Relationship between the Levels of Soluble Nonstarch Polysaccharides and the Apparent Metabolizable Energy of Wheats Assayed in Broiler Chickens

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The total arabinoxylan contents of 13 wheats grown in different regions of New South Wales, Australia, were determined. In addition, the wheats were sequentially extracted with cold 0.01 M NaCl, hot 0.01 M NaCl with a thermostable α-amylase, and cold 0.1 M NaOH. The nonstarch polysaccharide (NSP) content of each of the extracted fractions was determined. The nutritive quality of the wheats for broiler chickens was assessed by using a classical apparent metabolizable energy (AME) assay. The wheats ranged from 11.25 to 13.59 MJ/kg of dry matter. A highly significant negative correlation ($r = -0.91$, $P < 0.0001$) was found between AME values and the total NSP (which consisted mainly of arabinoxylan) removed from the wheats in the first two extractions. AME was not correlated with the alkaline-extracted NSP or total arabinoxylan levels. It was concluded that water-soluble NSP of wheat possesses antinutritive activity, while the water-insoluble NSP is probably biologically inert in the broiler chicken.

INTRODUCTION

The nonstarch polysaccharides (NSPs) of rye and barley possess antinutritive activities when these cereals are included at high levels in broiler chicken diets. Growth of the chickens is depressed, and feed conversion efficiency is reduced. In addition, the birds produce sticky, moist droppings (Halpin et al., 1936).

Rye contains high levels (approximately 10% dry matter) of pentosans which are primarily arabinoxylans (Antoniou et al., 1981). The antinutritive activity of these polysaccharides was demonstrated by Marquardt and coworkers (Antoniou and Marquardt, 1981; Ward and Marquardt, 1987), who showed that growth of broilers was depressed when pentosans isolated from rye were added to experimental diets. In a similar study White et al. (1981) added barley β-glucan to experimental diets and noted a depression of broiler chicken growth.

Further evidence for the antinutritive activity of the NSPs of rye and barley comes from studies which have demonstrated that the feed value of these cereals can be improved by supplementing diets with enzymes possessing glycanase activity. Addition of pentosanases to rye-based diets improves their nutritional value (Pettersson and Aman, 1988). In the case of barley diets, β-glucanases ameliorate the growth-depressing effects and allow the cereal to be used at high levels (Gohl et al., 1978; Hesselman and Aman, 1986; Classen et al., 1988).

Australian wheats are known to vary considerably in their nutritive quality. The results of two surveys (Mollah et al., 1983; Rogel et al., 1987) indicated that approximately 25% of Australian wheats, when included in broiler diets, have a lower apparent metabolizable energy value (AME < 13 MJ/kg of dry matter) than would be expected from proximate analysis and gross energy determinations. Wheat also contains appreciable amounts of NSPs. In a study examining 29 Australian wheats Annison (1990) reported arabinoxylan levels of 5.7–8.2% dry matter (DM). The wheat arabinoxylans are very similar to those of rye consisting of a (1–4)-β-xylan chain with α-arabinose substituted at the O2- and O3-positions (Fincher and Stone, 1986).

Choct and Annison (1990) have shown that wheat arabinoxylans also have antinutritive activity. When isolated wheat pentosans were added to experimental broiler diets at 3% dry matter, a depression in the AME resulted. In addition, reduced starch digestion, nitrogen retention, and a depression of broiler growth were noted in treatment groups compared to controls. It was suggested that variations in the levels of wheat pentosans may be responsible for the low-AME wheat phenomenon.

This paper details a study examining the relationship between the AME of wheats and the levels of their nonstarch polysaccharides.

MATERIALS AND METHODS

Wheat Samples. Wheat samples (13, 7 cultivars) were obtained during the 1989–1990 harvest from five registered seed producers in New South Wales, Australia. The wheats were free of fungicide dusts which are often applied to seed grains in Australia. Growers were situated in northern (2), central (2), and southern (1) New South Wales.

