Evaluation of Phosphorus Characterization in Broiler Ileal Digesta, Manure, and Litter Samples: ^{31}P-NMR vs. HPLC

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Using 31-phosphorus nuclear magnetic resonance spectroscopy (^{31}P-NMR) to characterize phosphorus (P) in animal manures and litter has become a popular technique in the area of nutrient management. To date, there has been no published work evaluating P quantification in manure/litter samples with ^{31}P-NMR compared to other accepted methods such as high performance liquid chromatography (HPLC). To evaluate the use of ^{31}P-NMR to quantify *myo*-inositol hexa*kis*phosphate (phytate) in ileal digesta, manure, and litter from broilers, we compared results obtained from both ^{31}P-NMR and a more traditional HPLC method. The quantification of phytate in all samples was very consistent between the two methods, with linear regressions having slopes ranging from 0.94 to 1.07 and r^2 values of 0.84 to 0.98. We compared the concentration of total monoester P determined with ^{31}P-NMR with the total inositol P content determined with HPLC and found a strong linear relationship between the two measurements having slopes ranging from 0.91 to 1.08 and r^2 values of 0.73 to 0.95. This suggests that ^{31}P-NMR is a very reliable method for quantifying P compounds in manure/litter samples.

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Phosphorus (P) characterization of ileal digesta, manure, and litter samples from broiler chickens (broilers) has an important application in both nutritional and environmental areas of research. The quantification of phytate in ileal and manure samples has been used to determine the impacts of dietary modification on phytate hydrolysis and P retention in poultry (Mohammed et al., 1991; Applegate et al., 2003; Tamim et al., 2004). In addition, the quantification of phytate and other organic P compounds in manure and litter samples can also be valuable from an environmental standpoint, as it provides information regarding the solubility of P in these samples and the potential reactivity of manure and litter P once land applied (Maguire et al., 2004; McGrath et al., 2005; Leytem et al., 2006a,b).

There are several methods available for quantifying organic P compounds in feeds, digesta, and manure samples. Ion-pair chromatography has been used for quantifying inositol phosphates in foods and digesta (Sandberg and Ahderinne, 1986; Sandberg et al., 1989). Several researchers have used 31-P nuclear magnetic resonance spectroscopy (^{31}P NMR) to identify organic P in foods, animal feeds, digesta, and manures (O'Neill et al., 1980; Kemme et al., 1999; Turner, 2004). The advantage of ^{31}P NMR analysis is that the entire range of P compounds (both organic and inorganic) present in samples can be identified with one simple extraction procedure providing better characterization of P in these samples. The main drawback to using ^{31}P NMR is that sample analysis can be costly and therefore less expensive means of analysis, such as high performance liquid chromatography (HPLC), are often preferred.

While there are several methods available, there has been no comparison of analytical methods or validation of these methods for characterizing the organic P fractions in ileal digesta, litter, or manure. In particular, there has been no validation of P quantification using the NaOH-EDTA extraction with ^{31}P NMR analysis (Turner, 2004) compared with other extraction and quantification techniques, such as acid extraction and HPLC analysis. Due to the extraction conditions and quantification techniques used in ^{31}P NMR analysis, there is some concern as to the validity of the

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quantification data obtained using this method. To address this, organic P compounds in ileal digesta, manure, and litter samples obtained from dietary modification studies in broilers were analyzed using ^{31}P NMR and HPLC analysis to evaluate the consistency of data obtained via different methods.

Materials and Methods

Sample Collection

Samples of ileal digesta, litter, and manure from broilers were collected from two experiments designed to evaluate effects of dietary Ca level, available P (AvP) level, and phytase enzyme on broiler performance and litter P concentrations. In Experiment 1 broilers were reared to 42 d of age in 72 floor pens (3.5 m^2) that contained fresh pine shavings at the onset of the study. At 41 d litter samples collected from three areas in each pen were pooled, thoroughly mixed, subsampled, and immediately frozen. At 42 d five broilers in each pen that weighed ± 100 g of the mean body weight of all birds in the house were killed by cervical dislocation and the terminal 13 cm of ileum removed 3 cm anterior to the ileo-cecal junction. Ileal contents were gently expressed, pooled per pen and immediately frozen. All samples were lyophilized and ground (2 mm) for analysis. To reduce analytical costs, two of the four replicate pens per treatment (18 treatments) were randomly selected for both ^{31}P NMR and HPLC analysis for a total of 36 samples per sample type (ileal digesta and litter).

