NSP concentration was the highest in the wheat batches from Austria (89.3 g/kg DM) and Ireland (86.9 g/kg DM).

Correlation analysis between different wheat characteristics

The results of the Pearson's correlation analysis between the different parameters for all wheat samples are shown in Table 4. Highly significant ($P < 0.001$) positive correlations ($r > 0.80$) were obtained between for example WU-AX and WU-NSP because the former constitutes a large part of the latter. Lower, but also highly significant ($P < 0.001$) positive correlations were obtained between for example WE-GLU and WE-NSP ($r = 0.37$). Because WE-GLU (presumably β-glucan) is part of the WE-NSP, these kinds of correlations were also evident.

Relatively high positive correlations ($r > 0.5$) were found between different fractions of the NSP, i.e. between WU-AX and WU-GLU ($r = 0.69$; $P < 0.001$) or between the total amount (TOT) of AX and TOT-GLU ($r = 0.57$; $P < 0.001$). For the WE fraction of the NSP, no significant correlation ($P > 0.05$) was detected between WE-AX and WE-GLU.

A relatively high negative correlation was obtained between the starch and protein content ($r = -0.63$; $P < 0.001$). In addition, a relatively high positive relation was found between the extract viscosity and WE-AX ($r = 0.59$; $P < 0.001$), while lower correlations were noted between extract viscosity and WE-NSP ($r = 0.46$; $P < 0.001$), TOT-AX ($r = 0.40$, $P < 0.01$) and TOT-NSP ($r = 0.33$; $P < 0.01$).

Low ($r > -0.5$), but highly significant negative correlations were found between starch and TOT-NSP ($r = -0.36$; $P < 0.001$) and between starch and the individual WU- and TOT-NSP fractions.

DISCUSSION

The ranges found for total NSP in the current study are comparable to literature data. Taking into consideration the variability in analytical techniques employed for the determination of NSP, values between 74.5 g/kg DM (Dusel et al., 1997) and 143.9 g/kg DM (McCracken et al., 2002) have been reported before for European wheats (Table 1) and even higher values, as high as 156.8 g/kg DM for Australian wheats (Choct et al., 1999) or 166 g/kg DM for Canadian wheats (Zijlstra et al., 1999). Furthermore, the ranges found for total AX were comparable to ranges reported in literature data, which were between 35 g/kg DM (Dusel et al., 1997) and 81 g/kg DM (Austin et al., 1999). The concentration of AX was, however, slightly overestimated due to the presence of an arabinogalactan-peptide in the endosperm (Loosveld et al., 1997). The study described by Dornez et al. (2008) took into account this variation by correcting the arabinose level for the presence of the arabinogalactan-peptide. For the current study this would mean that on average 0.84 g/kg DM of arabinose originated from the arabinogalactan-peptide rather than from AX (assuming that all WE-NSP originates from the endosperm and considering an arabinose to galactose ratio of 0.7 (Loosveld et al., 1997)). This is only a small concentration compared to the total concentration of AX. The small concentrations of mannose and galactose are comparable to other studies reporting individual sugar levels (Austin et al., 1999; Bach Knudsen, 1997; Jha et al., 2011; Zijlstra et al., 1999). Values between 14 and 22 g/kg DM have been reported for cellulose (Jha et al., 2011; Steenfeldt, 2001) and between 2.2 and 11.8 g/kg DM for β-glucan (Pritchard et al., 2011). This corresponds to the total GLU concentration in the current study.

It is difficult to compare WE-NSP concentrations between different studies. As mentioned before, most researchers utilize procedures based on the method described by Englyst et al. (1994) or the Uppsala method (Theander et al., 1995). In these methods, the concentration of

