Interactions between the concentration of non-starch polysaccharides in wheat and the addition of an enzyme mixture in a broiler digestibility and performance trial

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ABSTRACT Two broiler trials were designed to investigate the relationship between the concentration of non-starch polysaccharides (**NSP**) in wheat and 1) its nutritional value for broilers and 2) the efficacy of exogenous enzymes. In a balance trial, diets were formulated with 3 wheat cultivars (Rustic and Viscount—medium NSP, Centenaire—high NSP) and were tested with or without the addition of an exogenous enzyme mixture. The diets were fed to 144 male Ross 308 broiler chickens housed in digestibility cages. Total tract nutrient digestibilities and AME_n were measured from 18 to 22 d of age. In a performance trial, diets were formulated with wheat (medium NSP diet) or with wheat mixed with rye and barley (high NSP diet) and were tested with or without the addition of an exogenous enzyme mixture. The diets were fed to 960 male Ross 308 broilers housed in pens and broiler performance during starter, grower and finisher periods was measured.

In the balance trial, wheat cultivar did not affect nutrient digestibility or AME_n. Enzyme addition caused a significant increase in nutrient digestibilities and AME_n for the diet formulated with the high NSP wheat Centenaire only. In the performance trial, feeding the high NSP diet resulted in a higher feed conversion ratio and lower final body weight compared to the medium NSP diet. The largest improvements by enzyme addition were observed in the high NSP diet.

In conclusion, the study was not able to show a consistent relationship between the NSP concentration of wheat and its nutritional value, but did demonstrate that the effect of an enzyme mixture on nutrient digestibility or broiler performance depends upon the NSP concentration in the diet.

Key words: wheat, broiler, nutrient digestibility, AME_n, enzyme mixture

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INTRODUCTION

Wheat, the third most widely grown cereal in the world after maize and rice, is an important raw material in poultry feed (FAO, 2015). In Europe, wheat is the most important cereal, and about 40% of wheat production is used for animal feed (Global Agricultural Information Network, 2016). Wheat, however, contains non-starch polysaccharides (**NSP**), and a plethora of potential anti-nutritional effects are associated with the NSP fraction of wheat (Choct and Annison, 1992; Bedford, 2002). The water-extractable (**WE**) part of the NSP can increase the viscosity of the intestinal contents, hence disturbing digestion (Dusel et al., 1997; Van Campenhout, 2007). Furthermore, the water-unextractable (**WU**) NSP in the cell walls can 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 (Carré et al., 2002; Dornez et al., 2008; Smeets et al., 2014a). As a result, also the nutritional value of wheat can vary when fed to broiler chickens (Scott et al., 1998; Choct et al., 1999; Barteczko et al., 2009).

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To counter the possible anti-nutritional effects caused by NSP and to improve nutrient digestibility and animal performance, NSP-degrading feed enzymes are commonly added to wheat-based feed. The efficacy of feed enzymes is variable, however, and sometimes no effects are observed (Gutiérrez-Alamo et al., 2008; Svihus, 2011). In general, it is assumed that enzyme addition is more effective in low-quality wheat (Choct et al., 1995; Scott et al., 1998; Huyghebaert and Schöner, 1999; Bedford, 2002). The definition of low-quality wheat is, however, not straightforward. Earlier research showed a positive relationship between enzyme efficacy and NSP concentration (Huyghebaert et al. 1999; Smeets et al., 2014a; Smeets et al., 2015) and a negative relationship between enzyme efficacy and the arabinose to xylose ratio of arabinoxylan (Huyghebaert et al., 1999; Smeets et al., 2014a). More research on the relationship between the chemical composition of the substrate (in particular the NSP) and enzyme efficacy is needed to be able to predict the nutritional value of wheat-based diets and the response of a diet to the addition of NSP degrading enzymes.

The objectives in the present study were to study the relationship between the concentration of NSP in wheat and 1) its nutritional value for broilers and 2) the efficacy of NSP-degrading enzymes.

