Nutrition 32 (2016) 491–497

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

Soluble arabinoxylan enhances large intestinal microbial health biomarkers in pigs fed a red meat–containing diet

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ARTICLE INFO

Article history:
Received 29 April 2015
Accepted 12 October 2015

Keywords:
Wheat arabinoxylan
Soluble dietary fiber
Microbial biomass
Protein fermentation
Pig model

ABSTRACT

Objectives: The aim of this study was to investigate how moderately increased dietary red meat combined with a soluble fiber (wheat arabinoxylan [AX]) alters the large intestinal microbiota in terms of fermentative end products and microbial community profiles in pigs.
Methods: Four groups of 10 pigs were fed Western-type diets containing two amounts of red meat, with or without a solubilized wheat AX-rich fraction for 4 wk. After euthanasia, fermentative end products (short-chain fatty acids, ammonia) of digesta from four sections of large intestine were measured. Di-amino-pimelic acid was a measure of total microbial biomass, and bacterial profiles were determined using a phylogenetic microarray. A factorial model determined effects of AX and meat content.
Results: Arabinoxylan was highly fermentable in the cecum, as indicated by increased concentrations of short-chain fatty acids (particularly propionate). Protein fermentation end products were decreased, as indicated by the reduced ammonia and branched-chain ratio although this effect was less prominent distally. Microbial profiles in the distal large intestine differed in the presence of AX (including promotion of *Faecalibacterium prausnitzii*), consistent with an increase in carbohydrate versus protein fermentation. Increased di-amino-pimelic acid ($P < 0.0001$) suggested increased microbial biomass for animals fed AX.
Conclusions: Solubilized wheat AX has the potential to counteract the effects of dietary red meat by reducing protein fermentation and its resultant toxic end products such as ammonia, as well as leading to a positive shift in fermentation end products and microbial profiles in the large intestine.

Introduction

A range of factors are now known to lead to shifts in the large intestinal microbial community, including host stress [1], redox status [2], and diet [3]. Of these, diet is considered the easiest to manipulate.

Higher-protein diets, as is more commonly being consumed in modern society, result in increased amounts of protein reaching the large intestine (LI) [4,5]. Consequently, the trend of increasing dietary protein intake for weight control or muscle development in athletes, if not balanced by an appropriate amount of dietary fermentable carbohydrate content, is likely to predispose individuals to a range of diseases [6,7].

Generally, in the absence of sufficient fermentable carbohydrates, bacteria will ferment protein as an energy source, leading to the production of branched-chain fatty acids and potentially toxic compounds such as ammonia, amines, phenols, cresols, and hydrogen sulphide [8]. This increase of toxic compounds is thought to promote genetic instability and cancer risk, particularly in the distal colon [9,10].

The presence of fermentable carbohydrates in the LI (dietary fiber) from whole grains, fruits, and vegetables is associated with positive health benefits, including microbiota and immune development in young animals [11–13], microbiota stabilization in adults [3], production of short-chain fatty acids (SCFAs) [14],

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http://dx.doi.org/10.1016/j.nut.2015.10.008
0899-9007/

and prevention of protein fermentation [3], are related to dietary fiber fermentability. Other benefits include fecal bulking [15], water retention [16], and reduced cholesterol [17]. Of the multitude of potentially fermentable dietary carbohydrates, research has focused on very few. These include resistant starch [18–20], many examples from the oligosaccharide group [21–24], and various grain brans [25]. Barley and oats in particular [26–28], have proven beneficial, partly because of their readily solubilized (1,3; 1,4)-β-glucans (mixed-linkage glucans). However, worldwide, wheat is the most widely consumed grain (global per-capita food use of wheat from 2000–2001 to 2015–2016 is 67.2 kg/y versus 57.3 kg/y for rice [29]) but until recently it mainly has been considered a source of energy (or even some protein), but it has not been associated with soluble fiber–based health benefits.

Arabinoxylan [AX] is the major soluble fiber component in wheat, but it is incompletely solubilized during food processing [30]. It is unclear whether the solubility or the physico-chemical properties of the soluble fiber component is responsible for the varying health properties of wheat versus oats and barley. Previous studies of solubilized wheat AX have shown extensive fermentation in rats [31,32] and in vitro with human [33] and pig fecal inocula [34].

Since the 1970s, pigs have been increasingly used as an in vivo model for humans [35,36], particularly for nutritional studies, given their gastrointestinal anatomical and physiological similarities and eating habits. More recently, pigs have been used as a model for gut microbiota studies [37] because samples can be collected from the entire digestive tract rather than mainly from feces or from ileostomy patients, as reviewed recently [38].

Our hypothesis was that pigs fed solubilized wheat AX as part of a Western-style diet with two concentrations of red meat would show evidence of stimulated LI carbohydrate fermentation, modified microbial populations, and a reduction in potentially negative fermentative metabolites.

Materials and methods

The work reported here was part of a large animal experiment, some of the results of which have been reported previously, which detailed the materials and methods used. The following is a brief synopsis of that information [39]. All procedures were approved by the Animal Ethics Committee of the University of Queensland and CSIRO Food and Nutritional Sciences (AEC Approval Number SAS/181/09/CSIRO-NF).

Experimental design, animals, and housing

Two separate groups of 20 large white male pigs (average body weight: 19.1 ± 1.9 kg) were used. The animals were fed for 4 wk each, with 6 wk between groups. During each period, five pigs from each group were allocated to one of the four experimental diets. For each period, the pigs were matched by weight before diet allocation and were housed in pens in a temperature-controlled room in groups of five, but each was fed separately.