WE-NSP is calculated from the difference between concentration of total NSP and the concentration of WU-NSP. On the other hand, various extraction procedures have been used for the isolation of the WE-NSP fraction. For example, Saulnier et al. (1995) employed an extraction with distilled water whereas Carré et al. (2002) used a boiling ethanol:water treatment before aqueous extraction. In the current study, the concentrations of WE-NSP and WU-NSP were separated according to the dietary fiber method (AACC 32-07), which uses an aqueous extraction of WE-NSP and subsequent precipitation with ethanol after an enzymatic removal of starch. A study by Steenfeldt (2001) for 16 Danish wheat samples showed an even larger range (between 10 and 38 g/kg) for WE-NSP than the one reported in the present study. Regarding WE-AX, literature data report concentrations between 3.6 (Saulnier et al., 1995) and 16.4 g/kg DM (Dusel et al., 1997). AX consists of a linear backbone of (1→4) linked β-D-xylose to which single L-arabinose residues (on the *O*-2 or *O*-3 positions or both) are attached (Saulnier et al., 2007). Hence, the arabinose to xylose (A/X) ratio can be used for a rough characterisation of the structure of AX. The A/X ratios in the current study were within the ranges reported by Carré et al. (2002) and Kim et al. (2003). These researchers found ranges between 0.7 and 1.2 for WE-AX and between 0.5 and 1.2 for WU-AX.

The method for the determination of the protein content is more universal than for NSP. Values for European wheat have been reported between 72 g/kg DM (Yasar, 2002) and 144 (Nicol et al., 1993) (Table 1). Most researchers apply Kjeldahl-based methods to assess the nitrogen concentration, although combustion methods are also used (Dornez et al., 2008; Losada et al., 2009). There was, however, a difference in the conversion factor employed for the calculation of protein concentration from nitrogen concentration. Generally, a lower factor (i.e. 5.7 instead of 6.25) is preferred for the estimation of wheat protein, based on the presence of the proteins

gliadin and gluten in in wheat which have a high amount of nitrogen (Jones, 1931). Therefore, protein concentrations obtained from literature were recalculated to a conversion factor of 5.7 (Table 1).

As for protein, the method for the determination of starch is fairly common. Starch content is typically measured enzymatically, involving hydrolysis with α-amylase and amyloglucosidase. After this, the glucose released can be determined by a glucose oxidase and peroxidase method. This assay is provided as a commercial kit by Megazyme, as described for the current study.

The obtained ranges in the present study were comparable to literature data, which report values between 541 g/kg DM (Jha et al., 2011) and 744 g/kg DM (Nicol et al., 1993), or even higher for Australian wheats (769 g/kg DM) (Choct et al., 1999).

In contrast to the analysis of protein and starch, many different methods exist for the determination of extract viscosity. Bedford and Classen (1993) described a procedure involving a pretreatment with pepsin and pancreatin, whereas Carré et al. (2002) proposed a protocol using a boiling ethanol:water pretreatment. Other researchers tested the viscosity of aqueous extracts without pretreatment (Dusel et al., 1997; Pirgozliev et al., 2003; Rose et al., 2001; Saulnier et al., 1995; Svihus and Gullord, 2002). But even then, there are differences in wheat:water ratio, temperature during extraction, extraction time and centrifuging conditions (time, speed) prior to measuring the viscosity. This makes comparisons between literature data very difficult. Some results from other research are shown in Table 1. One of the hypotheses for the anti-nutritional effect of wheat NSP in broiler chickens is related to their ability to increase the viscosity of the intestinal content and thereby disturbing nutrient digestibility (Choct and Annison, 1992b). In this respect, several studies have found a negative correlation between the viscosity of a wheat extract and the AME (Barteczko et al., 2009; Carré et al., 2002; Dusel et al., 1997; McCracken et

al., 2002). This shows the importance of the extract viscosity and the consequences of its variation.

Currently, the NSP concentration of wheat is not analysed in the practice of feed formulation, because its analysis is costly and time-consuming. The current dataset was used to make correlations between the NSP and some more simple characteristics like the concentration of protein and starch or extract viscosity. The correlation analysis between the different wheat characteristics showed that a high concentration of AX is accompanied by a high concentration of β-glucan and cellulose or in general a higher concentration of fibre in these wheat samples. This can be expected because AX, β-glucan and cellulose are all important constituents of the plant cell wall. Correlations between different NSP fractions from wheat are rather scarce in literature and no other study was found that reports these correlations. In practice, this could mean that a measurement of total WE- or WU-NSP might be sufficient, instead of analysing the monosaccharide composition of these fractions. For the WE fraction of the NSP, however, no significant correlation ($P > 0.05$) was detected between WE-AX and WE-GLU. During storage of wheat, grain-associated glycanases can cause changes in its chemical composition (Choct and Hughes, 2000; Dornez et al., 2009; Rowe et al., 1999). For example grain-associated xylanases can degrade the NSP, resulting in a release of WU-NSP from the cell walls and an increase in the WE-NSP fraction. The WE-NSP can also be further degraded to smaller fragments or to free sugars which can no longer be precipitated by ethanol treatment. This was demonstrated in a study by Kim et al. (2003), who observed a decrease in WE-NSP and an increase in free sugar concentration in wheat grains stored for six months (in sealed metal bins in a grain shed at ambient temperature). In the current study, the wheat samples were analysed as quickly as possible after reception of the samples (within one month), to minimise changes during storage

