



Dietary carbohydrate composition can change waste production and biofilter load in recirculating aquaculture systems

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ABSTRACT

This study investigated the effect of dietary carbohydrate composition on the production, recovery and degradability of fecal waste from rainbow trout (*Oncorhynchus mykiss*) in recirculating aquaculture systems (RAS). Dietary carbohydrate composition was altered by substituting starch with non-starch polysaccharides (NSP) while keeping the diets isonitrogenous and isoenergetic. We tested a high starch, low NSP (LNSP) and a low starch, high NSP (HNSP) diet in six identical, small-scale RAS ($V = 460$ L). Each diet was tested in three independent systems over a period of six weeks. Shifting dietary carbohydrates from starch to NSPs resulted in a 50% increase in the production of chemical oxygen demand (COD) based on digestibility. Fecal waste recovery showed a 40% increase in HNSP treatments when compared with LNSP. Consequently, the COD output from HNSP systems doubled from 91 g to 194 g of COD per kg feed when compared with LNSP. Although COD production was higher in HNSP systems, the COD load on the biofilters was significantly lower when compared with LNSP systems. COD-to-nitrogen (COD/N) ratios in the biofilter load were 1.7 ± 0.2 and 2.2 ± 0.2 g COD/g N for HNSP and LNSP, respectively. Shifting the dietary carbohydrate composition from starch to NSPs decreased the biodegradability of fecal COD from 66.3% to 43.7% ($P < 0.001$). Fiber analyses revealed that approximately 40% of the COD in HNSP feces came from cellulose and hemicellulose. The increased COD production of HNSP diets could be exploited by using fecal COD as an internal carbon source in denitrification. Full denitrification would be theoretically possible with a measured COD/N ratio of 7.2 in the waste stream of HNSP systems. However, it is not clear if the low COD bioavailability of HNSP feces could be a limiting factor. This study shows that COD/N ratios in the biofilter load and system output can be manipulated by changing dietary carbohydrate composition. Although an increased dietary NSP content increased COD production, it also increased COD recovery, decreased COD load on the biofilters and generated sufficient carbon for denitrification on internal sources.

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1. Introduction

Over the past decades, many studies have shown how plant-based ingredients can be used to substitute fish meal in fish feeds without significant losses in fish performance (El-Saidy and Gaber, 2003; Fournier et al., 2004; Gomes et al., 1995; Kaushik et al., 1995; Lund et al., 2011). The process of feed formulation focuses almost entirely on protein efficiency and the allocation of energy, using the most economical combination of ingredients (Pillay, 1990). Depending on the ingredients used, the carbohydrate fraction in the feeds can contain different levels of non-starch polysaccharides (NSPs), which directly affect dry matter (DM) digestibility and fecal stability (Amirkolaie et al., 2005; Glencross, 2009; Hilton et al., 1983). Increasing the indigestible NSP content of a diet will thus increase the net production of chemical oxygen demand (COD) per unit of nitrogen (N) produced (Farhangi

and Carter, 2007; Glencross et al., 2012). The basic treatment of water in recirculating aquaculture systems (RAS) requires the removal of solid COD and the complete conversion of ammonia–nitrogen into nitrate (Timmons and Ebeling, 2007). An incomplete removal of solid waste can result in an excessive load of COD on the biofilter, hampering the nitrification process (Zhu and Chen, 2001). The effective load of biochemical oxygen demand (BOD) is determined by the degradability of the supplied COD. Lignocellulosic material originating from unpurified plant ingredients can significantly decrease COD degradability. A decreased COD degradability will restrict the amount of readily degradable carbon for possible waste treatment processes like denitrification. Several authors have proven that the solid organic waste generated in RAS can be used as a carbon source for denitrification (Gelfand et al., 2003; Kaiser and Schmitz, 1988; Schuster and Stelz, 1998; Shnel et al., 2002). Using denitrification on internal carbon sources does not only reduce COD and N output from RAS, but it even allows to lower the water exchange to 30 L/kg feed in RAS (Martins et al., 2009). To the best knowledge of the authors, there were no papers in literature which specifically discuss the effect of an increased NSP content in feeds on [1] COD production, composition and recovery, [2] COD and

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N load of the biofilters and system performance, and [3] COD/N ratios and COD bioavailability for denitrification. Therefore, we investigated the effect of a low starch, high NSP diet (HNSP) and a high starch, low NSP diet (LNSP) on COD and N mass balances on fish level and system level. The goal of this experiment was to determine the COD and N load on the biofilters (system load), in-situ losses (system losses) and the possible denitrification potential in the waste stream leaving the experimental RAS (system output).

2. Material & methods

2.1. Experimental design

We tested the effect of an NSP-rich diet on COD and N production, COD/N ratios and solid COD degradability in a total of six independent RAS stocked with rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792). The experimental period was six weeks, two experimental diets were used as treatments and each treatment was replicated three times. The two experimental diets were a low starch, high NSP diet (HNSP) and a high starch, low NSP diet (LNSP). The experiment was approved by the Ethics Commission for Animal Experiments of Wageningen University and is filed under the reference number 2011085.c.