Experiment 2 utilized 64 battery cages of 13 broilers each to evaluate effects of dietary Ca level, phytate level, and phytase enzyme on broiler performance and manure P concentrations from 16 to 19 d of age. Manure was collected in pans lined with clean plastic below each cage during a 24-hr collection period that commenced after birds had been acclimated to the experimental diets for 72 h. Samples were thoroughly homogenized, and subsampled, with the subsamples being immediately frozen. Samples were later lyophilized and ground (2 mm) for analysis. There were 16 treatments and one pen per treatment of the four replicate pens was randomly selected for both ^{31}P NMR and HPLC analysis.

Following sample preparation, a subsample of ileal digesta or litter (Experiment 1) and manure (Experiment 2) were analyzed independently by two separate laboratories. Samples analyzed by ^{31}P NMR were extracted in the facilities at North Carolina State University and sent to the University of Idaho for ^{31}P NMR analysis. The HPLC extraction and analysis of samples was performed by the Soybean and Nitrogen Fixation Research Unit of the Agricultural Research Service in Raleigh, NC.

Analytical Procedures

Total Phosphorus Determination

Total P in the ileal digesta, litter, and manure samples was determined by microwave-assisted digestion of a 0.5 g dried sample with 8 mL of concentrated HNO_3 and 2 mL of 30% H_2O_2 (v/v) with P quantified using inductively-coupled plasma optical-emission spectrometry detection (ICP–OES; 4300DV, PerkinElmer, Wellesley, MA, USA).

Phosphorus-31 Nuclear Magnetic Resonance Spectroscopy Analysis

The P composition of the ileal digesta, litter, and manure was determined by solution ^{31}P NMR spectroscopy as described by Turner (2004). Briefly, P was extracted in duplicate by shaking 2.00 ± 0.01 g of dried sample with 40 mL of a solution containing 0.5 mol L^{-1} NaOH and 0.05 mol L^{-1} EDTA for 4 h at 20°C. Extracts were centrifuged at 10,000 × *g* for 30 min and aliquots were analyzed for total P by inductively-coupled plasma atomic-emission spectrometry (ICP–AES; Optima 2000, PerkinElmer, Wellesley, MA, USA). The remaining solutions from the duplicate extracts were combined, frozen rapidly at –80°C, lyophilized, and ground to a fine powder.

Freeze-dried extracts were re-dissolved in 0.1 mL of D_2O (for signal lock) and 0.9 mL of a solution containing 1 mol L^{-1} NaOH and 0.1 mol L^{-1} EDTA, and then transferred to a 5-mm NMR tube. Solution ^{31}P NMR spectra were obtained using a Bruker Avance DRX 500 MHz spectrometer operating at 202.456 MHz for ^{31}P. A 5 μs pulse (45°), a delay time of 5.0 s, an acquisition time of 0.8 s, and broadband proton decoupling for all samples was used. The number of scans varied between 3797 and 16,091, and spectra were plotted with a line broadening of 1 Hz. Chemical shifts of signals were determined in ppm (ppm) relative to 85% H_3PO_4 and assigned to individual P compounds or functional groups based on literature values (Turner et al., 2003a). Signal areas were calculated by integration and P concentrations calculated by multiplying the proportion of the total spectral area assigned to a specific signal by the total P concentration (g P kg^{-1} dry feces) in the original extract. This NMR procedure detects concentrations of P compounds of approximately 0.1 mg P kg^{-1} of dry feces (Turner, 2004).