in the lab (stored in sealed plastic containers at ambient temperature). Nevertheless, the samples were obtained from the feed mills all over the year so some samples were stored at the feed mills. No information was available about the storage conditions at the feed mills, but it can be that the WE-NSP concentration had changed during storage, which can be an explanation for the lack of correlation between the different fractions of WE-NSP.

The correlation between starch and protein concentration was observed before by Choct et al. (1999) and Kim et al. (2003) and could be expected because they are both the major components of the wheat endosperm. The current research also confirmed the positive correlation between extract viscosity and WE-AX. The correlation was, however, too small to make any predictions about the NSP concentration of wheat. Carré et al. (2002) and Dusel et al. (1997) obtained positive correlations between WE-AX and viscosity with a correlation coefficient close to 0.90, whereas Rose et al. (2001) found a correlation coefficient of 0.73 with WE-NSP. Positive correlations with TOT-NSP (r = 0.43), TOT-AX (r = 0.54) and WE-NSP (r = 0.79) were reported by Gebruers et al. (2010). A positive correlation was therefore expected, although a negative correlation was also reported by Austin et al. (1999).

In the current study, negative correlations were found between starch and TOT-NSP and between starch and the individual WU- and TOT-NSP fractions. Although the relationships between NSP and starch was too small to make any predictions about the NSP concentration of wheat, a negative relationship between NSP and starch has also been observed by Coles et al. (1997) (for TOT-AX), Parsaie et al. (2006) (for WU-NSP and TOT-NSP), Pritchard et al. (2011) (for WU-NSP, WU-AX and WU-GLU) or Gebruers et al. (2010) (for TOT-NSP and TOT-AX). Part of this relation can be explained by the dilution of starch concentration with NSP. But, as

noticed by Coles et al. (1997), the total variation in NSP concentration is small compared to the variation in starch concentration (i.e. 36% in the present study), so it cannot solely be due to starch dilution. Coles et al. (1997) observed that starch (negative relation) and AX (positive relation) both responded to the same environmental effect, namely drought conditions. The latter was controlled by the use of a mobile rainshelter. In a study by Zhang et al. (2010), the increase in AX concentration and the reduction in starch concentration due to high temperature and water-deficit conditions were accompanied by a reduction in endosperm volume. They suggested that the increase in AX content can be explained by the shrinkage of the wheat kernel and the increase in the relative amount of seed coat. The seed coat contains most of the AX. The susceptibility of TOT-NSP and TOT-AX to environmental conditions was confirmed in an extensive study by Gebruers et al. (2010), who found that 30% of the variability in TOT-NSP and TOT-AX could be explained by environment, while 30% was explained by genotype. The interaction between genotype and environment explained another 20% of the variability.

Based on the data described in the current paper, three wheat batches which were representative for NSP rich and NSP poor wheat in Europe were selected for a broiler digestibility trial (Smeets et al., 2015). The data confirm the relationship between the concentration of NSP and the nutritional value of wheat and highlight the need for considering the concentration of NSP in the practice of feed formulation.

In general, the variations in chemical composition reported here are in accordance with the data described by other researchers for individual countries in Europe. By including wheat samples from 15 different European countries over five different years of harvest no larger ranges were observed. Nevertheless, the present study confirms the wide variability in wheat NSP in European wheats used for broiler diets, even when one single method was used. This

variability in NSP can affect the nutritional value of wheat as observed by several researchers (Annison, 1991; Choct et al., 1992; Choct et al. 1999; Dusel et al., 1997; Smeets et al., 2015) and should be considered in the practice of feed formulation.

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Table 1. Within country variation in chemical composition (total arabinoxylan (AX), total non-starch polysaccharide (NSP), starch and protein content) and extract viscosity in wheat.