Wheat NSP Fractionation. NSPs were removed from wheat samples by a series of aqueous treatments (termed extractions 1, 2, and 3) as follows. Wheat samples were ground (0.5-mm screen) and allowed to equilibrate to equal moisture contents in a desiccator over silica gel. The following procedure was carried out in duplicate. Exactly 1 g of each was transferred to a 50-mL glass centrifuge tube. Aqueous ethanol (80%, 10 mL) was added, and the tubes were heated to 100 °C for 10 min. The samples were centrifuged (2000*g*, 30 min), and the supernatant was removed and discarded. The pellet was resuspended in 0.01 M NaCl (10 mL) and held at 40 °C for 1 h with vigorous vortex mixing every 10 min. After centrifugation (2000*g*, 30 min), the supernatant was collected and filtered (Whatman No. 1), and an aliquot (1 mL) was transferred to a reaction vial (8 mL, screw cap, Teflon seal) for sugar analysis to determine the levels of NSP released in extraction 1. The pellet was resuspended in 0.01 M NaCl and heated to 85 °C. A thermostable α-amylase (0.5 mL, Thermamyl 120L, Novo Industries) was added, and the mixture was held at 85 °C for 1 h with vigorous vortex mixing every 10 min. After centrifugation (2000*g*, 30 min), the supernatant was collected and filtered (Whatman No. 1), and an aliquot (1 mL) was transferred to a reaction vial (8 mL, screw cap, Teflon

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0021-8561/91/1439-1252$02.50/0

seal). Ethanol (4 mL) was added, and the resultant precipitate was pelleted by centrifugation (bench centrifuge). The supernatant was discarded, and the precipitate was washed with ethanol (80% v/v) and collected by centrifugation. The pellet was dried under a stream of N_2 gas and dissolved in 1 mL of distilled water. The sugar composition was determined to estimate the levels of NSP released in extraction 2. The insoluble pellet from the hot-water extraction was resuspended in 0.1 M NaOH (10 mL) and held at 40 °C for 1 h with vigorous mixing every 10 min. The mixture was centrifuged (2000g, 30 min), and the supernatant was collected and filtered (Whatman No. 1); an aliquot (1 mL) was transferred to a reaction vial and neutralized with trifluoroacetic acid (4 M TFA, 0.025 mL). The sugar composition was determined to estimate levels of alkali-soluble NSP released in extraction 3.

Sugar Analysis. The sugar compositions of the wheats and polysaccharides released in each of the extractions were determined as follows. Subsamples of the ground wheats were ball-milled to a fine powder and dried in vacuo over P_2O_5. Aliquots (20 mg) were added to reaction vials (3.5 mL, screw caps, Teflon septa) and extracted twice at 80 °C for 5 min with ethanol (85% w/v) to remove soluble sugars. After drying, hydrolysis of the sample was carried out with trifluoroacetic acid (2 M, 1 mL) as describe by Olsen et al. (1988). The 1-mL aliquots of the extracts of the wheat samples were adjusted to 2 M TFA by addition of 1 mL of 4 M TFA. For hydrolysis the reaction mixtures were held at 120–125 °C for 1 h. After cooling and addition of an internal standard (D-allose, 3 mg/mL, 0.2 mL), the hydrolysate was centrifuged (bench centrifuge, 5 min) and 0.8 mL was transferred to a clean reaction tube. The excess TFA was removed by codistillation with water under a stream of N_2 gas. The dry residue was recovered in distilled water (0.2 mL), and the free sugars were reduced by treatment with dimethyl sulfoxide containing $NaBH_4$ (20 mg/mL) at 40 °C for 1.5 h as described by Blakeney et al. (1983). The alditols were acetylated by addition of acetic anhydride (2 mL) and 1-methylimidazole (0.2 mL). The excess acetic anhydride was decomposed with distilled water (10 mL), and the alditol acetates were extracted with dichloromethane (1 mL). The samples were analyzed by using a Varian 3400 gas chromatograph equipped with a Varian Series 8000 autosampler and a flame ionization detector and capillary column (DB1701, 15 m, J&W Scientific). During analysis the column was held at 200 °C for 1 min and then raised by 5 °C/min to 220 °C for 4 min. Duplicate samples were hydrolyzed, and the derivatized products were determined twice. The sugar composition of the samples was used to calculate polysaccharide levels. It was assumed that all the pentoses were present as arabinoxylans. The arabinoxylans were considered to be composed of a xylan backbone with single-residue arabinose side chains. The levels of arabinoxylans were calculated from the levels of the component sugars as follows:

$$\text{arabinoxylan} = (0.887 \times \text{arabinose}) + (0.887 \times \text{xylose})$$

Other polysaccharides (glucan, galactan, and mannan) were assumed to be linear polymers and were estimated by using the relationship

$$\text{polysaccharide} = 0.9 \times \text{monosaccharide}$$

Animal Experiments. The nutritive quality of the wheats for broiler chickens was assessed by using classical apparent metabolizable energy trials. Due to a restriction in the number of bird cages available, three trials were conducted over a 3-month period. In the first two trials five wheats were assayed, and in the third trial the remaining three were assayed. To ensure that the AME assay was reproducible and that the AME values of the wheats from different trials could be compared, a sample of sorghum was assayed with the wheats as a control. The trial diets were formulated by using the ingredients and proportions detailed in Table I.

