($p < 0.01$). The highest difference was observed on phosphorous ADC (63% vs. 40%, $p<0.000$) suggesting that water temperature can affect phosphorus digestibility. Temperature has been shown in previous studies to affect nutrient digestibility in fish (Arnesen et al., 1993; Azevedo et al., 1998; Jobling, 1997), however in salmonids, this effect is unclear (Bendiksen et al., 2003; Ng et al., 2004). Some authors found decreased nutrient digestibility at reduced temperatures in Atlantic Salmon, rainbow trout, and yellowtail (Azevedo et al., 1998; Bendiksen et al., 2003; Miegel et al., 2010), whilst others found no effect of temperature on nutrient digestibility in rainbow trout (Austreng, 1978; Cho and Kaushik, 1990). Dry matter, protein and energy digestibility were not affected by diet or temperature in European seabass, nevertheless the ADC of starch was higher at 25 °C than at 18 °C (Moreira et al., 2008). Despite water temperature being the only plausible explanation to the differences in ADC of DM and starch, faeces recovery and thus solid waste, as well as faecal physical characteristics in European sea bass between the two experiments, the influence of other factors cannot be overruled.

In the present study the chemical composition of the faeces varied strongly when fed diets having a different ingredient composition. In general, the major fraction of the faeces consisted mainly of carbohydrates, followed by ash, protein and fat. A large variability in the carbohydrate and ash fraction was observed similar to the findings in common carp (Prabhu et al., 2019), nevertheless, higher than the values of the present study. Fat content in faeces was generally low, but significantly higher in FeM diet due to the low fat digestibility (Bureau et al., 1999; Prabhu et al., 2019). The crude protein content of the faeces was significantly higher in FeM diet which was a result of the high dietary crude protein level, as observed in common carp (Prabhu et al., 2019). The chemical composition of faeces is related to digestibility, thus by improving nutrient digestibility the quantity and composition of the faeces can be altered. The dietary inclusion of exogenous enzymes like phytase and xylanase in diets containing raw materials rich in either insoluble or soluble NSP, has been shown to improve nutrient and DM digestibility in Nile tilapia (Maas et al., 2018) and sea bass (Fountoulaki et al. unpublished data). In this way, NSP rich raw materials in fish feeds can still be used with minimal effect to the composition of the solid waste produced.

The analysis of total Volatile Fatty Acids (VFA) in faecal waste in this study showed that the only VFA produced was acetic acid indicating the occurrence of microbial fermentation in the intestine in European sea bass. In previous studies with African catfish and Nile tilapia, the major VFA produced was acetic acid (Amirkolaie et al., 2006; Leenhouwers et al., 2007). The highest value was found in faecal pellets of fish fed the SFM diet which however, differed significantly only from the control diet. It has been suggested that high digesta viscosity might stimulate intestinal fermentation activity as in Nile tilapia and African catfish (Amirkolaie et al., 2006; Leenhouwers et al., 2007). However, this was not observed in the present study in European sea bass, where VFA concentrations remained constant in the gastrointestinal tract (Schrama et al., 2005). Properties such as density, sinking velocity, osmolality and viscosity of the faeces determines the removal efficiency and the type of waste associated, dissolved or solid (Unger and Brinker, 2013). Concerning the physical characteristics of faecal pellets, in both the trials, density was unaffected by the inclusion of different raw materials. Furthermore differences in water temperature in both experiments did not show any affect in the feacal density of the CON diet. In the present study, due to the great variability in faecal sizes, it was not possible to find a relationship between faeces pellet size and sinking velocity (Magill et al., 2006). In salmonids, faecal mass is reported as a poor predictor of settling rate, whereas density appears to be of greater significance with a positive relationship between sinking speed and density (Chen et al., 2003; Moccia et al., 2007; Ogunkoya et al., 2006). The results of the present study showed no differences concerning the viscosity of faecal pellets for all diets except for significantly higher viscosity when fed WDG containing soluble NSP’s. High viscosity values have been previously reported in the digesta of Nile tilapia fed on diets containing soluble NSP’s as starch or guar gum and in African catfish fed a basal diet containing soluble NSP (guar gum), or grains in order to obtain a range of dietary viscosities in soluble and insoluble forms (Amirkolaie et al., 2006; Leenhouwers et al., 2006, 2007). Fish fed the WDG diet were found to have the lowest dry matter possibly due to an increase in digesta viscosity driven reduction in the dry matter content of digesta (Leenhouwers et al., 2007; Refstie et al., 1999; Storebakken and Austreng, 1987). Faeces recovery as an indicator of faeces stability showed to be affected by the raw materials used in the experimental diets in sea bass in line with results reported in literature for Nile tilapia (Amirkolaie et al., 2006, 2005b), rainbow trout (Brinker, 2007; Meriac et al., 2014) and common carp (Prabhu et al., 2019). The measured recovery percentage ranged from low (52.6% FeM diet) to high (87.5% CON diet). Both insoluble and soluble NSP’s content in diets SFM and WDG respectively, increased faeces recovery in comparison to PEAS and FeM diet. Moreover WDG diet containing soluble NSP’s exhibited the highest recovery in accordance with Prabhu et al. 2019 in carp, rainbow trout (Brinker, 2007; Brinker and Friedrich, 2012) and contrary to the reduced ~~faeces~~ recovery in Nile Tilapia fed cellulose, an insoluble NSP’s (Amirkolaie et al., 2005b). Different species respond differently to dietary ingredients, consequently in order to achieve a better faecal stability and an effective solid waste recovery especially in land-based systems more research is needed in this direction.