Source	n	Origin	Chemical composition (g kg^{-1} dry matter)				Extract viscosity mPa·s
			Total AX	Total NSP	Starch	Protein (N x 5.7)[a]	
Austin *et al*. 1999	12	UK	37-81	55-104	-	-	2.1-8.4[b]
Barteczko *et al*. 2009	9	Poland	-	-	-	93-120	-
Carré *et al*. 2002	22	France	-	98-117	688-725	88-122	1.0-3.8[c]
Dornez *et al.* 2008	42	Belgium	58-76	-	-	-	-
Dusel *et al*. 1997	170	Germany	35-64	-	-	-	1.1-5.2
Gutierrez del Alamo *et al.* 2008	4	Spain	-	-	667-685	82-125	4.5-6.5
Hetland *et al*. 2007	20	Norway	-	85-128	536-648	98-126	-
Losada *et al*. 2009	13	Spain	-	98-112	550-611	93-124	-
McCracken *et al*. 2002	12	UK	-	83-129	612-656	106-134	5.2-17.5[b]
Nicol *et al.* 1993	10	UK	-	92-105	608-744	94-144	-
Parsaie *et al*. 2006	15	Iran	-	75-115	704-734	93-137	-
Pirgozliev *et al*. 2003	23	UK	-	106-144	594-732	78-129	2.0-7.1
Rose *et al.* 2001	6	UK	-	85-128	629-662	108-120	4.1-8.2
Saulnier *et al*. 1995	22	France	55-78	-	-	-	1.2-2.3
Steenfeldt 2001	16	Denmark	-	-	658-722	102-116	-
Svihus and Gullord 2002	20	Norway	-	-	614-712	99-140	0.9-3.1
Yasar *et al.* 2002	15	Turkey	-	-	-	72-129	1.8-2.7[b]

[a]The protein content from the different sources was recalculated to a conversion factor of 5.7 (AACC 46-10, specific factor for wheat). If no factor was mentioned, a factor of 6.25 was assumed (AACC 46-10, average conversion factor).

[b]*in vitro* viscosity

[c]Real Applied Viscosity

Table 2. Variation in non-starch polysaccharide (NSP) composition[a], arabinose to xylose (A/X) ratio, starch and protein content and extract viscosity in wheat originating from different European countries.

Composition (g/kg dry matter)	MINIMUM	MEAN	MAXIMUM	CV%[e]
Water-extractable NSP[b]				
Arabinose	2.1	3.3	4.9	18.2
Xylose	2.9	4.9	8.7	21.8
Mannose	1.2	2.0	2.9	13.7
Galactose	0.6	1.2	1.6	18.6
Glucose	0.6	1.6	3.5	34.0
Arabinoxylan	5.1	8.2	13.1	20.0
A/X-ratio	0.49	0.66	0.83	8.5
NSP	8.7	13.1	18.3	14.3
Water-unextractable NSP[b]				
Arabinose	13.0	18.8	24.8	11.0
Xylose	24.9	32.4	41.3	9.4
Mannose	0.9	1.4	2.5	18.5
Galactose	1.1	1.7	3.1	22.2
Glucose	17.6	24.9	33.2	14.0
Arabinoxylan	37.9	51.5	65.1	9.4
A/X-ratio	0.49	0.58	0.70	6.9
NSP	58.1	79.5	99.6	10.0
Total NSP[b]				
Arabinose	15.2	22.3	29.3	10.3
Xylose	28.2	37.7	46.3	9.0
Mannose	2.5	3.4	4.8	11.8
Galactose	1.8	2.9	4.3	15.5
Glucose	19.3	26.7	35.0	13.2
Arabinoxylan	43.4	59.9	73.6	9.0
A/X-ratio	0.51	0.59	0.71	6.5
NSP	68.4	93.2	112.2	8.8
Starch[c]	558	634	680	3.8
Crude protein (N x 5.7)[c]	92	124	173	11.3
Extract viscosity (mPa·s)[d]	1.26	1.61	3.22	21.0

[a] Values shown as anhydrosugars.
[b] Number of samples included in the analysis (n) = 153 (15 countries, 5 years of harvest).
[c] n = 122 (13 countries, 3 years of harvest).
[d] n = 69 (12 countries, 2 years of harvest).
[e] Coefficient of variation.