High Performance Liquid Chromatography Analysis

The inositol phosphate (IP) content of the ileal digesta, litter, and manure was determined using a modification of the method of Kwanyuen and Burton (2005). Briefly, P was extracted with 0.5 N HCl in a ratio of 1:20 (w/v) for 1 h while stirring at room temperature. Approximately 2 mL of crude extract from each sample was centrifuged at 18,000 × *g* for 10 min in a microcentrifuge. An aliquot of 1-mL supernatant was then filtered with a 1-mL tuberculin syringe and a 0.22-μm syringe filter (Durapore Membrane (PVDF)).

Chromatography was performed on a binary HPLC system (Agilent HPLC 1100 series, Agilent Technologies, Wilmington, DE) with a 4 × 250 mm IonPac AS7 column (Product #035393) equipped with a 4 × 50 mm IonPac AG7 guard column (Product #035394, Dionex Corp., Sunnyvale, CA). Elution of IP was achieved with a 20-min linear gradient of 0.01 mol L^{-1} 1-methylpiperazine, pH 4.0 to 0.5 mol L^{-1} $NaNO_3$ in 0.01 mol L^{-1} 1-methylpiperazine, pH 4.0 at a flow rate of 1 mL min^{-1} as previously described by Rounds and Nielsen with modifications. Wade's color reagent consisting of 0.015% (w/v) $FeCl_3$ and 0.15% (w/v) 5-sulfosalicylic acid (also at flow rate of 1 mL min^{-1}) and IPs eluted from the column were mixed in a mixing tee with inline check valves for both eluants installed before the mixing tee to prevent back flow. The post-column reaction was allowed to take place in a 250-μL sample loop (Part #1763, Upchurch Scientific, Oak

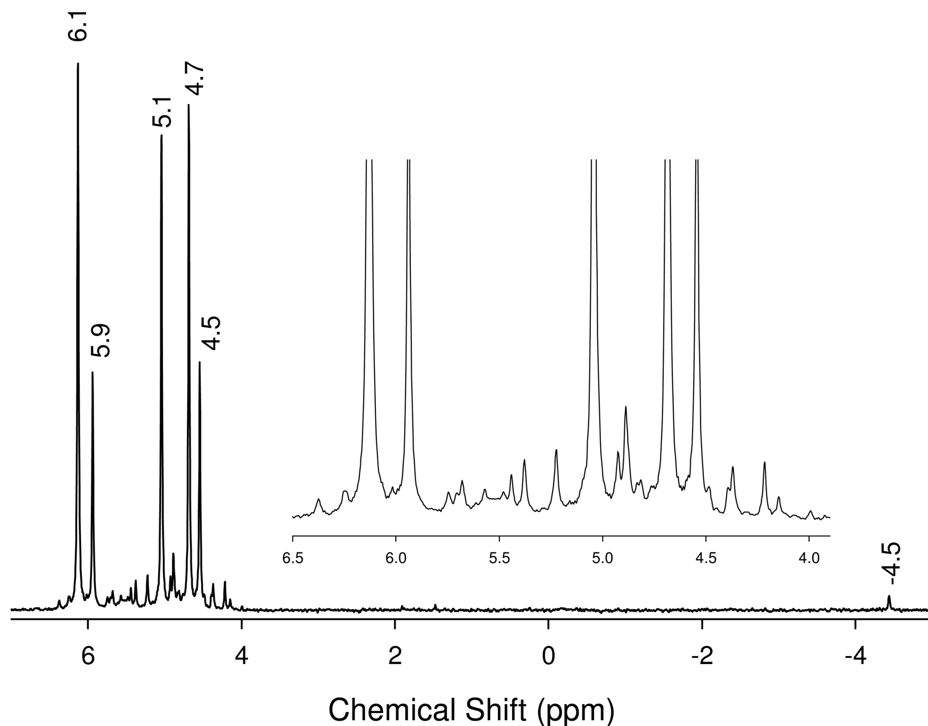

Fig. 1. A sample ^{31}P nuclear magnetic resonance spectroscopy (NMR) spectra obtained from one of the manure samples used in the study.

