Molecular weight changes of arabinoxylans of wheat and rye incurred by the digestion processes in the upper gastrointestinal tract of pigs

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ABSTRACT

Twenty cannulated-pigs were fed wheat flour (WFL), wheat whole grain (WWG), wheat aleurone flour (WAF) and rye aleurone flour (RAF) differing by their arabinoxylans (AX) proportions. After ileal collection of digesta (0800–1800), the soluble phase was extracted. The weight average molecular weight (MWw) of the ileal soluble phase and of the dietary water extracts were determined by high-performance size exclusion chromatography. The MWw value of the ileal soluble phase of digesta after feeding the RAF diet was reduced by approximately 25% compared with the dietary water extract whereas no difference was found between the water extract and the ileal soluble phase when the wheat based diets were fed. The viscosity of the ileal phase is the highest after feeding the RAF diet. In conclusion, the MWw of AX from wheat diets is not changed during passage of the upper intestinal tract of pigs, whereas that of AX from RAF diet is reduced by 25%.

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1. Introduction

Due to their structural characteristics, dietary fibre (DF) influences digestion and absorption processes along the gastrointestinal (GI) tract (Glitsø et al., 1999). Arabinoxylans, the most important DF of cereals, represent a heterogenic group of polysaccharides of high molecular weight which are comprised of xylan backbones substituted to various degrees with arabinose residues (Fincher and Stone, 1986) according to grain tissues and botanical origins. In our previous study, we have shown that the digestibility of macronutrients is influenced by the botanical source (rye and wheat) of AX from the aleurone layer and by origin tissues of AX (whole-grain vs. aleurone layer; Le Gall et al., 2009).

However, our knowledge about the resistance of AX from wheat and rye to digestion processes in the small intestine is limited. It is hypothesised that AX is resistant to the digestion process in the upper GI tract and that soluble AX is the main contributing factor for the variability in digesta viscosity and the impact of that on macronutrients digestibility observed among the WFL, the WWG, the WAF and the RAF diets. Here, we therefore investigated the MW change of AX in the soluble phase of digesta after passage of the upper GI tract in two periods (breakfast vs lunch).

2. Material and methods

The experiment, previously described (Le Gall et al., 2009), complied with the guidelines of the Danish Ministry of Justice with respect to animal experimentation and care of the animals under study. Briefly, twenty growing pigs, fitted with T-cannulae at the terminal ileum for collection of ileal digesta, were fed the experimental diets made as breads. The diets, made of standard white wheat flour and wheat purified fibre (WFL), whole wheat grain (WWG),
wheat aleurone flour (WAF) and rye aleurone flour (RAF), were balanced with regards of nutrients and differed by their DF proportion. After a 7-day recovery period, the pigs were fed at 0800, 1300 and 1800. Thus, when pigs received morning and afternoon meals, the time interval since last feeding was 14 and 5 h, respectively, and the expected impact of time since last feeding being more pronounced due to differences in portion size at the previous meal (20% of daily allowance at 1800, 40% at 0800). Ileal digesta were collected on days 15 and 17 from 0800 to 1300 (breakfast) and from 1300 to 1800 (lunch) after feeding and pooled for the two collection days within each pig. The supernatant phase of ileal digesta, after centrifugation (10,000×g, 20 min), was frozen until analysis.

The water extract from diets was obtained from five hundred milligrams of diet added in 20 mL of Millipore water, stirred for 1 h at 39 °C, then centrifuged (2,000 × g for 10 min).

After cationic exchange (SCX, 600 mg, Lida, Rochester, New York, USA), filtration over a 0.45 μm membrane (Minisart®, Sartorius, Taastrup, Denmark), the water extract from diet or the ileal soluble phase was injected on a high-performance size exclusion chromatography (HPSEC) system (Waters HPLC, Milford, USA) using the GMPWxl3285 column (Tosoh Corporation, Tokyo, Japan) and two Shodex HQ-pak SB 806 and B 806 M columns (Showa Denko K.K., Tokyo, Japan) eluted at 0.5 mL/min with acetate buffer (200 mM, pH 5.1). The elute was monitored using a refractive index detector (Waters 2410, U.S.A.). P-82 pullulan standards (Showa Denko K.K.) were used for calibration.

