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Increasing dietary oat fibre decreases the permeability of intestinal mucus

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ABSTRACT

This study investigates the influence of the dietary fibre β-glucan on nutrient composition and mucus permeability.

Pigs were fed a standard diet or a diet containing twice the β-glucan content for 3 days (n = 5 per group), followed by the collection of small intestinal mucus and tissue samples. Samples of the consumed diets were subjected to *in vitro* digestion to determine β-glucan release, nutrient profile and assessment of mucus permeability.

In vitro digestion of the diets indicated that 90% of the β-glucan was released in the proximal small intestine. Measurements of intestinal mucus showed a reduction in permeability to 100 nm latex beads and also lipid from the digested enhanced β-glucan diet.

The data from this study show for the first time that reducing mass transfer of bile and lipid through the intestinal mucus layer may be one way in which this decrease in bile re-absorption by soluble fibre is enabled.

1. Introduction

Dietary fibre is an important component in a healthy diet. However, current knowledge does not explain all the physiological benefits associated with dietary fibre consumption. In the UK most people do not consume the recommended average intake for adults of 18 g (NSP) per day (Scientific Advisory Committee on Nutrition, 2015); the average intake is 12.8 g/day for women and 14.8 g/day for men. This is important because of its association with lowering for cardiovascular disease (CVD). The rate of obesity is increasing in the developed world resulting in increases in morbidity and mortality from CVD and metabolic disorders such as type-2 diabetes. In 2013, it was estimated that more than 60% of adults in the UK were overweight including 25% obese. Diabetes alone costs the NHS £10b per year and is associated with 24,000 deaths, half of which are CVD related (Hex, Bartlett, Wright, Taylor, & Varley, 2012). Clinical and epidemiological studies have demonstrated that diet and lifestyle changes are essential parts of a multifaceted approach to prevent and/or limit disease progression.

The use of dietary fibre is one tool that can be used to lower risk factors for cardiovascular disease and type 2 diabetes mellitus (Anderson et al., 2009). Recently, the InterAct consortium, using 10.8 years of follow-up of 11,559 participants with type 2 diabetes, was able to conclude that the overall evidence indicated that the intake of total and cereal fibre is inversely related to the risk of type 2 diabetes (InterAct Consortium, 2015). In addition, a number of studies have shown efficacy for a range of different dietary fibres. For example the cereal dietary fibre (CDF) β-glucan has been shown to lower cholesterol and EFSA allows a health claim on this basis (EFSA-NDA, 2011). Although the

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precise mechanism is not known, it is thought to involve the sequestering of bile acids, probably through entrapment of mixed micelles (Brownlee, 2011). Bile acids have also been shown to increase secretion of the satiety hormone GLP-1 as a result of binding to TGR5 in the distal ileum and colon, even when bound to a sequestrant (Prawitt, Caron, & Steels, 2014). Finally, bile acids have been shown to alter cholesterol homeostasis and lipid metabolism through binding to the farnesoid X nuclear receptor (FXR), both in the GI tract and in the liver.

The ability of dietary fibres to alter digestion and metabolism depends upon their physical properties and there are clearly large differences between soluble and insoluble fibre. However, these are normally consumed together for instance in porridge or bakery products. The initial effects in the upper GI tract are thought to be a consequence of the increase in viscosity that can be induced by soluble fibre (Kristensen & Jensen, 2011). The viscosity that can be imparted by soluble fibre depends on a number of factors including the molecular weight and the extent of hydration. In general, higher viscosity meals tend to cause increased gastric retention in comparison to the equivalent lower viscosity meal (Juvonen et al., 2009). In a recent *in vitro* study, the impact on digestion of the addition of oat bran to biscuits was assessed. In particular, the viscosity of the chime was measured throughout digestion (Villemejane, Wahl, Aymard, Denis, & Michon, 2015), showing that the viscosity was maintained throughout the gut up to the ileal compartment and that the highest viscosity was obtained with biscuits containing soluble fibre. The effect of viscosity on digestion was also determined and protein hydrolysis decreased with fibre enrichment, i.e. increased viscosity (Villemejane et al., 2016).