Harbor, WA) at the combined flow rate of 2 mL min^{-1}. The absorbance was monitored at 500 nm while the detector signals and/or IP peaks were processed and integrated by the chromatographic data acquisition system. The calibration standards were purchased from Sigma Aldrich and were a mixture of phytate (IP6, 95% purity) and a mixed standard of IP3, IP4, IP5 (100% purity).

Statistics

Statistical analysis was performed using the Statistical Analysis System (SAS Institute, 1996). Regression analysis was performed using the generalized linear models function in SAS.

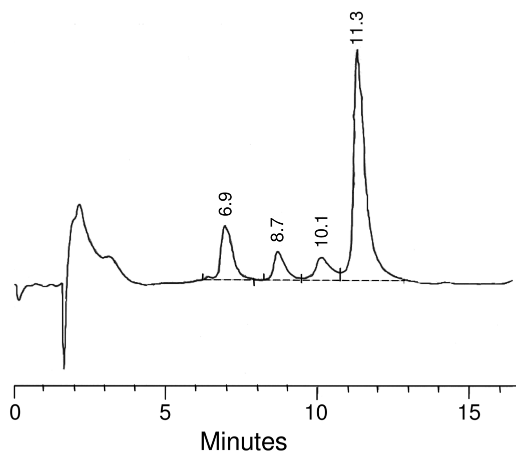

Fig. 2. A sample high performance liquid chromatography (HPLC) chromatograph showing the inositol ester peaks.

Results

Characterization of Ileal Digesta, Litter, and Manure Samples

Extraction of samples with NaOH-EDTA for ^{31}P NMR analysis recovered between 96 and 100% of total P measured by acid digestion, with the majority of samples recovering over 98% of total P. An example of a ^{31}P NMR spectrum is shown in Fig. 1. The strong signal appearing at 6.1 ppm was assigned to orthophosphate, while signals between 4.0 and 6.5 ppm were assigned to orthophosphate monoesters (organic phosphorus compounds with C-O-PO_3 bonds). The four strong signals appearing at 4.5, 4.7, 5.1, and 5.9 ppm in the ratio 1:2:2:1 were assigned to phytate. The other much weaker signals in the orthophosphate monoester region (4–6.5 ppm) likely represented lower IP esters, while the signal at −4.5 represented pyrophosphate. The majority of P in this and other samples analyzed was in the form of either orthophosphate or phytic acid. An example of an HPLC chromatograph is shown in Fig. 2. The esters of IP had retention times between 6.9 and 11.3 min. Inositol tri-phosphate (IP3), Inositol tetraphosphate (IP4), Inositol penta-phosphate (IP5), and Inositol hexa-phosphate (IP6, or phytate) had retention times of 6.9, 8.7, 10.1, and 11.3 min, respectively.

The P characteristics of the ileal digesta are shown in Table 1. Total P in ileal digesta ranged from 7.51 to 17.02 g kg^{-1}. The majority of P in the ileal digesta, measured by ^{31}P NMR, was in the form of phytate (4.89–10.31 g kg^{-1}, or 37–82% of total P), with only one sample containing less than 50% of its total P as phytate. Monoester P, which includes phytate and the lower inositol esters, comprised between 55 and 72% of total ileal P (4.90–10.46 g kg^{-1}). Orthophosphate concentrations ranged from 1.52 to 4.98 g kg^{-1} and only comprised between 15 and 30% of total P in the ileal digesta. There were small amounts of pyrophosphate in most of the samples which comprised only 1 to 2% of total P. The total IP concentration in the ileal digesta, measured by HPLC, ranged from 5.28 to 10.85 g kg^{-1}, the majority of which was phytate P (35–72% of total IP measured). Diets containing phytase had lower ileal phytate P, monoester P, and total IP concentrations as measured by both ^{31}P NMR and HPLC analysis. These samples also had higher concentrations of IP3, IP4, and IP5 than the non-phytase amended diets.

The P characteristics of the litter are shown in Table 2. Total litter P ranged from 5.91 to 11.32 g kg^{-1}. The majority of P in the litters as determined by ^{31}P NMR was in the form

Table 1. Phosphorus characterization of broiler ileal digesta via ³¹P nuclear magnetic resonance spectroscopy (NMR) and high performance liquid chromatography (HPLC) analysis.