The birds used in the AME trials were commercial-type male broiler chickens. All were obtained from the same commercial hatchery at 1 day old. The birds were housed initially in brooders (until 3 weeks of age) and then in group cages with wire floors until 4 weeks of age. Commercial starter diet was fed to the birds for the first 3 weeks, and a commercial finisher diet was fed during the fourth week. At 4 weeks of age the birds were transferred to individual metabolism cages and randomly allocated to experimental groups for the determination of the AME of trial diets. There were eight birds per group with one group being used to assay the AME of each wheat.

Table I. Composition of the Experimental Diets Used in the AME Assays

ingredient	g/kg	ingredient	g/kg
wheat or sorghum	820.0	vitamin premix[a]	5.0
casein	134.0	sodium chloride	3.6
dicalcium phosphate	26.0	choline chloride	0.4
calcium carbonate	11.0		

[a] The levels of active ingredients in the vitamin premix were as follows: vitamin A, 2.02 MIU/kg; vitamin D_3, 0.7 MIU/kg; vitamin E, 4.00 g/kg; vitamin K, 1.27 g/kg; vitamin B_2, 1.6 g/kg; pyridoxine hydrochloride, 1.0 g/kg; biotin, 0.02 g/kg; niacin, 6.00 g/kg; thiamine, 0.30 g/kg; D-calcium pantothenate, 3.00 g/kg; folic acid, 0.4 g/kg; ethoxyquin, 25.00 g/kg; Mn, 15 g/kg; Fe, 4.00 g/kg; Cu, 1.00 g/kg; I, 0.20 g/kg; Co, 0.06 g/kg; Se, 0.10 g/kg; Mo, 0.32 g/kg; vitamin B_{12}, 3.00 mg/kg.

Table II. Variety, Source, AME, and Pentosan Levels (% DM) of Wheat Samples[a]

no.[b]	variety	location[c]	AME, MJ/kg DM	pentosan	trial
1	Owlet	C.NSW	11.25 ± 0.64[d]	6.95[e]	1
2	Vulcan	C.NSW	11.35 ± 0.95	6.50	2
3	Sunbird	S.NSW	11.98 ± 0.85	6.64	3
4	Hartog	N.NSW	12.14 ± 0.40	6.62	3
5	Hartog	C.NSW	12.24 ± 0.52	7.00	1
6	Suneca	C.NSW	12.43 ± 0.51	6.26	2
7	Hartog	C.NSW	12.52 ± 0.98	6.39	1
8	Vulcan	S.NSW	12.74 ± 0.52	6.65	1
9	Sunco	N.NSW	12.89 ± 0.90	6.87	2
10	Vulcan	C.NSW	13.00 ± 0.83	6.35	1
11	Sunco	N.NSW	13.42 ± 0.22	6.69	2
12	Owlet	C.NSW	13.45 ± 0.45	6.29	2
13	Sunfield	C.NSW	13.59 ± 0.54	6.71	3
control	sorghum		15.35 ± 0.05		1
			15.15 ± 0.11		2
			15.37 ± 0.09		3

[a] The trial number (1, 2, or 3) in which each wheat was assayed and the values obtained for the sorghum control are also shown. [b] Sample identification number. [c] N.NSW, C.NSW, S.NSW: northern, central, and southern New South Wales, respectively. [d] Data are given as means ± SEM (n = 8). [e] Coefficient of variability for method was 3%.