The viscosity was measured in dietary water extracts and in ileal soluble phase following the procedure of Johansen et al. (1997).

The MW\(_w\) was calculated as:

\[
MW_w = \sum W_i M_i
\]

where \(W_i\) is the weight fraction, \(N_i M_i / \sum N_i M_i\), where \(N_i\) is the number of each molecule and \(M_i\) the molecular mass of each molecule. The concentration of soluble AX ([AX] corrected MW\(_w\)) was calculated as [AX] × MW\(_w\).

One-sample t-test was performed for testing whether the means of the MW\(_w\) data of water extract from diet and those of ileal soluble phase are different from each other. Differences were declared as significant when \(P < 0.05\).

Data were analysed by the Mixed procedure of SAS. The correlation between the log transformed ([AX] × MW\(_w\)) and log viscosity was calculated as Pearson’s product-moment correlation coefficient by using Proc Corr of SAS.

3. Results

In Fig. 1, three main peaks and two main peaks were detected for the wheat based diets and the RAF diet profiles, respectively. After feeding the wheat based diets, the peak detected at the highest MW\(_w\) was diminished and that at 4.2 was increased. After feeding the RAF diet, there was a shift in the retention time of the fraction of the highest MW\(_w\) toward longer retention times.

These modifications were also illustrated MW\(_w\) changes (Table 1). The MW\(_w\) of soluble digesta was increased \((P = 0.02)\) by 22% after feeding the WWG diet and it was reduced \((P = 0.001)\) by approximately 25% after feeding the RAF diet when compared with the MW\(_w\) of water extracts of diets. The MW\(_w\) of AX from digesta collected after the lunch

\[\text{Fig. 1. HPSEC-RI profiles of water extracts from diets (—) and ileal soluble phase from digesta (—) of pigs fed the WFL, the WWG, the WAF and the RAF diets.}\]
meal when feeding the WFL and the WWG diets was consistently lower \((P_{\text{meal}}=0.003)\) than after the breakfast meal but no effect of meal was observed after feeding the WAF and the RAF diets \((P_{\text{interaction}}=0.008; \text{Table 2})\). The concentration of soluble AX in ileal digesta was highest when feeding the RAF diet and increased \((P<0.05)\) between the breakfast and the lunch collection while no difference was observed among the wheat based diet groups. The PDI was highest \((P<0.05)\) in the WAF group, then in the RAF group and decreased \((P>0.05)\) between the breakfast and the lunch collection. The viscosity of ileal digesta was also significantly higher after feeding the RAF diet than the wheat diets \((P=0.001)\). The relationship between the log\((\text{AX})\times\text{MW}_{w}\), and the log viscosity of ileal digesta across all diets was linear \((P=0.001, \text{Fig. 2})\); that the higher AX concentration and MW\(_w\) coincide with increased ileal digesta viscosity.

### Discussion

The positive linear relationship between the log \([\text{AX}]\times\text{MW}_{w}\), and the log viscosity of ileal digesta across all diets and sampling periods inevitably point to soluble AX as the main factor responsible for the variation in the viscosity of ileal digesta. The results of this study further show that the bread making process acts on the polymers probably due to cross reactions between polysaccharides and proteins forming high MW\(_w\) complexes, which are shifted toward longer retention time in HPSEC (data not shown). These polymers, however, are degraded during the digestion processes in the upper GI tract of pigs. Finally, the MW\(_w\) of soluble AX from RAF diet was reduced after passing the upper GI tract whereas this was not the case when feeding the wheat based diets. Degradation of DF in the upper GI tract is caused by the microflora permanently colonising these sites of the tract (Jensen and Jørgensen, 1994) and leading to degradation of DF to different degrees depending on the molecular structure (Bach Knudsen et al., 2008). Indeed, AX from the rye aleurone, highly substituted by arabinose on the xylose backbone, is also more soluble than AX from wheat (whole grain and aleurone layer). This is also reflected in the higher concentration of soluble AX in the ileal digesta and in the higher viscosity of RAF ileal fraction compared to the wheat ileal fractions. Moreover, the viscosity increased from the diet to the ileal fraction due to digestion and absorption of sugars, starch, protein, and fat. However, although AX from rye aleurone flour is partly degraded during passage during the upper GI tract, its concentration and MW is high enough to impair the digestibility of macronutrients as previously reported (Le Gall et al., 2009). Indeed, the digestibility of starch was significantly lower in the RAF diet than the wheat diets and the digestibility of fat the lowest of all diets although it was only compared to the WFL diet the difference was significant. Finally, insoluble AX interacts with the digestion process by acting as a barrier against enzymes attack.

