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Short communication

Variability in the *in vitro* degradation of non-starch polysaccharides from wheat by feed enzymes

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ABSTRACT

Wheat is one of the major feed ingredients in poultry diets. Non-starch polysaccharides (NSP) from wheat can have a negative impact on animal performance and therefore NSP-degrading feed enzymes are commonly added to wheat-based diets. Variation exists in the amount of NSP present in wheat as well as in the composition of the NSP-fraction. This interferes with designing optimal enzyme applications. The objective of this study was to examine the effects of a single xylanase preparation on the degradation of water-extractable (WE) and water-unextractable (WU) NSP-fractions of wheat samples with variable NSP-characteristics. Treatment of nine wheat samples with the same xylanase resulted in varying degrees of hydrolysis (DH). Degradation was between 2.6% and 40.3% for the WE-NSP fraction and between 12.8% and 25.5% for the WU-NSP fraction. Correlations were found between the DH of WE-arabinoxylan and the arabinose to xylose (A/X) ratio of this fraction ($P<0.001$, $r=-0.82$) and between the DH of WE-NSP and the A/X-ratio ($P<0.001$, $r=-0.60$). It is concluded that *in vitro* the response of wheat to xylanase varies for different batches of the cereal and that the A/X-ratio can be used as a predictor for the degradation of the WE-NSP fraction by the xylanase used in this study.

1. Introduction

Wheat is an important feed raw material in poultry diets in Europe because of its relatively high starch and protein content compared to other important cereals such as maize and barley. Wheat, however, contains variable amounts of non-starch polysaccharides (NSP), which may limit nutrient utilisation in monogastric animals. These effects are based on two hypothetical mechanisms. First, the water-extractable (WE) fraction of the NSP can increase the viscosity of the intestinal contents, hence disturbing digestion (Dusel et al., 1997; Choct, 2001; Van Campenhout, 2007). Second, the water-unextractable NSP (WU-NSP) in the cell walls could impair nutrient availability to the animal by blocking the access to the cell contents for endogenous digestive enzymes, the so-called 'cage effect' (Bedford, 2002; Van Campenhout, 2007). The concentration, extraction properties (WE or WU) and structure (substitution of arabinoxylan) of the NSP in wheat can be highly variable, depending on many factors such as variety, growing conditions and post-harvest storage conditions (Kim et al., 2003; Gutiérrez-Alamo et al., 2008a). As a consequence, the nutritional value of wheat can vary when fed to broiler chickens (Annison, 1991; Austin et al., 1999; Choct et al., 1999; Wiseman, 2000; Steenfeldt, 2001).

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Table 1
Origin, year of harvest, concentration of glucan and arabinoxylan (AX), arabinose to xylose ratio (A/X) and concentration of non-starch polysaccharides (NSP) in the water-extractable (WE) and water-unextractable (WU)-NSP fractions of different wheat samples. The data are means of triplicate determinations.

	Origin	Harvest	WE-NSP fraction (g/kg DM)				WU-NSP fraction (g/kg DM)			
			Glucan	AX[a]	A/X	NSP[b]	Glucan	AX[a]	A/X	NSP[b]
Wheat 1	UK	2009	5.3	13.1	0.36	26.4	22.8	45.8	0.52	71.9
Wheat 2	Russia	2009	3.8	9.3	0.48	21.0	22.8	45.9	0.63	73.5
Wheat 3	Russia	2009	4.6	9.5	0.58	23.7	24.3	46.2	0.64	72.7
Wheat 4	Greece	2010	3.0	8.1	0.72	20.4	23.3	41.6	0.72	67.8
Wheat 5	Russia	2010	3.7	10.7	0.54	22.8	25.3	55.6	0.58	85.1
Wheat 6	Sweden	2010	2.7	7.6	0.53	16.7	20.6	45.5	0.59	69.5
Wheat 7	UK	2010	6.2	9.0	0.47	23.8	22.1	43.2	0.54	69.3
Wheat 8	Poland	2010	3.5	10.1	0.51	21.6	20.5	50.4	0.49	75.4
Wheat 9	Belgium	2010	4.6	8.8	0.55	21.9	23.4	50.2	0.59	78.0
Average			4.2	9.6	0.53	22.0	22.8	47.2	0.59	73.7
CV[c] (%)			26.9	17.0	18.3	12.2	6.8	9.0	11.7	7.2

[a] Calculated as the sum of arabinose and xylose.
[b] Calculated as the sum of arabinose, xylose, mannose, galactose and glucose.
[c] Coefficient of variation.