To conclude, different raw materials at 30% inclusion level significantly affected nutrient ADC values in European sea bass. The largest variability between diets was due to the carbohydrate fraction and, diets rich in NSPs in soluble or insoluble form resulted in higher recovery percentage. Soluble NSP’s resulted in higher DM digestibility and thus higher recovery percentage. Differences in faecal physical characteristics were observed in sea bass attributed to different test ingredients. Waste reduction strategies through dietary ingredient manipulation for marine fish aquaculture, land based or sea cages, is of primary importance and in combination with cost effectiveness of the feed, will contribute towards environmental and economical sustainability of aquaculture.

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Table 1. Proximate composition of the raw materials used in diet formulations for both feeding trials (as fed basis, %).

	Fish meal	Field Peas	FeM[1]	SFM[2]	WDG[3]	CGM[4]	SPC[5]
Moisture	7.40	6.80	7.00	7.60	6.30	6.5	5.0
Ash	17.00	4.70	2.60	6.80	5.50	0.9	0
Protein	67.80	24.40	83.90	34.60	32.80	82.1	64.7
Fat	7.50	0.70	7.40	1.90	7.10	1.3	1.0
Starch	0	41.6	0	1.00	1.00	7.4	0
NSP	0	8.3	0	41.16	44.2	1.0	3.5

[1] Hydrolyzed feather meal, [2] Sunflower meal, [3] Wheat DDGS, [4] Corn gluten meal, [5] Soy protein concentrate**.**

Table 2. Formulation of the experimental diets used in trial I and II (on as fed basis)

	Trial I					Trial II	
	CON	**PEA**	**FeM**	**SFM**	**WDG**	**SPC**	**CGM**
Fishmeal, >68%	32.00	22.40	22.40	22.40	22.40	24.00	24.00
Soybean meal, 48	12.00	8.40	8.40	8.40	8.40	9.00	9.00
Wheat	20.25	14.16	14.16	14.16	14.16	15.17	15.17
Wheat gluten	6.00	4.20	4.20	4.20	4.20	4.50	4.50
Corn gluten meal, 60	12.00	8.40	8.40	8.40	8.40	9.00	9.00
Fish oil	6.00	4.20	4.20	4.20	4.20	4.50	4.50
Rapeseed oil	9.00	6.30	6.30	6.30	6.30	6.75	6.75
Vitamins & mineral[1]	1.00	0.70	0.70	0.70	0.70	0.75	0.75
NaH2PO4 H2O	0.80	0.56	0.56	0.56	0.56	0.60	0.60
L-Lysine	0.90	0.63	0.63	0.63	0.63	0.68	0.68
Field peas		30.00					
Hydrolyzed feather meal			30.00				
Sunflower meal				30.00			
Wheat DDGS					30.00		
Soy protein concentrate						25.00	
Corn gluten meal, 60							25.00
Yttrium oxide	0.05	0.05	0.05	0.05	0.05	0.05	0.05

CON=control diet, common for both experiments; PEA=Pea diet; FeM=Feather meal diet; SFM=Sunflower cake diet; WDG=Wheat distillers grain diet. SPC soy protein concentrate diet; CGM corn gluten meal diet.