MATERIALS AND METHODS

Birds, Diets, and Management

The broiler balance and performance trial both started at the same time at the Institute for Agricultural and Fisheries Research (**ILVO**), Melle, Belgium and were approved by the ILVO Ethical Committee (EC no. 2012/186). In total, 1,104 day-old male broiler chickens (Ross 308) were obtained from a commercial hatchery (Belgabroed NV, Merksplas, Belgium). The broilers were vaccinated at 1 d of age against Newcastle disease (Clone 30, spray, Intervet International BV, Boxmeer, the Netherlands) and infectious bronchitis (Poulvac IB Primer, spray, Pfizer Animal Health, Louvain-La-Neuve, Belgium). On d 16, vaccination against Newcastle disease was repeated (Clone 30, drinking water).

Balance Trial The balance trial was carried out with 144 birds, housed in 36 digestibility cages (6 dietary treatments × 6 replicates, 4 birds/cage during the main balance period). Three basal diets were formulated with 3 different wheat cultivars: Viscount, Rustic, and Centenaire. The cultivars were chosen to differ mainly in NSP concentration. Diets were formulated to meet breeder's recommendations (Aviagen, 2009) and included a coccidiostaticum (0.5 g/kg Sacox; Salinomycin sodium; Huvepharma NV, Antwerpen, Belgium) and a phytase (0.2 g/kg Ronozyme® NP; DSM, Heerlen, The Netherlands). The ingredients and the chemical compositions of the diets and wheat cultivars are shown in Table 1. One part of each diet was used without enzyme addition, the other part was supplemented with a commercial enzyme mixture (KEMZYME® Plus dry, Kemin Europa NV, Herentals, Belgium) at a dose rate of 500 g/T of feed. Drinking water and feed (in mash form) were provided for ad libitum consumption. The balance trial was carried out according to the EU-reference method (Bourdillon et al., 1990) and consisted of a 5-d period of adaptation (to the respective experimental diets containing titanium dioxide, d 13 to 17) and a 5-d main balance period (d 18 to 22). Before the adaptation and main balance period (d 1 to 13), the chickens were housed together and fed the same commercial mash starter diet (based on Aviagen, 2009). Feed intake was recorded and all excreta were collected in a quantitative way using plastic trays under the cages. A homogeneous sample of the mixed wet excreta was freeze-dried until analysis.

Performance Trial The trial was performed with 960 birds, housed in 32 pens (4 dietary treatments × 8 replicates, 30 birds/pen). The birds were fed using a 3-phase feeding scheme with periods of 13 d each: starter (d 1 to 13), grower (d 14 to 26) and finisher (d 27 to 39). Drinking water and feed (in mash form) were provided ad libitum. Two basal diets for each of the 3-phase feeding periods were formulated using either wheat (unknown cultivar) alone or wheat mixed with rye and barley (83.8% wheat, 13.0% rye, 3.2% barley, further referred to as wheatmix), to mimic a wheat high in NSP. Diets were formulated to meet breeder's recommendations (Aviagen, 2009) and included a coccidiostaticum (0.5 g/kg Salinomycin) and a phytase (0.2 g/kg Ronozyme NP). The ingredients and the chemical compositions of the grower diets and the wheat or wheatmix are shown in Table 1. One part of each diet was used without enzyme addition, the other part was supplemented with KEMZYME Plus dry at a dose rate of 500 g/T of feed.

Collection of Intestinal and Blood Samples

At the end of the balance trial, 2 animals per cage were randomly selected and euthanized following the principles for care of animals in experimentation. Intestinal contents were collected from the ileum (between Meckel's diverticulum and the ileocecal junction) of the 2 animals by gently finger-stripping the intestinal segment. The digesta contents collected from 2 animals per cage were pooled and stored on ice until analysis.

At the end of the performance trial, blood samples were taken from one bird per pen. The blood samples were collected in 5 mL Vacuette® tubes containing Heparin (456,083, Greiner Bio-One GmbH, Kremsmünster, Austria), stored on ice until centrifugation (1,500 × *g* for 10 min) to obtain the plasma and then stored at –21°C until analysis.