In addition to viscosity effects in the intestinal lumen and the consequent impact on gastrointestinal motility and rates of hydrolysis, the soluble fibre may interact with the intestinal mucus and decrease its permeability (Mackie, Macierzanka, Aarak, Ridout, & Bajka, 2015) or secretion rate. Previous studies in rats have shown that low-methoxyl pectin did not affect the number of goblet cells but could interact directly with the epithelium and stimulate small intestinal mucin secretion (Hino et al., 2013), although no explanation of the mechanism was provided. Two studies undertaken in pigs showed that the addition of cereal dietary fibre to a standard diet increased the flow and presumably the secretion of intestinal mucin (Morel, Melai, Eady, & Coles, 2005; Morel, Padilla, & Ravindran, 2003). More specifically, the addition of β-glucan to a diet containing cellulose increased both mucin secretion and endogenous amino acid and nitrogen losses in the small intestine. The explanation given in these and other articles is that the dietary fibre increased the abrasion of the mucus layer (Montagne, Piel, & Lalles, 2004), presumably as a result the increase in luminal viscosity. Although it is unclear from these articles whether the secretion of mucin is upregulated it seems highly likely. The same trend has also been observed in rats fed on a range of dietary fibres (Brownlee, Havler, Dettmar, Allen, & Pearson, 2003), where the thickness of both the tightly and loosely adherent colonic mucus layers were found to increase as a result of the fibre in the diet.

In the work reported in this article we have used two different diets containing different amounts of oat meal as the fibre in order to assess the effect on the rheological properties and permeability of the intestinal mucus and mucin gene transcription.

2. Materials and methods

2.1. Animals and diets

Ten pigs in two groups of five raised at Easton Otley College were fed *ad libitum*, a standard commercial pig finisher diet (Easey Pigs, Eye, UK) either with or without supplementation with 10% oat bran (Suma Wholefoods, Elland, UK) for 3 days. The five OM10 fed animals were ear-tagged to allow carcass identification at the abattoir. Following feeding, the animals were transported to a local abattoir (Cranswick Country foods, Watton, Norfolk, UK) for conventional slaughter. Immediately following slaughter, porcine intestines were obtained and samples removed as outlined below.

The macronutrient composition of the two diets is described in Table 1. Water was also available to all of the animals throughout the three days. The control diet contained 0.7% cereal β-glucan, while the oat bran contained 8.7% oat β-glucan, giving a final value of 1.5% in the OM10 diet. The nutritional composition of the oat bran was 9.4% fat, 13.4% protein and 47.3% carbohydrate including 18.2% fibre. The average molecular weight of the β-glucan extracted from the oat bran by *in vitro* digestion and determined by HPLC-SEC was 2.3 MDa, subsequent treatment of the sample with laminarinase, completely abolished the peak, confirming it to be the β-glucan. The SEC used dextran Mw standards at 0.1, 0.18, 0.35 and 0.85 MDa. The differences between the conformation of the dextran and β-glucan may lead to a slight overestimation of the molecular weight of the β-glucan.

2.2. Sample collection

Porcine mucus was prepared as described previously (Macierzanka et al., 2011). Briefly, the fresh porcine small intestine obtained as described above, was stored on crushed ice for transport to the laboratory. The gut was rinsed through with ice cold phosphate buffer (10 mM phosphate pH 6.5, 5 mM EDTA) followed by a further rinse with the same buffer containing a protease inhibitor (0.5 mM Pefabloc, AEBSF). The gut was then opened out flat and mucus was collected by gently scraping the jejunal surface. Porcine jejunal mucus was isolated less than 45 min after slaughter. Samples were frozen in liquid nitrogen and stored at –80 °C for further use. Proximal small intestinal mucosal tissue samples were collected into RNALater (Sigma, Poole, UK), frozen in liquid nitrogen and stored at –80 °C for gene expression analysis.

Table 1 – The macronutrient of the control (55–100 finisher diet) and enhanced β-glucan diet composition (%).

	Control	OM10 (10% oat bran)
Crude protein	16.59	16.03
Crude fat	4.00	4.40
Carbohydrate	74.75	73.28
Fibre (NDF)	15.92	23.33
β-Glucan	0.7	1.4
Gross energy (MJ/kg DM)	13.35	13.52