In conclusion, the MW of soluble AX from rye was reduced by approximately 25% whereas the MW of AX from wheat as a whole did not change during passage of the upper GI tract. However, the concentration of soluble AX was

### Table 1

| Weight average MW (MW\(_w\)) of AX (×10\(^5\)) of the water extract from diets and of ileal soluble phase after passage of digesta through the stomach and small intestine of pigs fed the four experimental diets (WFL, WWG, WAF and RAF). |
|-----------------|--------|--------|--------|--------|--------|--------|--------|--------|
|                  | WFL\(^1\) | WWG\(^2\) | WAF\(^2\) | RAF\(^2\) | T-test  |
| Water extract from diet | 2.2 b   | 1.8 b   | 2.6 a   | 3.8 b   | P = 0.05 |
| Ileal soluble extract from digesta | 2.2 a   | 2.2 a   | 2.6 a   | 2.8 ab  | P = 0.02 |
| MW\(_w\) = 1 × 10\(^5\) |        |        |        |        |        |

\(^1\) MW\(_w\), weight average MW.

\(^2\) WFL, wheat flour and cellulose; WWG, whole wheat grain; WAF, wheat aleurone flour; RAF, rye aleurone flour diets.

\(^a,b\) Values within the same column are significantly different.

### Table 2

| Concentration of soluble AX, viscosity of soluble phase, weight average MW (MW\(_w\)) of AX (×10\(^5\)) and poly dispersity index (PDI) of polymers in ileal digesta collected from 8:00 to 18:00 h after feeding the four experimental diets. (Least square means with standard errors of ANOVA for five pigs by dietary treatment). |
|-----------------|--------|--------|--------|--------|--------|--------|--------|--------|
|                  | WFL\(^1\) | WWG\(^2\) | WAF\(^2\) | RAF\(^2\) | Sem  |
| Bread\(^3\)     |        |        |        |        |      |
| Soluble AX, g/kg digesta | 51 b   | 47 b   | 28 b   | 77 a   | 9 b   | 47 b   | 54 a   | 4 a   |
| Viscosity, mPa.s | 1.7 b   | 1.7 b   | 1.4 b   | 4.7 a   | 0.4   | 2.6   | 2.2   | 0.3   |
| MW\(_w\) = 2 ×10\(^5\) | 2.2 b   | 2.2 b   | 2.6 a   | 2.8 a   | 0.2 b  | 2.7 b   | 2.2 a   | 0.1 b  |
| PDI\(^3\), %    | 38.6 b  | 43.3 c  | 75.7 a  | 58.2 b  | 4.9   | 58.2 b  | 49.6 a  | 3.0 a  |
|                   |        |        |        |        |      |

\(^1\) WFL, wheat flour and cellulose; WWG, whole wheat grain; WAF, wheat aleurone flour; RAF, rye aleurone flour diets.

\(^2\) MW\(_w\), weight average MW.

\(^3\) Poly dispersity index.

\(^a,b,c\) Values within the same row is significantly different.
at a level that it impacted the digestion processes in the upper GI tract.

Conflict of Interest

Authors declare that no actual or potential conflict of interest, including financial transactions, no personal or other relationships with other people or organizations within three years of beginning the submitted work that could influence or be perceived to influence their work.

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