Chemical Analyses

Samples of feeds and freeze-dried excreta were analyzed at ILVO in an accredited lab for dry matter

Table 1. Composition of the experimental grower diets used in the balance trial and the performance trial.

Item	Diets with only wheat	Mixed diet
Ingredient (g/kg)		
Wheat	640.0	540.0
Rye	-	80.0
Barley	-	20.0
Soybean meal (49% CP)	267.8	265.0
Soya oil	24.0	45.9
Animal Fat	30.0	10.0
Limestone	5.2	6.8
Sodium bicarbonate	1.1	0.7
Sodium chloride	2.6	2.7
Monocalcium phosphate	11.3	8.2
L-Lysine hydrochloride	2.1	2.2
DL-Methionine	2.3	2.9
L-Threonine	0.6	2.7
Titanium dioxide	3.0	3.0
Vitamin/mineral premix[1]	10.0	10.0
Calculated diet composition (g/kg)		
ME_n broilers (kcal/kg)	2844	2844
Crude protein (N x 6.25)	195	195
Crude fat	67	69

Analyzed diet composition (g/kg)	Viscount	Rustic	Centenaire	Wheat	Wheatmix[2]
Dry matter	889	889	887	890	888
Crude protein (N × 6.25)	202.1	206.2	209.2	195.6	191.9
Crude fat (ether extract)	6.67	6.64	7.09	73.9	77.0
Gross Energy (kcal/kg)	4111	4135	4135	4135	4135
Analyzed wheat composition (g/kg DM)					
Water-extractable NSP	11.5	13.6	13.2	15.8	20.1
Water-unextractable NSP	80.3	82.7	98.2	80.4	83.8
Total NSP	91.8	96.3	111	96.1	103.9
Starch	629	641	604	666	657
Protein	126	121	128	106	104
Extract viscosity (mPa·s)	1.75	1.80	1.38	1.58	2.69
Xylanase inhibition activity (IU/g)[3]	331.3	290.7	275.4	287.1	NA
Grain-associated xylanase activity (XU/g)[4]	0.800	0.398	0.628	0.665	NA

[1]Supplied per kilogram of diet: manganese, 99 mg; zinc, 60 mg; iron, 49 mg; copper, 20 mg; iodine, 1.2 mg; selenium, 0.4 g; vitamin A, 13,500 IU (retinyl acetate); vitamin E, 55 IU (DL-α-tocopheryl acetate); cholecalciferol, 75 μg; vitamin B_1, 2 mg; vitamin B_2, 5 mg; vitamin B_3, 30 mg; vitamin B_5, 15 mg; vitamin B_6, 4 mg; vitamin B_{12}, 2 mg; vitamin K, 2.5 mg; folic acid, 1 mg; biotin; 0.2 mg; choline,600 mg; etoxyquine, 33 mg; butylated hydroxytoluene (BHT), 0.4 mg; propyl gallate, 0.12 mg; citric acid, 0.69 mg; sepiolite, 4 mg.

[2]Calculated from the analyzed values of wheat, rye and barley (83.8% wheat, 13% rye, 3.2% barley), except for the extract viscosity, which was determined on a mixture made from wheat, rye and barley in the appropriate concentrations.

[3]1.0 IU = amount of inhibitor that results in 50% inhibition of a xylanase under the conditions of the assay.

[4]1.0 XU = amount of enzyme needed to increase the A590 by 1.0 per hour of incubation under the conditions of the assay.

NSP: non-starch polysaccharides, NA: not analyzed.

