Aquaculture 356-357 (2012) 141–146

Contents lists available at SciVerse ScienceDirect

Aquaculture

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

The influence of various starch and non-starch polysaccharides on the digestibility of diets fed to rainbow trout (*Oncorhynchus mykiss*)

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ARTICLE INFO

Article history:
Received 8 April 2012
Received in revised form 13 May 2012
Accepted 15 May 2012
Available online 1 June 2012

Keywords:
Plant proteins
Starch
Fibre
Anti-nutritional
Lignin
Cellulose

ABSTRACT

This study examined the effect of increasing inclusion levels of various polysaccharides on the dry matter, protein and energy digestibility of diets fed to rainbow trout (*Oncorhynchus mykiss*). The different polysaccharides included pregelatinised starch, cellulose, lignosulphonate, pectin and mannan. There were significant differences among the digestibility parameters of the diets with the different inclusion levels of each of the different polysaccharide types. Using a MANOVA analysis effects were noted for polysaccharide type, inclusion level and interaction terms on the digestibilities of dry matter, protein and energy. Cellulose addition resulted in a reduction in both dry matter and energy that was largely commensurate with its inclusion level, but its effect on protein digestibility was marginal. Starch had the least effect on any of the digestibility parameters of all the polysaccharide types examined. At low inclusion levels lignosulphonate was observed to have the greatest impact on all digestibility parameters, particularly on protein digestibility. These results show that different polysaccharide classes can have distinctly different effects on diet digestibility parameters. The results also show that some classes of polysaccharide have greater effects than others.

1. Introduction

The use of grains in carnivorous fish feeds is now widespread throughout the world. A range of feed grains is routinely used in these feeds including; wheat, soybean, lupins, peas and rapeseed (Aslaksen et al., 2007; Gatlin et al., 2007; Hardy, 2010). The different grains each have advantages and disadvantages with their use, such as the presence of anti-nutritional factors or the improvement to functional characteristics to pellets (Glencross et al., 2010; Krogdahl et al., 2010). However, most grains result in the inclusion of carbohydrates of non-nutritive value, in the form of non-starch polysaccharides (NSP) and this raises another potential issue.

The introduction of NSP with grain meals presents a problem in that different chemical classes of NSP may have different biological effects on the digestion process in animals. While some types of starch can be well digested (Amirkolaie et al., 2006; Bergot and Breque, 1983; Enes et al., 2008; Glencross et al., 2012; Moreira et al., 2008), there are few NSP that succumb to the digestion processes in monogastric animals, fish included (Hansen and Storebakken, 2007; Kraugerud et al., 2007). There have been various reports on the effects of different NSP classes in fish diets (Amirkolaie et al., 2005; Glencross, 2009; Glencross et al., 2003; Hansen and Storebakken, 2007; Leenhouwers et al., 2004). The inclusion of purified oligosaccharides (guar gum) in the diet was shown to significantly reduce the digestive function of *Dicentrarchus labrax* (European seabass) at even low inclusion levels by changing digesta viscosity (Leenhouwers et al., 2004). In a study with tilapia (*Oreochromis niloticus*) the addition of oligosaccharides (guar gum) and cellulose to the diet was observed to significantly reduce diet energy digestibility, but not protein digestibility (Amirkolaie et al., 2005). The inclusion of cellulose in diets for rainbow trout also did not affect the protein digestibility of the diet, but was similarly shown to reduce the energy and dry matter digestibility (Glencross, 2009; Hansen and Storebakken, 2007). Other key NSP-classes include pectin and the lignin, but there is even less information available on the discrete effects of the use of these carbohydrate classes on the digestibility of protein and energy in fish diets, although some inferred effects have been reported (Glencross et al., 2008).

Due to the acknowledged effect of different NSP types on the digestion process, one of the key elements in understanding the implications of the use of different plant protein meals is to understand the carbohydrate complexity being added with the use of each raw material. Based on earlier work it is hypothesised that some classes of NSP will largely act as a bulking agent, similar to cellulose, but others may have interactive effects when fed to fish and reduce protein and energy digestibility at a greater degree than that seen by a bulking agent like cellulose (Glencross, 2009). Distinctly non-additive effects on digestibility were observed with the inclusion of soluble NSP compared to insoluble

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0044-8486/$ – see front matter.
doi:10.1016/j.aquaculture.2012.05.023

NSP and cellulose (Glencross, 2009). Therefore this study aims to examine the effects of the dietary inclusion of incremental levels of different classes of NSP (cellulose, lignosulphonate, pectin, mannan/oligosaccharides) and starch on the digestible value of diets fed to rainbow trout, *Oncorhynchus mykiss*.

2. Materials and methods

2.1. Diet preparation

The experiment design was based on a diet formulation strategy that used a diet-substitution approach, although the assessment of the digestible value of those ingredients was not the intent of the experiment (Glencross et al., 2007). To achieve this, a basal diet was formulated and prepared to include approximately 500 g/kg DM protein, 210 g/kg DM fat and an inert marker (yttrium oxide at 1 g/kg) (Table 2). Each polysaccharide-class ingredient was added to the test diets at 25, 50, 100 or 200 g/kg inclusion to a reciprocal-sample of the basal mash (see Table 2). The diets were made by the addition of water (about 25% of mash dry weight) to the mash while mixing to form a dough which was subsequently screw pressed using a pasta maker through a 4 mm diameter die. The moist pellets produced were then oven dried at 60 °C for around 12 h before being allowed to cool to ambient temperature in the oven. The basal diet was prepared in a similar manner, but without the addition of any test ingredient. The source and composition of all ingredients are presented in Table 1.

