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Short communication

Changes in relative molecular weight distribution of soluble barley beta-glucan during passage through the small intestine of pigs

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ABSTRACT

The relative molecular weight distribution of soluble barley beta-glucans (SBB) was monitored through the small intestine in pigs by analyzing water extracts of duodenal- and ileal digesta with HPLC-SEC. Variations among four diets, based on four different barley varieties, were documented as well as variations between animals fed the same diet. The results showed depolymerization of the SBB throughout the whole small intestine independent of diet. The average molecular weight of the SBB was reduced to approximately 50% in duodenum in all the experimental animals.

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1. Introduction

Dietary fiber will affect digestive physiology in pigs and influence digesta flow, voluntary feed intake and thus nutritional absorption and feed digestibility (Bach Knudsen et al., 2012), in addition to manure odor and ammonia emissions (O'Shea et al., 2010). Thus, different factors such as grain type and their chemical composition as well as cereal derived endogenous enzyme activities will affect gastrointestinal function, bacteria population and microbial metabolites in the gut (Högberg and Lindberg, 2004; Högberg et al., 2004; Bindelle et al., 2008; Pieper et al., 2008). These effects will further depend on the size, solubility and molecular structure of the dietary fiber (Bach Knudsen et al., 1993, 2012; Glitsø et al., 1998).

Dietary fiber, here/often referred to as non-starch polysaccharides (NSP), is depolymerized in the gastrointestinal (GI) tract in different biological systems (Bach Knudsen and Canibe, 2000; Coles et al., 2005). It is evident that cereal beta-glucans are digested in the upper GI tract of pigs at various degrees, and especially in the distal part of the small intestine (ileum). Digestibility of the cereal beta-glucans will depend on different factors; not only particle size or the feed matrix is important, but also source of beta-glucan and diet composition. Also different grain types and varieties with parallel variation in the fiber content, as well as different biological systems and individual biological differences between subjects will influence

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the monitored experimental results. However, not only digestibility is important, but physiological properties of beta-glucans are also significant for both animal nutrition and health. Despite different reports on digestion of cereal beta-glucans based on quantitative recovery (Fadel et al., 1988; Bach Knudsen et al., 1993), there is less information on quantitative changes in their molecular weights (Mw). There is a few studies showing changes in the molecular size of oat beta-glucans and of wheat and rye arabinoxylans during digestion in the upper GI tract (Johansen et al., 1993, 1997; Le Gall et al., 2010). However, there is scarce information in the literature regarding specific information on the Mw changes of soluble barley beta-glucans during passage in the GI tract and possible variations among/with different barley varieties. This is important since changes in Mw will affect the physico-chemical properties of the beta-glucans significant for their possible influence on gut health in both human and animals.

The main objectives of the present experiment were to measure and document the degree of depolymerization (changes in Mw) of soluble barley beta-glucans in the small intestine of pigs, and study possible differences between different dietary treatments using four barley varieties.

2. Material and methods

2.1. Dietary treatments

Four pelleted diets were produced at the Centre for Feed Technology, Ås, Norway. These were based on four Norwegian barley varieties: Olve (normal starch), Marigold (normal starch), Karmosè (high amylose starch) and Magdalena (waxy starch). The barley varieties were grown at the same location (Landvik, Norway) under the same growth conditions in 2010. The diets were formulated to meet the requirements for all nutrients (Subcommittee on Swine Nutrition et al., 1998). The composition of the diets is given in Table 1.

2.2. Experimental animals

The feeding experiment was performed at the Experimental Farm, Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Ås, Norway. All pigs were cared for according to laws and regulations controlling experiments with live animals in Norway (Animal Protection Act of December 20, 1974, and the Animal Protection Ordinance concerning experiments with animals of January 15, 1996).

A total of 16 female pigs ((Norwegian Landrace × Yorkshire) × (Norwegian Landrace × Duroc)) from 4 litters were used in the experiment with an average initial weight at 29.8 kg and an average final weight at 37.6 kg. They were blocked by litter and by live weight, and groups of four animals were fed each experimental diet.

Table 1
Composition of the four diets and their amount of soluble beta-glucan (%).

	Diet 1	Diet 2	Diet 3	Diet 4
Barley *Marigold*	83.47			
Barley *Magdalena*		83.47		
Barley *Karmosè*			83.47	
Barley *Olve*				83.47
Soybean meal (HiPro)	15.0	15.0	15.0	15.0
Limestone meal ($CaCO_3$)	1.3	1.3	1.3	1.3
Mineral premix	0.16	0.16	0.16	0.16
Vitamin premix	0.06	0.06	0.06	0.06
Y_2O_3[a]	0.01	0.01	0.01	0.01
Soluble beta-glucan	1.6	3.0	2.6	2.6

[a] Yttrium oxide was used as the indigestible dietary marker.

2.3. Experimental procedure

The total experimental period lasted for 14 days; a 5-day adaptation period followed by a 9-day experimental period with collection of feces the last four days. The pigs were given feed twice daily according to a restricted Norwegian feeding scale (Øverland et al., 2000). The experimental animals were fed in pens designed for individual feeding in a room with an average temperature of 20.4 °C, and had free access to water.

2.4. Sample collection

The pigs were slaughtered at a commercial slaughter house three hours after the last meal. The digestive tract was separated from the animal at the slaughter line, and the collection of digesta from duodenum and ileum was performed immediately. The duodenal samples were collected from the pyloric ring and 64 cm distally, and the ileal samples from the ileacaecal opening and 64 cm proximally. The samples were put in closed boxes and kept on ice until being frozen at −20 °C. The samples were freeze dried and ground homogenously before being analyzed.

2.5. Analytical methods

The four diets were analyzed for yttrium by inductively coupled plasma mass spectrometry (ICP-AES analysis, Perkin-Elmer Optia 3000DV; Perkin-Elmer, Wellesley, MA, USA) at 371 nm, after mineralization and solubilization in acid of the pooled sample.

2.5.1. Extraction of soluble barley beta-glucans for molecular weight determination

β-Glucans were extracted as described by Rieder et al. (Rieder et al., 2012). The initial step involved adding 10 mL of 50% ethanol to a 200 mg sample of the ground diets and of freeze dried duodenal and ileal samples. The mixture was boiled for 15 min, cooled and centrifuged (2000 g, 15 min; Heraeus Multifuge 4 KR). The supernatant was discarded before 20 mL 2.5 mM $CaCl_2$ and 50 μL thermostable α-amylase (Termamyl, Novozymes A/S, Denmark) was added to each sample. The samples were boiled for 90 min with mixing every 15 min. After cooling, samples were centrifuged (2500 g, 15 min; Heraeus Multifuge 4 KR) and the supernatants collected. Another 10 mL of 2.5 mM $CaCl_2$ was added and the procedure repeated with boiling for 60 min. The supernatants were combined with the

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