(**DM**), gross energy, protein, and fat. In addition, the titanium dioxide concentration in the feed was analyzed as described before (Smeets et al., 2015). Crude protein, moisture, starch and NSP content and extract viscosity of the wheat were measured as described by Smeets et al. (2015). The intestinal samples were centrifuged for 10 min at 10,000× *g* and the supernatants were analyzed for ileal viscosity, xylanase inhibition activity, xylanase activity and titanium dioxide concentration. Ileal viscosity was determined at 40°C using a Brookfield cone and plate viscometer. For the estimation of xylanase inhibition activity, the supernatant was diluted 2 times with sodium acetate buffer (pH 5.0, 0.025 M) and the assay was performed as described by Smeets et al. (2014b). Xylanase activity in the intestinal samples was evaluated by diluting the supernatant 10 times (in sodium acetate buffer, pH 5.0, 0.1 M) and adding a Xylazyme AX tablet (Megazyme International, Bray Co. Wicklow, Ireland) to 1 mL of diluted digesta sample. This mixture was then incubated at 50°C for 2 h. Hereafter, 4 mL ethanol (95% v/v) was added, the mixture was centrifuged at 1,500 × *g*, and the absorption was measured spectrophotometrically at 585 nm. A portion of the intestinal samples was freeze-dried and the concentration of titanium dioxide was quantified as described before.

Calculations and Statistical Analyses

Total tract digestibility of DM, fat, starch, WE-NSP, WU-NSP, and TOT-NSP, N retention, and apparent metabolizable energy, corrected for nitrogen (**AME_n**), were calculated using the concentrations of titanium dioxide tracer in the excreta and the feed (Smeets et al., 2015). Performance results (daily feed intake, body weight, feed conversion ratio [**FCR**]) were calculated for each sub-period (starter, grower and

Table 2. Effect of wheat cultivar and enzyme addition on total tract digestibility.

	Digestibility (%)							AME_n (kcal/kg DM)	Ileal viscosity (mPa·s)	Ileal XA (abs)[2]	Ileal XIA (IU/g)[3]
	DM	N ret	fat	starch	WE-NSP	WU-NSP	TOT-NSP				
Treatment[1]											
1. wheat Viscount	69.5[b,c]	56.5[b]	70.3	96.1	1.9	21.9	19.8	3,160[b,c]	6.5[b]	0.075[b]	8.34
2. wheat Rustic	68.9[b,c]	57.6[b]	71.7	96.1	-21.9	21.3	17.6	3,165[b,c]	6.4[b]	0.074[b]	13.1
3. wheat Centenaire	69.5[b,c]	57.8[b]	72.0	95.7	-8.6	31.5	27.5	3,186[b,c]	10.6[a]	0.071[b]	8.99
4. wheat Viscount 500 g/T enzyme	68.0[c]	55.6[b]	71.3	96.2	-2.2	13.8	12.1	3,107[c]	3.4[c]	0.202[a]	13.3
5. wheat Rustic 500 g/T enzyme	70.8[b]	58.6[b]	77.4	96.4	-0.9	28.5	25.9	3,264[a,b]	3.0[c]	0.173[a]	11.2
6. wheat Centenaire 500 g/T enzyme	73.6[a]	63.4[a]	77.3	97.3	-3.7	29.9	26.6	3,362[a]	4.3[b,c]	0.200[a]	13.9
Average	70.0	58.2	73.4	96.4	-5.9	24.5	21.6	3207	5.7	0.134	11.1
Pooled SEM	0.9	1.3	1.9	0.7	5.9	4.9	4.6	39	0.8	0.018	3.10
Main effect											
Wheat											
Viscount	68.7[b]	56.1[b]	70.8	96.2	-0.2	17.9	15.9	3,134[b]	5.0[b]	0.139	10.9
Rustic	69.9[a,b]	58.1[a,b]	74.5	96.2	-11.4	24.9	21.8	3,214[a,b]	4.7[b]	0.122	11.6
Centenaire	71.5[a]	60.6[a]	74.7	96.5	-6.1	30.7	27.0	3,274[a]	7.5[a]	0.136	10.9
Enzyme addition											
No enzyme	69.3[b]	57.3	71.3[b]	96.0	-9.6	24.9	21.6	3,170[b]	7.9[b]	0.072[b]	9.8
500 g/T	70.8[a]	59.2	75.3[a]	96.6	-2.2	24.0	21.5	3,244[a]	3.6[a]	0.192[a]	12.4
P-value											
Wheat	0.016	0.005	NS (0.08)	NS	NS	NS (0.07)	NS (0.09)	0.009	0.002	NS	NS
Enzyme	0.050	NS (0.08)	0.01	NS	NS	NS	NS	0.041	< 0.001	<0.001	NS
Wheat × enzyme	0.014	0.044	NS	NS	NS	NS	NS	0.019	NS	NS	NS