2.2. Fish handling and faecal collection

Hatchery-reared rainbow trout (*O. mykiss*) were transferred from grow-out ponds to experimental tanks (200 L). Freshwater (salinity < 1 PSU; dissolved oxygen 8.2 ± 0.5 mg/L) of 16.1 ± 0.1 °C (mean ± S.D.) at a flow rate of about 4 L/min was supplied to each of the tanks. Each of the tanks was stocked with 20 trout of 201.6 ± 18.7 g (mean ± S.D.; n = 40). Treatments were randomly assigned among 48 tanks, with each treatment having three replicates.

Fish were hand fed the diets once daily to apparent satiety as determined over three separate feeding events between 1500 and 1600 each day. The fish were allowed to acclimatise to the allocated dietary treatment for seven days before faecal collection commenced consistent with earlier studies by this group (Glencross, 2011). Faeces were collected using stripping techniques based on those reported by Glencross (2011). Fish were netted from their respective tank, placed in a smaller aerated tank containing isoeugenol (0.002 mL/L) until they lost consciousness. The faeces were then removed from the distal intestine using gentle abdominal pressure. Care was maintained to ensure that the faeces were not contaminated by urine or mucous and hands were rinsed with freshwater between each fish. After collection of faeces from the fish, the sample was transferred to a small plastic vial and stored in a freezer at −20 °C. Stripped faeces were collected between 0800 and 1000 over a four-day period, with each fish only being stripped twice and not on consecutive days. Faecal samples from different days were pooled within tank, and kept frozen at −20 °C before being freeze-dried in preparation for analysis.

Table 1
Nutrient composition of the experimental ingredients (all values are g/kg DM unless otherwise indicated).

Nutrient	Pregelled wheat starch[a]	Cellulose[b]	Pectin[b]	Lignosulphonate[c]	Locust bean gum (mannan)[b]
Dry matter content (g/kg)	907	938	898	917	889
Crude protein	1	1	1	1	1
Total lipid	0	0	0	32	15
Ash	6	5	49	98	8
Total carbohydrate*	993	994	950	869	976
Lignin	0	12	0	99	0
Cellulose	1	827	0	21	8
Hemicellulose	2	129	0	11	18
Gross energy (MJ/kg DM)	17.4	17.4	16.6	16.3	17.5

[a] Pregelatinised wheat starch: Manildra, Auburn, NSW, Australia.
[b] Cellulose, pectin and mannan: Sigma Chemical Company, St Louis, MO, USA.
[c] Calcium lignosulphonate: Dustex, Canningvale, WA, Australia.
* Calculated based on dry matter–(protein + ash + lipid).

2.3. Chemical and digestibility analysis

Diet and faecal samples were analysed for dry matter, yttrium, nitrogen and gross energy content. Diets and ingredients were analysed for these same parameters in addition for ash, total lipid, lignin, neutral-detergent fibre and acid-detergent fibre. Dry matter was calculated by gravimetric analysis following oven drying at 105 °C for 24 h. Total yttrium concentrations were determined after mixed acid digestion using inductively coupled plasma atomic emission spectrophotometry (ICP–AES) based on the method described by McQuaker et al. (1979). Protein levels were calculated from the determination of total nitrogen by LECO auto-analyser, based on N × 6.25. Gross ash content was determined gravimetrically following loss of mass after combustion of a sample in a muffle furnace at 550 °C for 12 h. Total lipid content of the diets was determined gravimetrically following extraction of the lipids using chloroform:methanol (2:1). Gross energy was determined by adiabatic bomb calorimetry. Dietary fibres were determined by digesting the defatted sample with multiple washes of acetone and ethanol. The resulting residue was corrected for undigested protein and ash according to the method of Champ et al. (2003). Neutral-detergent fibre (NDF) samples were boiled with buffered NDF solution. The residue was collected on a coarse sintered glass crucible (Van Soest and Robertson, 1981). The acid-detergent fibre (ADF) was determined following a sample being reacted in 0.5 M acid detergent solution and the residue was collected on a coarse sintered glass crucible after the method of Van Soest and Goering (1970). Lignin was determined by reacting the ADF residue with cold 72% sulphuric acid. The sample was ashed and the residue measured gravimetrically (Van Soest and Robertson, 1981). Total carbohydrate content was determined based on dry matter–(protein + lipid + ash) content. Cellulose content was determined based on the ADF–lignin. Hemicellulose content was determined based on NDF–ADF content.

Differences in the ratios of the parameters of dry matter, protein or gross energy to yttrium, in the feed and faeces in each treatment were calculated to determine the apparent digestibility coefficients (ADC_{diet}) for each of the nutritional parameters examined in each diet based on the following formula (Maynard and Loosli, 1979):

$$AD_{diet} = 1 - \left(\frac{Y_{diet} \times Parameter_{faeces}}{Y_{faeces} \times Parameter_{diet}} \right)$$

where Y_{diet} and Y_{faeces} represent the yttrium content of the diet and faeces respectively, and $Parameter_{diet}$ and $Parameter_{faeces}$ represent the nutritional parameter of concern (organic matter, protein or energy) content of the diet and faeces respectively.

2.4. Statistical analysis

All values are means ± standard error of the mean (SEM) unless otherwise specified. Effects of fibre type and inclusion level on the digestibility of dry matter, protein and gross energy in each of the diets were examined by MANOVA (Table 3). Levels of significance were determined using a least significant difference (LSD) test. Curve fitting and regression