Diet properties		Ileal	P characteristics of ileal digesta								
			NMR Determination				HPLC Determination				
AvP†	Phytase	Total P	Ortho P‡	Phytate P	Mono P§	Pyro P¶	IP3#	IP4#	IP5#	IP6#	TIP††
		g kg⁻¹									
0.26	–	12.71	1.91	9.18	9.19	0.08	0.14	0.06	0.58	8.56	9.33
0.26	–	12.87	3.02	8.23	8.31	0.16	0.07	0.06	0.61	8.04	8.78
0.26	–	13.58	1.97	9.99	9.80	0.14	0.10	0.10	0.85	9.80	10.85
0.31	–	12.39	2.02	8.85	8.79	0.11	0.14	0.07	0.62	8.91	9.75
0.31	–	13.49	2.81	9.05	8.97	0.20	0.10	0.09	0.73	8.52	9.43
0.31	–	13.88	2.19	9.89	9.90	0.14	0.11	0.09	0.72	8.96	9.87
0.36	–	12.35	2.07	10.15	8.60	ND	0.14	0.08	0.59	8.62	9.43
0.36	–	14.17	2.79	9.46	9.67	0.13	0.15	0.09	0.62	9.03	9.90
0.36	–	17.02	4.55	10.31	10.46	0.29	0.09	0.07	0.73	9.87	10.75
0.26	+	7.51	1.72	4.91	4.90	0.09	0.16	0.25	0.66	4.21	5.28
0.26	+	8.79	2.22	4.94	5.77	ND	0.09	0.33	0.75	4.41	5.58
0.26	+	9.42	1.88	6.21	6.51	ND	0.15	0.40	1.05	5.34	6.93
0.31	+	9.36	2.24	5.35	6.09	0.13	0.21	0.41	0.96	5.48	7.07
0.31	+	9.83	1.52	6.74	7.11	0.07	0.16	0.41	1.05	5.74	7.36
0.31	+	10.56	3.07	5.62	6.37	0.17	0.15	0.60	1.14	4.82	6.70
0.36	+	10.24	2.37	5.98	6.73	0.15	0.17	0.40	1.22	5.61	7.40
0.36	+	11.98	3.03	6.48	7.81	0.06	0.15	0.51	1.37	6.17	8.19
0.36	+	13.18	4.98	4.89	7.18	0.20	0.19	0.86	1.27	4.67	6.99

† AvP = the dietary available P percentage.

‡ Ortho P = orthophosphate.

§ Mono P = monoester P.

¶ Pyro P = pyrophosphate.

IP3, 4, 5, 6 = inositol tri-phosphate, tetra-phosphate, penta-phosphate, and hexa-phosphate.

†† TIP = Total inositol phosphates (sum of IP3-IP6).

of monoester P (3.93–7.61 g kg⁻¹, 53–76% of total litter P). The phytate content of the litter ranged from 2.83 to 6.32 g kg⁻¹, which comprised 37 to 63% of total litter P, and was generally higher in diets without phytase supplementation. There were only small amounts of pyrophosphate present that constituted approximately 1% of total litter P. Total IP measured by HPLC analysis ranged from 4.13 to 8.24 g kg⁻¹, the majority of which was phytate (70–86% of total IP). Lower inositol phosphates ranged from 1 to 16% of total IP. As with the ileal digesta, litter from broilers fed diets with phytase had greater IP3, IP4, and IP5 and less phytate (IP6) than litter from non-phytase amended diets.

Manure P characteristics are shown in Table 3. Total manure P ranged from 2.96 to 13.72 g kg⁻¹, with the majority of P being in the form of monoester P (37–77% of total P), as measured by ³¹P NMR. The manure phytate P ranged from 0.65 to 7.90 g kg⁻¹, while orthophosphate P ranged from 1.20 to 5.28 g kg⁻¹. Total IP ranged from 0.82 to 9.82 g kg⁻¹, with the majority present as phytate (49–86% of total IP). In the manure, there was less phytate P vs. in manure from birds fed phytase and non-phytase amended diets.