[1] Vitamin & mineral premix contained (mg/kg diet): vitamin A 6; vitamin D 0.05; vitamin E 100;vitamin K 25; Thiamine 30; Riboflavin 30; pantotenic acid 100; nicotinic acid 200; pyridoxine 20; folic acid 15; vitamin B12 0.1; vitamin C 1000; inositol 500; choline 1000; betaine 500; Co 065; Cu 9; Fe 6; I 0.5; Mn 9.6; Se 0.01; Zn 7.5; Ca 18.6%; Cl 2.41%.

Table 3. Proximate composition (%) of the experimental diets used in trials I and II (on dry matter basis)

	Trial I					Trial II	
	CON	**PEA**	**FeM**	**SFM**	**WDG**	**SPC**	**CGM**
Dry matter, %	93.6	90.7	91.1	94.2	89.3	96.8	95.0
Ash, %	8.49	7.06	7.12	8.34	7.88	7.19	5.62
Protein, %	46.55	40.55	58.52	41.39	43.73	51.58	52.3
Fat, %	19.74	13.98	15.95	14.49	16.42	17.98	20.92
Starch, %	14.1	20.0	9.2	8.9	8.2	10.27	13.47
Rest carb, %	7.32	10.74	7.83	15.45	15.72	ND	ND
Phosphorus, %	1.04	0.79	0.78	0.87	0.82	0.71	0.66
Energy MJ/Kg	23.14	21.74	23.30	21.68	22.34	23.30	24.26
Yttrium, mg/100g	42	41	41	41	39	44	43

CON=control diet, common for both experiments; PEA=Pea diet; FeM=Feather meal diet; SFM=Sunflower cake diet; WDG=Wheat distillers grain diet. SPC soy protein concentrate diet; CGM corn gluten meal diet. NFE (Nitrogen free extract). NSP, non-starch polysaccharides; ND, not determined. Rest carb, includes carbohydrates other than starch (NSP, Fibre, etc).

Table 4. Nutrient digestibility (ADC) values (in %) of feeds in trial I with European sea bass

	CON	PEA	FeM	SFM	WDG	P-value
Dry matter	79.0±1.4[c]	70.7±1.3[b]	75.7±1.95[c]	64.1±1.1[a]	71.5±1.0[b]	***
Crude protein	94.3±0.5[c]	92.3±0.2[b]	91.5±0.4[a]	93.5±0.01[bc]	92.8±0.2[b]	***
Fat	95.8±0.2[d]	94.4±0.8[c]	91.0±0.6[a]	92.7±0.4[b]	94.0±0.1[bc]	***
Starch	96.13±0.3[c]	78.67±1.3[a]	97.88±0.9[d]	94.45±0.4[b]	96.04±0.3[c]	***
Phosphorus	66.9±1.0[b]	66.3±0.6[b]	72.5±3.1[c]	57.1±2.1[a]	67.4±2.2[bc]	**

CON=control diet; PEA=Pea diet; FeM=Feather meal diet; SFM=Sunflower cake diet; WDG=Wheat distillers grain diet. ns=not significant P>0.1; *P<0.05; **P<0.01; ***P<0.001. Means along a row lacking a common superscript letter differ significantly, P<0.05.

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Table 5. Nutrient digestibility (ADC) values (in %) of feeds used in trial II with European sea bass

	CON	SPC	CGM	P-value
Dry matter	71.9±1.9[a]	68.1±1.5[b]	71.7±1.0[a]	*
Crude protein	92.2±0.5	91.7±0.7	92.5±0.3	ns
Fat	95.2±1.0	91.8±1.8	93.1±1.2	ns
Starch	82.48±4.1	85.71±0.4	81.53±1.34	ns
Phosphorus	40.0±2.6[b]	26.8±4.1[a]	41.0±1.7[b]	**

CON=control diet; SPC, soy protein concentrate ; CGM, corn gluten meal. ns=not significant P>0.1; *P<0.05; **P<0.01; ***P<0.001. Means along a row lacking a common superscript letter differ significantly, P<0.05.