To improve nutrient utilisation and bird performance, non-starch polysaccharide-degrading enzymes, such as xylanases (EC 3.2.1.8), are commonly used as feed additives in wheat based feed formulations (Bedford, 2000; Adeola and Cowieson, 2011). The effect of adding these enzymes is the largest for batches of cereals with a low nutritional value (Choct et al., 1995; Scott et al., 1998). The addition of enzymes can thus be used to reduce the variation in ingredient quality (Bedford, 2000). It has been shown, however, that cultivar differences cannot be completely eliminated by using enzymes (Gutiérrez-Alamo et al., 2008b) and that sometimes no effects can be observed (Svihus, 2011). Therefore, there is a need to predict the nutritional value and the response of a diet to the addition of NSP-degrading enzymes (Adeola and Cowieson, 2011). It is not clearly known to what extent the variation in the chemical composition of the substrate, in particular the NSP, affects the efficacy of the enzymes (Cowieson et al., 2006). Only a few studies are published so far that investigate the degradation of NSP by enzymes *in vitro* (*e.g.* Tervilä-Wilo et al., 1996; Meng et al., 2005). The current paper describes a series of experiments, based on an *in vitro* NSP-digestion model, to assess the effect of the NSP-variability on the efficacy of xylanase.

2. Materials and methods

2.1. In vitro digestion and isolation of water-extractable and water-unextractable fractions

Nine different batches of wheat were selected from a collection of about 150 samples so as to cover a wide range in NSP-content. The samples (variety not known) were obtained from commercial feed mills. The country of origin and the year of harvest can be found in Table 1.

The *in vitro* enzyme degradation model was based on the NSP-analysis procedure described by Englyst et al. (1994). The wheat samples were hammermilled (1 mm screen size). Starch was removed by incubating triplicate samples of 0.3 g at 100 °C for 30 min. in acetate buffer (0.1 M, pH 5.0, 4 mL) and thermostable alpha-amylase (Termamyl 120 L, Novozymes A/S, Bagsvaerd, Denmark, 100 μl). Further degradation of starch was achieved by incubation at 60 °C for 30 min. with amyloglucosidase (A9913, Sigma–Aldrich NV/SA, Bornem, Belgium, 100 μl). Subsequently, 1 mL buffer (control treatment) or 1 mL of xylanase (produced by *Trichoderma longibrachiatum*, 970,000 units/kg wheat) was added and the samples were incubated at 40 °C for 4 h. Hereafter, the samples were centrifuged at 1500 × *g* for 10 min. to separate the WE-NSP (supernatant) from the WU-NSP (residue). The WE-NSP in the supernatant were precipitated by adding 20 mL of ethanol 95% (v/v) and keeping the samples one night at 4 °C. The next day, the WE-NSP were isolated by centrifuging at 1500 × *g* for 10 min and discarding the liquid. The remaining residue was washed by mixing it with 25 mL ethanol 85% (v/v) and centrifuging at 1500 × *g* for 10 min. This was repeated with 25 mL ethanol 95% (v/v) to further purify the WE-NSP fraction. The residue containing the WU-NSP was washed with demineralised water followed by ethanol 95% (v/v).

2.2. Measurement of the constituent monosaccharides

The remaining residues of WE- and WU-NSP were subjected to acid hydrolysis. The constituent monosaccharides were determined as alditol acetates with gas liquid chromatography (GLC) as described by Englyst et al. (1994). The constituent monosaccharide values were converted to the equivalent polysaccharide values using the conversion factor of 0.88 for pentoses (arabinose and xylose) and 0.90 for hexoses (mannose, galactose and glucose) (Bach Knudsen, 1997). The WE- and WU-NSP fractions were calculated as the sum of the constituent monosaccharides in the respective fractions, while arabinoxylan was defined as the sum of arabinose and xylose. The amount of glucose was defined as glucan (deriving from both beta-glucan and cellulose). The degree of hydrolysis of NSP (DH_{NSP}) was calculated as the difference in the amount of NSP (expressed as the sum of its constituent monosaccharides) between the control and the enzyme treated samples,