[1]Reported values are means of 6 replicates, Viscount and Rustic: medium concentration of NSP, Centenaire: high concentration of NSP.
[2]absorbance (585 nm) of the final assay solution.
[3]IU/g tracer, 1.0 IU = amount of inhibitor that results in 50% inhibition of a xylanase under the conditions of the assay
[a–c]means within a column and main effects lacking a common superscript differ significantly ($P \leq 0.05$).
Abbreviations: AME_n, apparent metabolizable energy corrected for nitrogen; DM, dry matter; NS, not significant; NSP, non-starch polysaccharides; TOT, total; WE, water-extractable; WU, water-unextractable; XA, xylanase activity; XIA, xylanase inhibition activity.

finisher) and for the entire period. A balance cage (6 replicates per treatment) or floor pen (8 replicates per treatment) was the experimental unit. Data were analyzed by 2-way analysis of variance (**ANOVA**) using Statgraphics Centurion XVI software (Statpoint Technologies, Inc., Warrenton, VA). When interactions were observed ($P \leq 0.05$), the least significant differences procedure was used to separate individual treatment means. The balance trial results were analyzed as a 3 × 2 factorial arrangement of dietary treatments involving 3 wheats (Viscount, Rustic, and Centenaire) and 2 enzyme dosages (0 and 500 g/T). The performance trial results were analyzed as a 2 × 2 factorial arrangement of dietary treatments involving 2 raw material sources (wheat alone or wheatmix) and 2 enzyme dosages (0 and 500 g/T). All statements of significance were based on a *P*-value less than or equal to 0.05.

RESULTS

Wheat Cultivars

The results of the composition analysis for the different wheat samples and wheatmix are shown in Table 1. Centenaire wheat had the highest concentration of total NSP (111 g/kg), and Rustic (96.3 g/kg) and Viscount (91.8 g/kg) had comparable and lower values. The wheat used in the performance trial was a standard wheat with a total NSP concentration of 96.1 g/kg DM. This wheat was mixed with rye and barley to mimic wheat high in NSP. This mixture indeed had a high NSP concentration (103.9 g/kg DM), most pronounced in the WE part of the NSP.

Balance Trial

The average recovery of xylanase (main enzyme activity present in the enzyme mixture) from the enzyme-treated feed for both the balance and performance trial was 104% (data not shown). The effect of different wheat cultivars and enzyme addition on total tract digestibilities, AME_n, ileal viscosity, ileal xylanase activity, and ileal xylanase inhibition activity is shown in Table 2. No effect of wheat cultivar on nutrient digestibility or AME_n was observed without enzyme addition. Inclusion of the enzyme mixture significantly reduced the ileal viscosity for all diets. In the diets with the Rustic and Viscount wheat cultivars, this reduction in ileal viscosity did not result in any improvements in nutrient digestibility. In the diet with the high NSP wheat cultivar Centenaire, enzyme addition increased DM digestibility, nitrogen retention, and AME_n. No

Table 3. Daily feed intake, body weight and feed conversion ratio during grower, finisher and the whole period in the performance trial with wheat.