2.2. Replicated recirculating aquaculture systems

System configuration: Each replicated RAS (Fig. 1) was composed of a circular fish tank ($V = 300$ L), a settling cone ($V = 75$ L, hydraulic surface load (HSL) $150 \text{ m}^3/\text{m}^2/\text{d}$; Fleuren & Nooijen, Nederweert The Netherlands), a sump ($V = 75$ L) equipped with a UV unit (UV-C 36 W, Phillips, Eindhoven, The Netherlands) and two trickling filters of equal size and surface area ($A = 15.8 \text{ m}^2$ for each filter, cross-flow medium, $242 \text{ m}^2/\text{m}^3$ specific surface area, Fleuren & Nooijen, Nederweert, The Netherlands). One biofilter was operated in bypass across the sump with a flow of $6\text{--}7 \text{ L/min}$ (HSL: $\sim 85 \text{ m}^3/\text{m}^2/\text{d}$), the other filter was located above the fish tank and loaded with the main flow of 20 L/min (HSL: $264 \text{ m}^3/\text{m}^2/\text{d}$, see Fig. 1). The total volume of each RAS was 460 L . Each fish tank was equipped with a double stand

pipe for solids removal from the bottom of the tank. The fish tank effluent passed the settling cone with minimal head loss. The feces settled into a water-cooled glass bottle ($T = 4^\circ \text{C}$), which was attached to the bottom of the cone. The water supply of the systems was connected to the sump and the exchange water was discharged using a tap at the bottom of the sump. Each of the six RAS was randomly assigned to either the HNSP or LNSP diet. **System acclimatization:** To develop the necessary nitrification capacity in the experimental systems, pre-cultivated cross-flow biofilter media was supplied with NH_4Cl for 10 days. Afterwards, each RAS was stocked for 12 days with 24–25 (non-experimental) trout to adapt the biofilters to the feed load of the experiment. The conductivity of all systems was gradually increased with artificial sea water (Instant Ocean, Aquarium Systems; Sarrebourg, France) to $\sim 2.1\text{--}2.2 \text{ mS/cm}$. **System operation:** The systems were operated at a temperature of $15\text{--}16^\circ \text{C}$, sodium bicarbonate was added when necessary to keep the pH between 7 and 8 and the photoperiod was set to 12:12 h light/dark. Water losses due to evaporation were compensated in the mornings before water exchange and feeding. Water was exchanged daily before the morning feeding at a rate of 450 L/kg feed, the exchange volume was based on the feed load of the previous day. Conductivity was maintained between 1 and 2 mS/cm by the addition of artificial sea water during the experiment.

2.3. Fish

Rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) were obtained from a trout farm in Germany (Mohnen GmbH, Stolberg, Germany) and housed at the experimental facilities of “De Haar Vissen” (Wageningen, The Netherlands). The fish arrived at an age of ~ 6.5 months and a bodyweight of $\sim 75 \text{ g}$. Upon arrival, fish were acclimated in circular tanks on flow-through supplied with well water of $13\text{--}15^\circ \text{C}$. Two weeks prior to the start of the experiment, the fish were divided into an HNSP and a LNSP group and housed in separate tanks. Each group was fed with their respective diet at the same levels as during the experiment for acclimatization, and feeding was ceased one day prior to the start of the experiment. Experimental fish from either the HNSP or LNSP adapted group were stocked in recirculation systems assigned to their respective diet. The fish had an individual

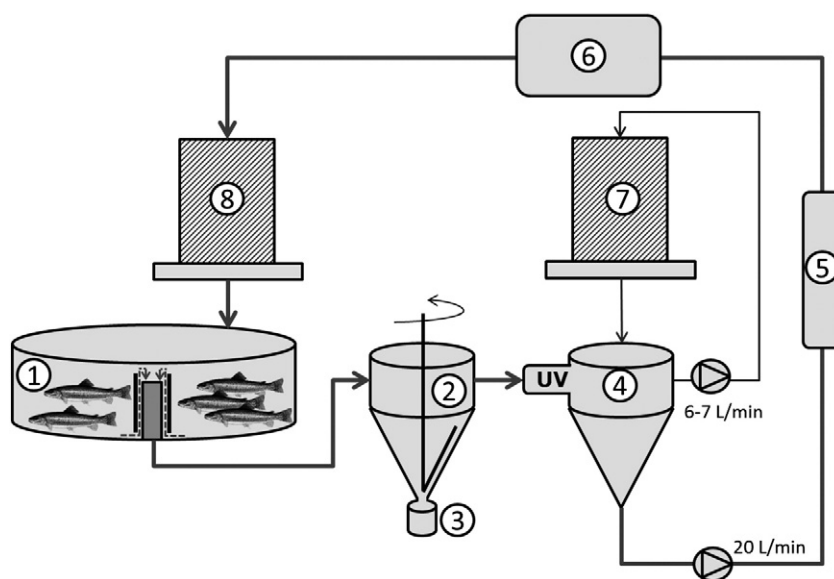


Fig. 1. RAS layout; [1] fish tank with double stand pipe ($V = 300 \text{ L}$, $A = 0.72 \text{ m}^2$); [2] settling tank/w stirrer to clean tank walls every 30 min ($V = 75 \text{ L}$, HSL: $150 \text{ m}^3/\text{m}^2/\text{d}$); [3] Collection bottle connected to settling tank and cooled to 4°C ($V = 250 \text{ ml}$); [4] Sump ($V = 75 \text{ L}$) and UV (UV-C 36 W, Phillips, Eindhoven, The Netherlands), water exchange and sampling point; [5] flow meter; [6] cooler-heater (TC20, Teco, Ravenna, Italy); [7] & [8] trickling filter/w cross flow medium ($V = 0.059 \text{ m}^3$, $A = 15.8 \text{ m}^2$ each).

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