Diets and feeding procedures

The base Western-style diet consisted of human food ingredients and was formulated to meet the pigs' macronutrient needs. Diet formulation achieved lower (~23%) and higher (~30%) meat contents, with or without added AX in the form of an AX-rich fraction (AXRF) from a gluten extraction plant (Penfords, Tamworth, New South Wales, Australia) that contained 36% AX, with the remainder being starch (32%), protein (11%), cellulose (5%), and water and other components (16%) [39].

The meat was beef steak that was trimmed of fat, cooked on a hotplate, minced, and dried. This product contained 915 g/kg dry matter (DM), with 830 g/kg DM protein and 166 g/kg DM crude fat. The AX-containing diets contained 25% of the dried AXRF, resulting in ~8% AX. The AX was exchanged for extra starch in the non-AX diets. Wheat bran (4%) was added to all diets to maintain laxation (diet compositions shown in Supplementary Table 1). The four experimental diets fed were low (LM) and high (HM) meat without AXRF and low (LMAX) and high (HMAX) meat with AXRF.

Pigs were adapted to their assigned diet over 7 d and then fed that diet for ~4 wk. Daily diet allocation increased weekly according to body weight. The animals received two meals in individual pens and had free access to water. The mash diets were fed with water added at feeding (~500 mL).

Animal anesthesia and LI preparation for sampling

Each pig was fed at 4 and 2 h before anaesthesia to standardize sample collection with feeding. The pigs were premedicated, and isoflurane was used to ensure full anesthesia while maintaining blood perfusion to the intestines [39]. After abdominal dissection, the LI was ligated with plastic strips at regular intervals to avoid digesta mixing in situ. After LI removal, the animal was sacrificed without regaining consciousness.

The LI was separated as previously described [39]. Briefly, the LI was divided into four parts: cecum (CAE) and proximal (PC), middle (MC), and distal (DC) colon.

Digesta sampling

Digesta was removed from each section by gentle squeezing into a preweighed beaker. It was then weighed, mixed, pH measured, and sampled and weighed into tared sample bottles before being stored at –20C pending analysis.

Chemical analyses

Dry matter was determined by drying the sample to a constant weight at 105°C using ISO standard 6496 [40]. The pH of digesta samples was determined using a pH Cube (TPS, Springwood, Queensland, Australia) and an IJ44-BNC pH Combination probe (Ionode, Tennyson, Queensland, Australia). Because of the viscous nature of the LI digesta in particular, the electrode was calibrated daily or as required.

For SCFA analysis, digesta samples were thawed, prepared by vacuum distillation, and analyzed by gas chromatography (Agilent 6890 Series GC, Agilent Technologies, Wilmington, DE, USA) using a fused silica column (Agilent) with a 1-um coating. The carrier gas was helium at a flow rate of 6 mL/min with a split injector and flame ionization detector held at a temperature of 250°C. For each run, the oven was held at 90°C for 1 min, then increased to 190°C at a rate of 10°C/min and held for 1 min. Results are reported as mmol/L digesta water, excluding DM content, as SCFAs are contained within the liquid rather than solid phase of digesta.

Ammonia was analyzed using a previously modified method [41]. Ammonia nitrogen was determined colorimetrically, using the chemical reaction of ammonia ions $(NH_4)^+$ with sodium salicylate and nitroprusside in a weakly alkaline buffer at a wavelength of 650 nm, using a ultraviolet/visible spectrophotometer (Automated Discrete Analyzer Model AQ2+, SEAL Analytical).

Diaminopimelic acid (DAPA) is known to be a component of the peptidoglycan layer of the bacterial cell wall [42] and has been used for many years in ruminant nutrition to provide a quantitative estimate of the amount of microbial protein reaching the small intestine from the rumen [43]. Recently it has been shown to be an effective marker to determine microbial protein in pigs [44]. For the work reported here, and as described previously [39], DAPA was analyzed in combination with other amino acids in digesta, using standard ion-exchange column chromatographic separation techniques with fluorimetric detection after postcolumn derivitization with *O*-phthaldialdehyde [45].

Bacterial profiles of distal mucosa

A custom phylogenetic microarray developed and validated for gut bacteria was used to analyze the microbiota. The development of the custom microarray and its associated methods used in this study were previously described [46], with the relevant microarray probe information and other methodological details also accessible at the National Center for Biotechnology Information's GEO DataSets under the search term "GPL9353."

The bacterial profiles of the LI distal mucosa fractions from the four dietary groups (n = 5 per group) were collected, and DNA was extracted and hybridized in the custom microarray as reported previously [14]. Briefly, microbial 16 S ribosomal RNA genes were amplified using the primer sets 27 F (5′–AGAGTTTGATCMTGGCTCAG–3′) and T7/1492 R (5′– TCTAATACGACT CACTATAGGG GGYTACCTTGTTACGACTT -3′; the underlined region is modified to include a T7 promoter sequence). The polymerase chain reaction amplicons were purified with the MinElute polymerase chain reaction purification kit (Qiagen Inc N.V., Venlo, Netherlands) and then added as a template for in vitro transcription-based synthesis of complementary RNA (cRNA) using the MEGAScript T7 in vitro transcription kit (Ambion/ThermoFisher Scientific, Grand Island, NY, USA).

After purification with a MEGAclear Kit (Ambion), 1 μg of the sample cRNA and 140 ng of standard cRNA were labeled at the same time using *Label* IT μArray Cy5 reagent (Mirus Bio LLC, Madison, WI, USA) for 1 h at 37°C while protected from light. Subsequently, 0.1 volumes of the 10 × stop reagent were added