Table 6. Proximate composition (%) of faecal waste from European sea bass fed different diets in trial I

	CON	PEA	FeM	SFM	WDG	P-value
Dry matter	95.9±0.7[a]	96.1±0.4[ab]	96.6±0.4[ab]	97.2±0.3[b]	96.9±0.3[ab]	*
Crude protein	12.7±0.4[c]	10.0±0.5[b]	20.6±1.3[d]	7.5±0.2[a]	11.0±0.3[bc]	***
Fat	4.2±0.5[b]	3.2±0.4[a]	5.4±0.4[c]	3.1±0.2[a]	3.9±0.1[ab]	***
Starch	2.8±0.2[b]	16.1±0.3[c]	0.9±0.3[a]	1.45±0.1[a]	1.3±0.1[a]	***
Rest carb	27.1±1[b]	21.6±0.9[a]	20.2±1.2[a]	34.7±1.9[d]	39. 2±0.8[c]	***
Ash	46.3±1.2[b]	33.4±0.4[a]	47.0±2.7[b]	33.3±1.8[a]	33.9±1.0[a]	***
Phosphorus	2.0±0.3[b]	1.0±0.03[a]	1.0±0.1[a]	1.1±0.1[a]	1.1±0.1[a]	***

ns=not significant P>0.1; *P<0.05; **P<0.01; ***P<0.001. Means along a row lacking a common superscript letter differ significantly, P<0.05. Rest carb, includes carbohydrates other than starch (NSP, Fibre, etc)

Table 7. Proximate composition (%) of the faecal waste from European sea bass fed different diets in trial II

	CON	SPC	CGM	P-value
Dry matter	94.9±0.5[a]	96.1±0.4[b]	96.6±0.4[b]	**
Crude protein	13.0±0.5	13.1±0.6	14.1±1.7	ns
Fat	4.1±0.6	4.5±0.8	5.2±1.3	ns
Starch	22.2±3.8[b]	11.2±0.6[a]	20.2±1.3[b]	**
Rest carb	27.4±1.6	37.4±0.7	30.0±2.6	ns
Ash	31.4±1.2[b]	32.5±0.5[b]	28.9±0.6[a]	**
Phosphorus	1.8±0.2	1.4±0.4	1.7±0.5	ns

ns=not significant P>0.1; *P<0.05; **P<0.01; ***P<0.001. Means along a row lacking a common superscript letter differ significantly, P<0.05. Rest carb, includes carbohydrates other than starch (NSP, Fibre, etc)

Table 8. Properties of faeces from European sea bass in trial I

	CON	PEA	FeM	SFM	WDG	P-value
Density, g/ml	1.2±0.2	1.3±0.2	1.2±0.2	1.2±0.1	1.2±0.2	ns
Dry matter, %	19.8±0.3[c]	17.0±0.3[b]	16.8±0.3[b]	16.8±0.3[b]	15.5±0.3[a]	***
Sinking velocity, cm/sec	4.5±0.4	4.8±0.5	4.8±0.5	5.3±0.2	4.7±0.6	ns
Osmolality, Osmol/kg	1.26±0.04[b]	1.23±0.05[ab]	1.23±0.03[ab]	1.19±0.05[ab]	1.15±0.09[a]	*
Viscosity, cP	2.61±0.38[a]	2.42±0.22[a]	2.59±0.16[a]	2.55±0.14[a]	3.62±0.16[b]	***

ns=not significant P>0.1; *P<0.05; **P<0.01; ***P<0.001. Means along a row lacking a common superscript letter differ significantly, P<0.05.

Table 9. Properties of faces from European sea bass in trial II

	CON	SPC	CGM	P-value
Density, g/ml	1.1±0.1	1.1±0.1	1.2±0.1	ns
Dry matter, %	15.8±2.1^{b}	13.3±0.7^{a}	16.1±1.8^{b}	*
Sinking velocity, cm/sec	4.6±0.6	4.2±0.3	4.3±0.4	ns
Osmolality, Osmol/kg	1.2±0.02	1.2±0.05	1.2±0.03	ns
Viscosity, cP	2.1±0.3	2.1±0.3	1.8±0.3	ns

ns=not significant P>0.1; *P<0.05; **P<0.01; ***P<0.001. Means along a row lacking a common superscript letter differ significantly, P<0.05.