The AME values of the wheat samples were determined by using a classical total collection method. Experimental diets were fed for 7 days. For the last 4 days feed intake was monitored, and the excreta of each bird was collected, dried overnight, and pooled for analysis. The gross energy of diets and the excreta samples was determined in duplicate by using an adiabatic bomb calorimeter (Gallenkamp). The AME values of the wheats were calculated from the AME values of the diets (not reported) by assuming an additive model for the diet ingredients and with appropriate corrections made for moisture content. Thus

$$\text{AME}_{\text{wheat}} = [\text{AME}_{\text{diet}} - (\text{AME}_{\text{casein}} \times \text{casein level})]/\text{wheat level}$$

The AME value of the casein was assumed to be 15.96 MJ/kg of DM. This value was initially determined by Mollah (1982) in a series of experiments in our laboratory examining locally obtained casein. The AME of the same commercial casein product has been shown to be of low variability following a series of further studies (unpublished data).

Statistical Analysis. To determine whether the nutritive quality of the wheats was influenced by their polysaccharide composition, the levels of different NSP fractions removed by each of the extractions were individually regressed by using a linear model against the AME values of the wheats. As arabinoxylans form the greater part of NSPs, this fraction was considered separately. The sum of arabinoxylan, glucan, galactan, and mannan values (termed NSP) was also regressed against AME. The extractions were considered individually and combined (i.e., the sum of extractions 1 and 2 and extractions 1–3) for the regression analyses. Statistically significant corre-

Table III. Levels (Percent Dry Weight) and Composition of Polysaccharides Released from Wheat in Three Sequential Extractions

wheat no.	ar-xylan[a]	A/X[b]	glucan	galactan	mannan	total
Extraction 1 (0.01 M NaCl, 40 °C, 1 h)						
1	0.52	0.85	0.68	0.12	tr[c]	1.32
2	0.51	0.87	0.40	0.16	0.01	1.08
3	0.50	0.85	0.51	0.14	tr	1.15
4	0.44	0.89	0.49	0.14	tr	1.07
5	0.47	0.74	0.53	0.13	tr	1.13
6	0.51	0.86	0.54	0.14	0.04	1.23
7	0.53	0.88	0.42	0.14	tr	1.09
8	0.50	0.90	0.44	0.17	tr	1.10
9	0.46	0.94	0.44	0.13	tr	1.04
10	0.50	0.93	0.49	0.16	tr	1.15
11	0.46	0.94	0.43	0.14	tr	1.02
12	0.43	0.88	0.41	0.12	tr	0.96
13	0.40	1.01	0.37	0.13	tr	0.90
Extraction 2 (0.01 M NaCl + α-Amylase, 85 °C, 1 H)						
1	0.24	0.54	tr	0.04		0.28
2	0.24	0.50	tr	0.16		0.40
3	0.25	0.60	tr	0.09		0.33
4	0.29	0.57	tr	0.10		0.39
5	0.25	0.57	tr	0.08		0.33
6	0.22	0.54	tr	0.04		0.26
7	0.22	0.53	tr	0.12		0.34
8	0.23	0.55	tr	0.09		0.32
9	0.25	0.60	tr	0.09		0.34
10	0.22	0.49	tr	0.05		0.27
11	0.21	0.76	tr	0.09		0.30
12	0.25	0.53	tr	0.09		0.35
13	0.25	0.53	tr	0.11		0.36
Extraction 3 (0.1 M NaOH, 40 °C, 1 h)						
1	0.71	0.80	5.19		0.11	6.00
2	0.90	0.67	5.57		0.15	6.66
3	0.76	0.75	4.68		0.10	5.54
4	0.86	0.78	5.15		0.11	6.11
5	0.78	0.81	5.17		0.10	6.04
6	0.86	0.73	5.23		0.12	6.20
7	0.82	0.76	5.23		0.10	6.22
8	0.90	0.96	4.71		0.09	5.70
9	0.95	0.72	4.64		0.12	5.70
10	0.81	0.72	5.17		0.11	6.08
11	0.83	0.86	5.13		0.11	6.07
12	0.72	0.74	5.63		0.12	6.47
13	0.94	0.71	5.63		0.12	6.70

[a] Sum of arabinose and xylose. [b] Arabinose:xylose ratio. [c] Trace amount.