Table 3. Variation in the concentration of water-extractable (WE) and water-unextractable (WU) non-starch polysaccharides (NSP, shown as anhydrosugars), concentration of starch and protein and extract viscosity in wheat originating from different European countries over different years of harvest. Numbers shown are mean values and the coefficients of variation (in parentheses). Glu: glucose, AX: arabinoxylan, n: number of wheat samples.

	Composition (g/kg dry matter)													Extract viscosity
		WE-NSP			WU- NSP			Total NSP					Crude	
	n	Glu	AX	NSP	Glu	AX	NSP	Glu	AX	NSP	n	Starch	protein	n	(mPa·s)
Origin															
Austria	2	1.1	11.0	14.2	26.9	59.0	89.3	28.0	70.0	103.4	2	610	130	2	1.69
		(6.7)	(7.1)	(5.0)	(17.9)	(7.2)	(10.9)	(17.0)	(7.4)	(10.1)		(2.4)	(9.8)		(13.4)
Belgium	35	1.8	7.7	12.5	23.5	49.6	76.2	25.3	57.3	88.7	27	633	122	10	1.59
		(28.8)	(20.2)	(13.4)	(15.8)	(13.3)	(13.3)	(14.9)	(12.9)	(12.3)		(4.0)	(10.5)		(12.1)
Czech Republic	8	1.2	9.2	13.2	25.0	50.5	79.1	26.2	59.6	92.3	8	658	116	8	2.02
		(19.9)	(16.0)	(13.4)	(11.0)	(7.5)	(8.4)	(10.8)	(7.8)	(8.2)		(1.2)	(8.2)		(15.0)
Germany	21	1.7	7.8	12.7	24.8	52.2	80.0	26.5	60.0	92.7	11	631	132	3	1.48
		(19.0)	(19.2)	(13.8)	(15.0)	(6.9)	(8.3)	(14.2)	(4.9)	(5.8)		(2.1)	(8.0)		(4.8)
Denmark	1	2.3	7.4	13.1	28.8	54.2	86.2	31.1	61.6	99.2	0	-	-	0	-
		-	-	-	-	-	-	-	-	-		-	-		-
France	10	1.9	7.7	12.9	24.7	50.4	78.5	26.6	58.1	91.4	10	644	117	2	1.48
		(21.8)	(14.1)	(9.1)	(14.8)	(7.4)	(7.6)	(13.4)	(7.9)	(7.4)		(2.5)	(12.7)		(2.4)
Hungary	2	2.2	7.9	13.6	22.5	45.7	71.4	24.7	53.6	85.0	2	642	121	0	-
		(12.9	(7.2)	(5.2)	(8.8)	(2.8)	(4.9)	(9.2)	(3.3)	(4.9)		(0.0)	(8.2)		-
Ireland	5	2.1	6.9	13.0	29.7	53.8	86.9	31.9	60.7	99.9	0	-	-	0	-
		(13.4)	(4.9)	(5.2)	(8.7)	(3.7)	(3.4)	(8.4)	(3.7)	(3.1)		-	-		-
Italy	9	1.0	8.4	12.4	24.5	50.6	78.1	25.4	59.0	90.5	9	633	124	9	1.61
		(17.4)	(20.7)	(15.7)	(10.7)	(6.7)	(7.1)	(10.6)	(6.4)	(6.1)		(2.4)	(5.6)		(13.2)
Norway	14	1.5	9.4	14.2	22.5	51.9	77.5	24.0	61.3	91.7	12	618	132	3	1.48
		(25.7)	(16.0)	(13.5)	(11.2)	(4.8)	(6.1)	(10.4)	(5.1)	(5.6)		(2.2)	(7.3)		(11.7)
Poland	8	1.0	9.4	13.4	24.2	50.3	7.7.4	25.2	59.6	90.8	8	636	133	8	1.61
		(18.3)	(15.5)	(10.3)	(13.6)	(10.4)	(11.1)	(13.1)	(7.9)	(8.7)		(2.9)	(8.6)		(19.4)
Portugal	4	1.4	11.2	16.1	25.8	43.4	82.9	27.1	64.6	99.0	4	622	126	4	2.26
		(19.1)	(10.7)	(10.8)	(5.6)	(6.0)	(5.7)	(5.0)	(4.8)	(4.5)		(2.5)	(11.2)		(11.4)
Russia	18	1.5	8.0	12.7	28.2	54.6	86.3	29.7	62.6	99.0	15	607	135	6	2.04
		(21.6)	(12.3)	(9.2)	(7.8)	(7.6)	(7.1)	(6.7)	(7.1)	(6.0)		(4.3)	(12.5)		(28.9)
Sweden	3	1.1	8.1	12.5	25.0	50.0	77.7	26.1	58.0	90.1	3	652	124	3	1.46