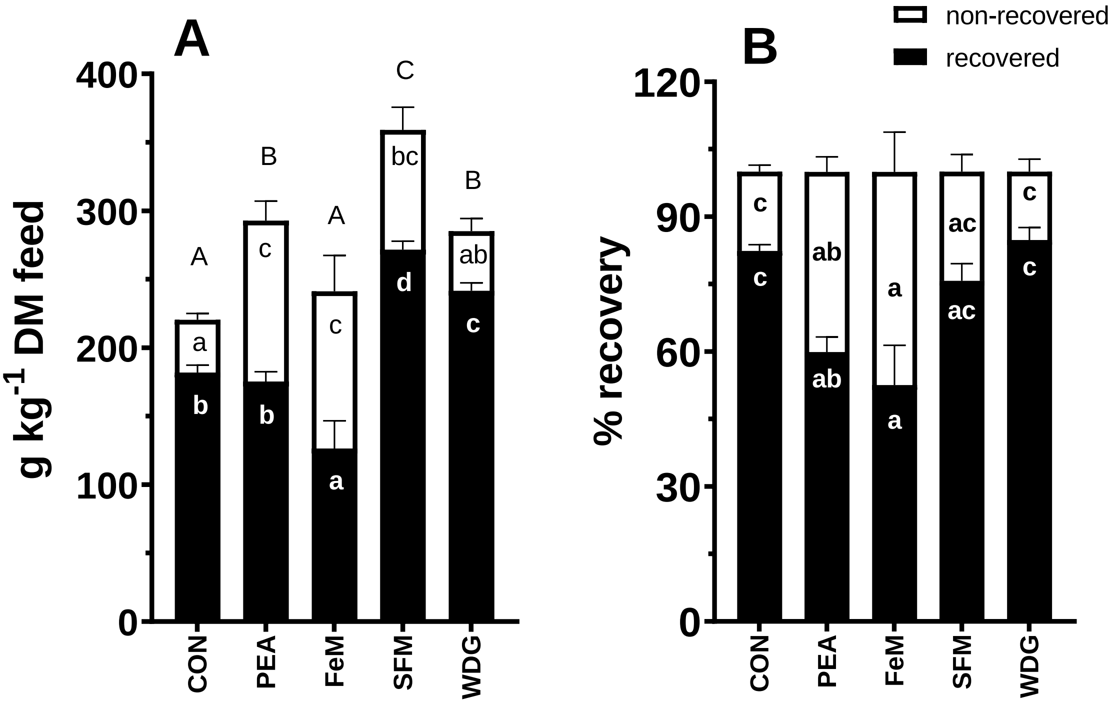

Figure 1. Faeces production (A) and % recovery (B) in diet groups of trial I

Legend: Total amount of faeces produced (entire bar), recovered (black) and non-recovered (white) in each dietary group presented as g kg^{-1} DM feed (Fig. 1A). Recovery percentage as a proportion of total faeces produced (Fig. B). Different superscripts (lower case) labelled within black and white bars indicate dietary difference for recovered and non-recovered faeces ($p<0.05$). Different superscripts (upper case) labelled above the bars indicate dietary différence for total faeces produced ($p<0.05$).