	Daily feed intake (g)				Body weight (g)			Feed Conversion Ratio (g/g)			
	Starter (1–13d)	Grower (14–26d)	Finisher (27–39d)	Total (1–39d)	Starter (d13)	Grower (d26)	Finisher (d39)	Starter (1–13d)	Grower (14–26d)	Finisher (27–39d)	Total (1–39d)
Treatment[1]											
1. wheat	31.7[a]	104.9	192.3	106.6	336[b]	1,345[b]	2,607[b]	1.286[a]	1.456[c]	1.678	1.537[b]
2. wheat 500 g/T enzyme	32.7[a]	107.5	189.1	106.9	364[a]	1,379[a,b]	2,646[a,b]	1.208[b]	1.484[b]	1.641	1.518[b,c]
3. wheatmix	30.3[b]	103.7	193.0	105.7	324[b]	1,281[c]	2,530[c]	1.287[a]	1.517[a]	1.699	1.570[a]
4. wheatmix 500 g/T enzyme	32.0[a]	108.2	192.0	107.8	357[a]	1,396[a]	2,684[a]	1.212[b]	1.458[c]	1.639	1.508[c]
Average	31.7	106.1	191.6	106.7	345	1350	2617	1.248	1.479	1.664	1.533
Pooled SEM	0.4	0.9	1.9	0.9	5	12	18	0.013	0.007	0.015	0.008
Main effect											
Diet											
Wheat	32.2[a]	106.2	190.7	106.8	350[a]	1362	2627	1.247	1.470[b]	1.660	1.527
Wheatmix	31.2[b]	106.0	192.5	106.7	340[b]	1338	2607	1.249	1.488[a]	1.669	1.539
Enzyme addition											
No enzyme	31.0[b]	104.3[b]	192.6	106.1	330[b]	1,313[b]	2,569[a]	1.286[a]	1.487[b]	1.688[a]	1.554[a]
500 g/T	32.3[a]	107.9[a]	190.5	107.3	361[a]	1,387[a]	2,665[b]	1.210[b]	1.471[a]	1.640[b]	1.513[b]
P-value											
Diet	0.010	NS	NS	NS	0.040	NS (0.06)	NS	NS	0.023	NS	NS
Enzyme	0.002	< 0.001	NS	NS	< 0.001	< 0.001	< 0.001	< 0.001	0.043	0.003	< 0.001
Diet x enzyme	NS	NS	NS	NS	NS	0.003	0.003	NS	< 0.001	NS	0.016

[1]Reported values are means of 8 replicates, wheat: medium concentration of non-starch polysaccharides, wheatmix: mixture of wheat, rye and barley to mimic a high concentration of non-starch polysaccharides.

[a–c]means within a column lacking a common superscript differ significantly ($P \leq 0.05$). NS: not significant.

effects of the dietary treatments on WE-NSP digestibility could be observed. WU-NSP were the most digestible in the Centenaire wheat diet and the least digestible in the Viscount wheat diet, when averaged across enzyme levels ($P \leq 0.10$). The results shown in Table 2 show some xylanase activity left in the ileum of the enzyme-treated feed. By use of the results of the tracer analyses in the feed and intestinal content, it could be calculated that on average 12% of the original xylanase activity in the feed was present in the ileum. Also, some xylanase inhibition activity was present in the ileal samples, which was on average 1.3% of the inhibition activity in the feed.

Performance Trial

The mortality during the performance trial was on average 3.1% and was not affected by the dietary treatments ($P > 0.05$; data not shown). The effect of the 2 diet formulations (wheat or wheatmix) and the addition of an enzyme mixture on daily feed intake, body weight, and FCR during the starter, grower, finisher, and the total period is shown in Table 3. During the starter and grower period, feed intake was higher with the addition of the enzyme mixture (when averaged across diets). The body weight was lower after the grower and finisher period with the wheatmix diet than with wheat alone. At the end of the grower and finisher periods, enzyme addition only improved the body weight in the wheatmix diet. The FCR was higher for the wheatmix diet compared to wheat alone during the grower period and the whole period. Enzyme addition improved the FCR in the diet with wheat alone during starter and grower period, whereas the FCR was improved by the enzyme in the wheatmix diet during each period.