		(5.4)	(13.0)	(7.2)	(2.8)	(6.1)	(5.3)	(2.5)	(6.6)	(5.0)		(1.5)	(2.5)		(20.8)
UK	13	1.6	9.8	15.4	24.0	48.9	76.0	26.3	58.8	91.4	11	660	106	11	1.67
		(34.3)	(16.5)	(14.7)	(9.5)	(6.4)	(10.0)	(8.7)	(6.7)	(7.0)		(2.4)	(6.6)		(20.5)
Year of harvest															
2009	9	1.2	8.3	12.2	25.0	50.8	79.2	26.2	50.8	7.9	8	643	129	8	1.73
		26.4	26.4	20.0	17.5	7.3	10.6	17.2	7.3	10.6		5.0	16.2		21.7
2010	52	1.4	9.3	13.8	25.2	51.3	79.9	26.6	51.3	8.0	52	640	121	52	1.75
		51.4	17.2	14.5	10.9	8.4	8.9	10.1	8.4	8.9		3.7	12.1		21.6
2011	70	1.8	8.0	13.0	23.5	50.0	7.7	25.3	50.0	7.7	62	626	126	9	1.52
		20.2	19.1	12.5	14.9	10.2	10.3	14.0	10.2	10.3		3.4	9.6		11.8
2012	16	1.6	7.8	12.4	27.5	55.6	8.6	29.1	55.6	8.6	0	-	-	-	-
		25.2	15.8	13.6	8.3	6.5	6.0	8.5	6.5	6.0		-	-	-	-
2013	6	2.1	7.3	13.3	28.9	53.6	8.6	31.0	53.6	8.6	0	-	-	-	-
		13.1	13.4	6.6	10.7	3.6	4.3	10.4	3.6	4.3		-	-	-	-

Table 4. Pearson correlation coefficients for different fractions from all wheat samples[a]

	WE GLU[b]	WE AX[b]	WE NSP[b]	WU GLU[b]	WU AX[b]	WU NSP[b]	TOT GLU[b]	TOT AX[b]	TOT NSP[b]	Starch[c]	Prot[c]	Visc[d]
WE GLU	1.00											
WE AX	-0.03	1.00										
WE NSP	0.37***	0.90***	1.00									
WU GLU	-0.04	-0.09	-0.08	1.00								
WU AX	-0.13	0.13	0.06	0.69***	1.00							
WU NSP	-0.11	0.05	0.01	0.90***	0.94***	1.00						
TOT GLU	0.12	-0.09	-0.02	0.99***	0.67***	0.87***	1.00					
TOT AX	-0.13	0.43***	0.34***	0.60***	0.95***	0.87***	0.57***	1.00				
TOT NSP	-0.02	0.25**	0.24**	0.85***	0.93***	0.97***	0.84***	0.92***	1.00			
Starch	0.05	-0.08	-0.08	-0.22**	-0.43***	-0.36***	-0.21**	-0.39***	-0.36***	1.00		
Prot	-0.31**	0.01	-0.09	-0.02	0.15	0.08	-0.08	0.13	0.05	-0.63***	1.00	
Visc	-0.11	0.59***	0.46***	0.14	0.23	0.22	0.12	0.40**	0.33**	-0.22	0.14	1.00

[a] *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

[b] Number of samples included in the analysis (n) = 153 (15 countries, 5 years of harvest).

[c] n = 122 (13 countries, 3 years of harvest).

[d] n = 69 (12 countries, 2 years of harvest).

WE: water-extractable, WU: water-unextractable, TOT: total, GLU: glucose, AX: arabinoxylan, NSP: non-starch polysaccharides, Prot: protein, Visc: extract viscosity