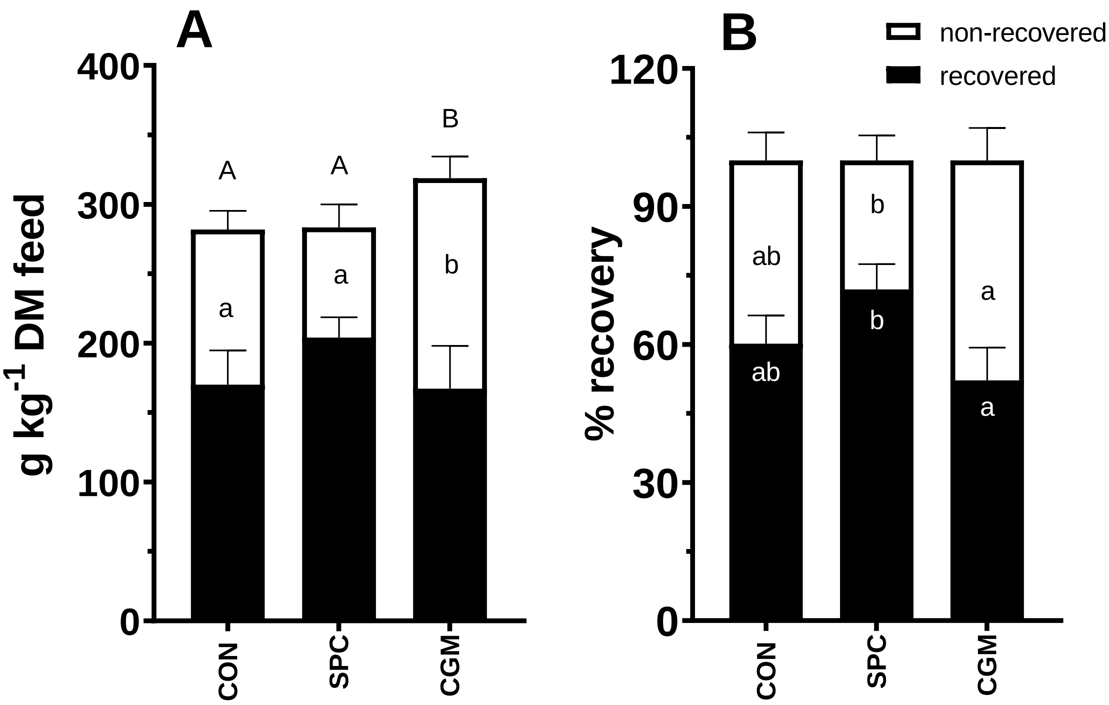

Figure 2. Faeces production and recovery in different diet groups of trial II

Legend: Total amount of faeces produced (entire bar), recovered (black) and non-recovered (white) in each dietary group presented as g kg^{-1} DM feed (Fig. A). Recovery percentage as a proportion of total faeces produced (Fig. B). Different superscripts (lower case) labelled within black and white bars indicate dietary difference for recovered and non-recovered faeces ($p<0.05$). Different superscripts (upper case) labelled above the bars indicate dietary difference for total faeces produced ($p<0.05$).

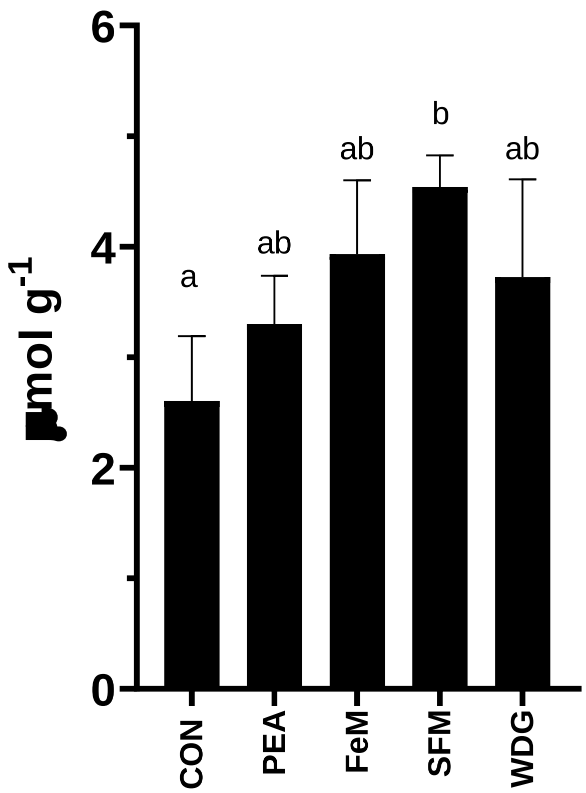

Figure 3 Volatile fatty acids of faecal waste in trial I

Legend: Volatile fatty acids in faces collected by stripping expressed as micromole per gram units. Different superscripts labelled above the bars indicate dietary differences ($p<0.05$). Units, µmol per g of wet facees.

Plate 1: Appearance of faeces pellets (collected by the faecal trap) from European seabass fed the different deits in trial I and trial II

Trial I

CON

PEA

FEM

SFM

WDG

Trial II

CON

SPC

CGM