DISCUSSION

A first aim of the animal trials described in this paper was to examine the effect of the variable NSP concentration and composition of wheat on broiler digestion, similar to the previous experiment described by Smeets et al. (2015). Unlike what was expected, based on the NSP composition of the wheat cultivars, and the higher ileal viscosity in the Centenaire diet, no differences in nutrient digestibilities or AME_n were observed between the diets in the balance trial. Other reports also indicate no relationship between AME_n and NSP concentration (Nicol et al., 1993; Austin et al., 1999; Parsaie et al., 2006), although there are a lot of researchers who do find a correlation (Dusel et al., 1997; Choct et al., 1999; Smeets et al., 2015). A possible explanation for this lack of relationship could be related to the NSP digestibility, as will be explained further in this discussion. In the performance trial, differences were observed in performance measures between the 2 diets (wheat: medium NSP—wheatmix: high NSP) without enzyme addition. These differences were most clear in the grower and finisher periods. The birds fed the wheatmix diet were less heavy and had a higher FCR than the birds fed the wheat diet. These results indicate that the relationship between AME_n and animal performance is not always clear, as also observed by other researchers.

The second aim of the trials described in this paper was to study the relationship between the NSP composition of wheat and the effect of enzyme addition. In the balance trial, addition of the enzyme caused a significant increase in nutrient digestibilities and AME_n for the high NSP wheat Centenaire, whereas in the Viscount cultivar no differences were observed. In the performance trial, inclusion of the enzyme mixture improved the body weight (grower and finisher period) and FCR (finisher and total period) only for the wheatmix diet. These observations confirm the hypothesis that the effect of enzyme addition is related to the NSP concentration of the diet, as observed earlier (Choct et al., 1995; Dusel et al., 1998; Huyghebaert et al., 1999) and as also demonstrated in vitro by Smeets et al. (2014a). The concentration of NSP should therefore be considered in the practice of feed formulation.

In addition, the remaining xylanase activity in the small intestine was measured in the current study. Only a few other researchers report intestinal enzyme activities (Silva and Smithard, 2002; Choct, 2006). It was calculated that on average 12% of the original xylanase activity was present in the ileum. This corresponds to the study by Silva and Smithard (2002) who estimated that between 15 and 20% of the xylanase added to a rye-based feed was active in the small intestine. In addition to grain-associated xylanases, wheat also contains xylanase inhibitors, which are also believed to be still active in the small intestine (Smeets et al., 2014b). In the latter study, it was observed that the xylanase inhibitors were not affected by the in vitro simulation of intestinal conditions. In the present study, however, only 1.3% of the original inhibition activity was present in the ileum. Nevertheless, the inhibition activity of wheat is considerable (Smeets et al., 2014b) and it is therefore conceivable that even a portion, as small as 1.3% could inhibit the exogenous xylanases present.

The digestibility of NSP in the intestinal tract was low, as observed in previous studies (Dusel et al., 1998; Meng et al., 2004), although the amount of studies that report intestinal NSP digestibility is limited. No significant differences were observed for NSP digestibility between the wheat cultivars. It should be noted, however, that the experimental errors were rather high, as the analytical measurement of NSP in fecal samples is difficult. There were some trends observed ($P \leq 0.10$). The NSP from the high NSP wheat Centenaire were generally more digestible (when the data were averaged across enzyme levels). Choct et al. (2004) also noticed a higher NSP-digestibility in a low-ME wheat. They suggested that the NSP of the low-ME wheat were structurally different from those in the high-ME wheat. In the current study, only the arabinose/xylose-ratio gives some estimation of structure, but the latter was not different between the 3 wheat samples. The arabinose/xylose ratio only gives information about the amount of arabinose side-chains on the xylose backbone, but not about the distribution of the arabinose side-chains. The presence of large fragments of unbranched xylose residues could lead to the formation of "junction zones" between different AX molecules (Izydorczyk and Biliaderis, 1995). It can be expected that these kinds of AX molecules are not readily solubilized and not fermented by the microbiota in the intestines. Furthermore, the presence of other side chains such as ferulic acids can also affect the water-extractability and hence possibly the digestibility of the NSP (Izydorczyk and Biliaderis, 1995). The observation that the NSP from the Centenaire wheat were more digestible can explain the lack of depression in nutrient digestibility observed with this wheat.

In conclusion, the study was not able to show a consistent relationship between the NSP concentration of wheat and its nutritional value, but did demonstrate that the effect of an enzyme mixture on nutrient digestibility or broiler performance depends upon the NSP concentration in the diet.

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