Table IV. Linear Correlations between AME Values and Levels of Extracted Polysaccharides from Wheat Samples

	extractions					
	1		1 + 2		1 + 2 + 3	
	AX[a]	NSP[a]	AX[b]	NSP[b]	AX[c]	NSP[c]
AME	−0.65	−0.71	−0.86	−0.91	0.13	−0.07
P	<0.05	<0.05	<0.001	<0.0001		

[a] AX, arabinoxylans; NSP, nonstarch polysaccharide from extraction 1. [b] Sum of extractions 1 and 2. [c] Sum of extractions, 1, 2, and 3.

lation coefficients are shown in Table IV. Also shown is the correlation coefficient calculated for the linear regression of total extracted polysaccharides against AME values. The methods used to determine the correlation coefficient and to calculate the levels of significance were those described by Steel and Torrie (1982).

RESULTS

The AME values of the wheats ranged from 11.25 to 13.59 MJ/kg of dry matter (Table II). Because not all of the wheat varieties were available from the three different locations in New South Wales, no attempt was made to assess whether the nutritive quality of the wheats as measured by the AME assay was subject to significant varietal or environmental influences. The AME values obtained for the sorghum were very similar (Table II) in each of the three trials. There was no relationship between the AME of the wheats and the total levels of pentosan, which did not vary greatly (6.26–7.00% DM).

With each extraction of the wheats different amounts of polysaccharides with different sugar profiles were released (Table III). In the first extraction (0.01 M NaCl, 40 °C, 1 h) polymers containing arabinose, xylose, glucose, and galactose with traces of mannose were detected. The second extraction (0.01 M NaCl, 85 °C, 1 h with α-amylase) released less material with polysaccharides being composed of arabinose, xylose, and galactose only. The final extraction (0.1 M NaOH, 40 °C, 1 h) removed further arabinose- and xylose-containing polysaccharides and a large amount of glucan (4.64–5.63% dry weight). Relatively high levels of mannose were also detected, indicating the release of a mannan-type polysaccharide. Assuming that a great majority of the arabinose is derived from arabinoxylans, the levels of arabinose in the polysaccharides removed from the wheat in the second extraction were less then those removed by the first and third extractions as indicated by the lower arabinose:xylose ratio.

Highly significant negative linear correlations between the AME of the wheats and the levels of arabinoxylans ($r = 0.65$, $P < 0.05$) and NSP ($r = -0.71$, $P < 0.05$) removed by extraction 1 and the sum of the polysaccharides from extractions 1 and 2 (arabinoxylans, $r = -0.86$, $P < 0.001$; NSP, $r = -0.91$, $P < 0.001$) were found (Table IV). The levels of the polysaccharides extracted by alkali (extraction 3) did not correlate with the AME of the wheats.

DISCUSSION

The very similar AME values obtained for the sorghum control demonstrated the reproducibility of the AME assay and confirmed that the AME values of wheats obtained in different trials could be compared. Most of the wheats in this study would be classified as low-AME wheats (<13 MJ/kg), although the range of AME values (11.25–13.59 MJ/kg of dry matter, Table II) is much less than has previously been recorded in Australia (10.72–14.59 MJ/kg; Rogel et al., 1987). The total arabinoxylan contents were similar to previously reported values (Henry, 1986; Annison, 1990), and no significant correlation with AME existed. Examining 29 wheats, Annison (1990) was unable to correlate total arabinoxylan levels with starch digestibility in broiler chickens; thus, the data from this study confirm that total arabinoxylan levels are poor predictors of nutritive value.

When soluble polysaccharides are added to poultry diets, depression of growth occurs (Vohra and Kratzer, 1964). Insoluble polysaccharides, however, such as cellulose are inert. Subjecting cereals to a series of extractions beginning with very mild conditions employing neutral solutions and progressing to strong basic or acidic solutions removes different NSP fractions (Pomeranz, 1961; Ahluwalia and Fry, 1986; Saini and Henry, 1989). It is difficult to precisely mimic the environment in which NSPs are extracted from cereals in the broiler chicken, but certainly some will be dissolved and others will remain insoluble. The aim of this study was to determine whether a relationship exists between the nutritive quality of the wheat, as assessed by the AME assay, and the level of NSP fractions that can be removed from wheats with aqueous extractions.

