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Aquacultural Engineering

journal homepage: www.elsevier.com/locate/aqua-online



Effects of diet composition and ultrasound treatment on particle size distribution and carbon bioavailability in feces of rainbow trout

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ARTICLE INFO

Article history:
Available online xxx

Keywords:
Fiber
Feces
Ultrasound
Microscreen
Bioavailability
Particle size

ABSTRACT

The effect of a high and low non-starch polysaccharide diet (HNSP and LNSP diet) and ultrasound treatment on particle size distribution and carbon bioavailability in fecal waste of rainbow trout (*Oncorhynchus mykiss*) was studied. Feces were collected from four flow-through fish tanks, two tanks fed the HNSP diet and two the LNSP diet. The collected feces were sonicated (disintegrated) in duplicate with high-intensity (0.6 W/ml), low-frequency ($f=20$ Hz) ultrasound at five different energy levels (0.6 W/ml for 0, 0.25, 1, 4, and 16 min). The particle size distribution of the treated feces samples was measured by wet sieving (1000, 500, 200, 100, 63, 36, 1.2 μm screen size) and total suspended solids (TSS) measurement. Carbon bioavailability in sonicated fecal waste samples was determined with oxygen uptake rate (OUR) tests. The results showed that: (1) feces from the HNSP diet contained significant more particulate material and bigger particles; (2) carbon bioavailability was almost three times higher in untreated LNSP feces when compared with HNSP feces; (3) almost 50% of HNSP feces could have been recovered on a micro-screen of 36 μm after wet sieving, whereas it was only 10% for LNSP feces; (4) the production of small particles (1.2–36 μm), which could pass a drum filter screen and potentially accumulate in RAS, was approximately 50 g/kg feed, showing no significant differences between diets; (5) sonication increased fecal dry matter below 36 μm ($p=0.015$), but it had no significant effect on the median particle size; (6) sonication increased carbon bioavailability with 7–10% for the HNSP feces ($p=0.037$); (7) fecal particles withstood up to 16 min sonication at an intensity of 0.6 W/ml and a frequency of 20 Hz corresponding to specific energy input of 20,000 kJ/kg DM without major changes in particle size distribution. The results of this study indicate that the applied ultrasound treatment of fecal waste is not an effective method to increase short-term carbon bioavailability.

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1. Introduction

Modern fish feeds are being formulated with increasing amounts of alternative plant protein sources (Naylor et al., 2009). Increasing the amount of unpurified plant ingredients in feeds will introduce fibers (i.e. non-starch polysaccharides, NSPs), which are considered indigestible in fish (Lovell, 1998). Due to their low digestibility and high structural integrity, fibers could potentially change the particle size distribution of fecal waste and its subsequent recovery with microscreens in recirculating aquaculture systems (RAS). Furthermore, the low degradability of fibers could hamper the use of feces as a carbon source in denitrification (Meriac

et al., 2014). Innovative RAS use the organic carbon (expressed as chemical oxygen demand, COD) originating from fecal waste for denitrification, effectively reducing water demand and nutrient emissions in RAS (Gelfand et al., 2003; Martins et al., 2009). However, the total removal of nitrate can be limited by the bioavailability of carbon (Kaiser and Schmitz, 1988; Klas et al., 2006). To overcome a limited carbon bioavailability, low frequency, high intensity ultrasound is commonly applied as a pre-conditioning method in waste water treatment and anaerobic digestion (Pilli et al., 2011; Tiehm et al., 1997). Several studies have shown how ultrasound can increase carbon bioavailability by decreasing particle size and COD solubilization (Show et al., 2007; Tiehm et al., 1997; Yagci and Akpınar, 2011). The goal of our research was to determine (i) if differences in diet formulation can affect the particle size distribution in feces and subsequent recovery with microscreens, and (ii) if ultrasound can break down fecal particles

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Table 1

Feed composition, including analyzed proximate composition. FW: fresh weight, DM: dry matter, NSP: non-starch polysaccharides. Nitrogen-free extracts (NFE) was calculated as dry matter – crude protein – crude fat – crude ash. Non-starch polysaccharides (NSP) were calculated as NFE – starch.

Parameter	Unit	Diet	
		HNSP	LNSP
<i>Proximate composition^a</i>			
Dry matter	g/kg FW	964	948
Crude protein	g/kg DM	409	389
Crude fat	g/kg DM	132	92
Crude ash	g/kg DM	95	82
Nitrogen-free extracts	g/kg DM	342	417
NSP	g/kg DM	198	8
Starch	g/kg DM	143	410
Acid-insoluble ash	g/kg DM	19	18
Energy	MJ/kg DM	21.2	20.2
Digestible Energy	MJ/kg DM	17.9	17.8
Digestible Nitrogen	g/kg DM	60.9	57.1
<i>Ingredients</i>			
Fish meal ^b	g/kg FW	220	410
Fish oil ^c	g/kg FW	102	22
Soy bean meal ^d	g/kg FW	180	–
Wheat flour ^e	g/kg FW	95	530
Sunflower seed meal ^f	g/kg FW	180	–
Rape seed meal ^g	g/kg FW	180	–
Monocalciumphosphate ^h	g/kg FW	5	–
L-Lysine HCl ⁱ	g/kg FW	44	4
DL-Methionine ^j	g/kg FW	44	4
Diamol ^k	g/kg FW	2020	20
Premix ^l	g/kg FW	1010	10

^a Feed was analyzed for proximate composition according to methods described in Meriac et al. (2014).