The amounts of arabinoxylans removed by the first and second extraction were similar to the those recorded by Saini and Henry (1989) following a sequential extraction

of wheat, the first parts of which were similar to the procedures used in the current study. The polysaccharides removed by extraction 1 (0.01 M NaCl) were probably arabinoxylans, β-(1–3,1–4)-glucans, and arabinogalactans which have previously been identified in neutral extractions of wheat (Mares and Stone, 1973; Izydorczyk et al., 1990). No attempt was made to distinguish between arabinose associated with the galactan from that associated with the xylan. Izydorczyk et al. (1990) reported that arabinose accounts for 40% of the residues in water-soluble wheat arabinogalactans. Low levels of galactose were found in the wheats in the current study (Table III). Therefore, most of the arabinose must have been associated with the xylan. Because the correlation between the total NSP removed by extraction 1 and AME was greater than when the arabinoxylans were considered alone (Table IV), it appears that other types of soluble NSP contribute to the antinutritive activity. Wheat β-glucan is very similar in structure to the β-glucan of barley and so would be expected to contribute antinutritive activity.

A high-temperature incubation was used in extraction 2 to gelatinize the starch to enhance its degradation by the α-amylase. It is unclear whether the elevated temperature or the action of the α-amylase was responsible for the further release of arabinoxylans and galactans. The negative correlation between AME and the sum of NSP released in extractions 1 and 2 suggests that these extractions simulate the digestive activities in the chicken, whereby NSPs are released and subsequently exert antinutritive activity, as detected by the AME assay.

The third extraction of wheat released further arabinoxylans and glucans. Mannans were also detected in significant amounts for the first time. Mares and Stone (1983) reported that the arabinoxylans from wheat released by water and mild alkali extractions are very similar and the differences in their solubility are probably due to the alkali-soluble species being esterified to other structural components which renders them water insoluble. The large amount of glucan released may have been derived from residual β-(1–3,1–4)-glucans or from glucomannans which are also present in wheat (Fincher and Stone, 1986). It is also possible that some residual starch remained which dissolved in the alkaline conditions of extraction 3 and subsequently contributed to the high glucose levels. As the levels of NSP in this fraction do not correlate with the AME of the wheats, it is likely that these polysaccharides remain insoluble and inactive during their passage through the chicken.

Evidence for the antinutritive activity of cereal NSP is derived primarily from experiments investigating the activity of isolated NSP preparations added to trial diets or alternatively from studies examining the effect of degrading the NSP in vivo with feed enzymes. Correlations between the levels of NSP and the nutritive value of particular cereals have not previously been demonstrated, although it has been shown that the AME of different cereals correlates well with total levels of NSP (Choct and Annison, 1990). Studies have shown (Campbell et al., 1989; Rotter et al., 1989) that the performance of barleys in broiler chicken diets is negatively correlated with the viscosity of aqueous extractions which are assumed to be related to the levels of soluble β-(1–3,1–4)-glucans, although these were not determined. The extracts may also have contained soluble arabinoxylans. Pentosans are also present in barley at levels similar to those found in wheat (Henry, 1986).

The results of this study demonstrate that the AME of wheat is closely correlated with the levels of NSP which are released from wheat by extensive extraction at neutral pH, and it is likely that this is also true for other cereals. Fengler and Marquardt (1988a) reported that the water-soluble pentosans of rye are considered to be the active antinutritive component of rye. The mechanism of the antinutritive activity of the cereal NSP is yet to be described. Isolated wheat arabinoxylans added to experimental diets have been shown to reduce ileal starch, protein, and lipid digestibility (Hesselman and Aman, 1986; Ward and Marquardt, 1987; Fengler and Marquardt, 1988b; Choct and Annison, 1990) which depresses diet AME. Gut-modeling systems using dialysis tubing have demonstrated that nutrient diffusion and absorption is inhibited by solutions of polysaccharides (Fengler and Marquardt, 1988b). There is also evidence that the antinutritive activity is mediated by the gut microflora because the growth-depressing effects of cereals with high levels of NSP can be reversed in part by addition of antibiotics to the diet (Wagner and Thomas, 1978).

The data from this study provide further evidence that, in common with the water-soluble NSP of rye and barley, the water-soluble NSPs of wheats have an antinutritive activity in broiler chickens. Successful formulation of commercial broiler rations relies upon accurate information regarding the nutritive quality of major feed ingredients. The determination of soluble NSP levels of wheats and other cereals, using a method based on those used in extractions 1 and 2, should be added to the proximate analyses that are already performed as part of quality control procedures.

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Received November 7, 1990. Revised manuscript received January 22, 1991. Accepted March 4, 1991.

Registry No. Arabinoxylan, 9040-27-1; β-(1-3,1-4)-glucan, 55965-23-6; galactan, 9037-55-2; mannan, 9036-88-8.