^b TripleNine Fish Protein, Esbjerg, Denmark.

^c Coppens International, Helmond, The Netherlands.

^d Cargill, Amsterdam, The Netherlands.

^e Meneba, Weert, The Netherlands.

^f Arkervaat-Twente, Leusden, The Netherlands.

^g ADM, Spycyk, Germany.

^h Tessenderlo Chemie, Rotterdam, The Netherlands.

ⁱ Sewon Paik Kwang Industrial Co. Ltd., Jeollabuk-do, South Korea.

^j Evonik, Hanau, Germany.

^k Damolin A/S, Fur, Denmark.

^l Premix, includes vitamins, minerals and trace elements.

to increase carbon bioavailability for a waste treatment process like denitrification.

2. Materials and methods

2.1. Feed and feeding

The two experimental feeds were formulated to be isoenergetic and isonitrogenous on digestible energy and nitrogen, and to produce feces with a contrast in fiber content between the treatments. Rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) were fed a low starch, high NSP (HNSP) diet and a high starch, low NSP (LNSP) diet (Table 1). We used extruded diets, which were produced by Research Diet Services (Wijk bij Duurstede, The Netherlands). Acid insoluble ash was used as a marker to determine digestibility (Atkinson et al., 1984; Vandenberg and De La Noue, 2001). The fish were fed a floating pellet of 3 mm, and the daily ration (~1.5% body weight/d over the whole experimental period) was equally divided between morning (9:30 h) and afternoon feeding (16:30 h). Feeding was done by hand and under close observation to avoid leftover pellets in the feces collection. In case a tank would not eat the whole ration, the leftover pellets would be collected and counted. In the subsequent feeding, the ration for the remaining tanks would be reduced by the amount of uneaten feed recovered to ensure homogenous growth between tanks and diets. Feed samples were collected weekly and analyzed as a pooled

sample at the end of the experiment to determine proximate composition.

2.2. Fish husbandry

Rainbow trout were obtained from Mohnen GmbH (Stolberg, Germany) and kept in a flow-through set-up comprising four circular 130 L-tanks. Each tank was equipped with a hydrocyclone for feces collection ($V = 17$ L, Aquaoptima AS, Trondheim Norway). The flow rate of the tanks was ~6.5 L/min, resulting in a hydraulic surface load of ~200 m³/m²/d on the hydrocyclones. The photoperiod was set to 12:12 light/dark. Two weeks prior to the start of the experiment, each tank was stocked with 15 fish with an individual weight of ~230 g for acclimatization. The fish were weighed at stocking, before and at the end of each experimental period. Details on fish performance and feeding rate over the whole experiment, and the respective experimental periods can be found in Table 2. Water temperature and oxygen was measured two times a day in the tank effluent with a handheld meter (WTW multi 340i, WTW GmbH, Weilheim, Germany). The average temperature and O₂ concentration in the effluent of all four tanks over the whole experiment was 15.5 ± 0.2 °C ($n = 652$) and 6.6 ± 0.9 mg/L ($n = 651$), respectively ($n = 4$).

2.3. Feces collection

Feces were collected twice a day before feeding. The feces collection bottles were connected to the bottom of the hydrocyclones and cooled with ice water (Fig. 1), similar as described in Saravanan et al. (2012). Morning and afternoon collections were pooled for each system to give a 24 h composite sample for the sonication experiments. To determine proximate composition of the fecal waste, feces were collected during the first experimental period for five subsequent days. The collected feces were stored in aluminum trays at –20 °C for later analyses.

Dry matter digestibility of the feed was used to determine the production and recovery of solid waste in the experimental systems for HNSP and LNSP ($n = 2$). The efficiency of fecal recovery in sedimentation was determined by relating the total amount of collected dry matter to the amount of feces produced based on dry matter digestibility of the feeds (Table 3). The resulting feces recovery efficiency was $68.1 \pm 0.1\%$ and $49.1 \pm 3.6\%$ for HNSP and LNSP, respectively ($n = 2$).

2.4. Experimental periods

The experiment consisted of two experimental periods, in which we focused on two different research questions. In the first experimental period, we measured the differences in particle size distribution in fecal waste produced on a high fiber (HNSP) and a low fiber (LNSP) diet. Furthermore, we determined how ultrasound treatment affects the particle size distribution in HNSP and LNSP feces. In the second experimental period, we investigated the effect of ultrasound treatment on short-term carbon bioavailability in feces using an oxygen uptake rate (OUR) test.

Since fecal pellets will disintegrate within an upflow sludge blanket denitrification reactor due to shear forces caused by the stirring of the sludge bed, it was necessary to determine the particle size distribution of resilient, primary particles forming the fecal pellet. In this study, we used wet sieving as an invasive method to determine the size distribution of the resilient particles forming the fecal pellet. Thus, the presented particle size distributions refer to the size distribution of primary particles in fecal waste, and not to secondary particles as feces fragments, flocs and other aggregates.

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