Comparison of Phosphorus-31 NMR and HPLC Data

The comparison of phytate determination by both ³¹P NMR and HPLC analysis for ileal, manure, and litter samples is shown in Fig. 3. There was a strong linear correlation between analyzed phytate concentrations determined by ³¹P NMR and HPLC analysis for all three sets of samples. The slopes of the regression (NMR vs. HPLC) ranged from 0.94 to 1.07 and had an r^2 of 0.84 to 0.98 that was dependent on the sample group analyzed. The analyzed phytate concentrations in litter from the two replicate pens in each treatment were averaged for both the ³¹P NMR and HPLC analysis; the slope did not change from 1.07, but the r^2 improved from 0.84 to 0.96. Both analytical methods produced very similar results for phytate content in ileal digesta and manure with slopes close to 1.0 and r^2 of 0.94 to 0.98. There was only a very small improvement in the r^2 from 0.94 to 0.96 when the ileal phytate concentrations from the two replicate pens were averaged for both the ³¹P NMR and HPLC analysis.

The total monoester P content (which includes all the esters of inositol phosphate) by ³¹P NMR determination was estimated by summing the signal areas between 4.0 and 6.5 ppm, excluding the orthophosphate peak at 6.1 ppm. The monoester P concentration determined using ³¹P NMR analysis was then compared to the total IP concentrations determined by HPLC analysis to ascertain how closely the total IP measured by HPLC matched with monoester P determined by ³¹P NMR (Fig. 4). There was a linear relationship with slopes of 0.91 to 1.08 between monoester P determined by ³¹P NMR analysis and total IP determined by HPLC analysis with r^2 values of 0.73 to 0.95. This suggested that the majority of organic P compounds present in these samples was in the form of IP esters. When results of the litter analysis from the two replicate pens were averaged for both methods an improved r^2 from 0.73 to 0.90 was obtained.

Table 2. Broiler litter phosphorus characterization with 31P nuclear magnetic resonance spectroscopy (NMR) and high performance liquid chromatography (HPLC) analysis.

Diet properties		Litter	P Characteristics of litter								
			NMR Determination				HPLC Determination				
AvP†	Phytase	Total P	Ortho P‡	Phytate P	Mono P§	Pyro P¶	IP3#	IP4#	IP5#	IP6#	TIP††
		g kg⁻¹									
0.26	–	6.99	1.88	4.23	5.23	0.05	0.41	0.06	0.41	4.97	5.84
0.26	–	7.29	2.19	5.45	6.64	0.05	0.31	0.08	0.59	5.77	6.75
0.26	–	8.21	1.91	5.19	6.18	0.05	0.19	0.10	0.58	5.57	6.44
0.31	–	7.93	2.60	5.23	6.29	0.05	0.58	0.15	0.68	5.90	7.32
0.31	–	8.19	2.60	5.12	6.16	0.07	0.24	0.07	0.59	5.15	6.06
0.31	–	8.56	3.57	4.55	5.87	0.07	0.43	0.25	0.84	5.34	6.86
0.36	–	9.21	4.20	6.01	7.09	0.06	0.50	0.16	0.74	6.17	7.57
0.36	–	10.56	4.10	6.23	7.61	0.09	0.41	0.10	0.74	6.69	7.94
0.36	–	11.32	2.79	6.32	7.17	0.07	0.59	0.16	0.77	6.72	8.24
0.26	+	5.92	2.51	2.83	3.93	0.02	0.64	0.26	0.59	2.64	4.13
0.26	+	6.35	2.17	2.97	4.14	0.03	0.57	0.23	0.64	2.92	4.35
0.26	+	6.60	2.35	3.46	4.63	0.08	0.20	0.19	0.67	3.41	4.47
0.31	+	5.91	3.64	2.93	4.24	0.06	0.47	0.20	0.65	3.02	4.33
0.31	+	6.66	2.50	3.24	4.28	0.07	0.41	0.26	0.66	3.30	4.63
0.31	+	7.61	2.36	3.12	4.14	0.04	0.56	0.36	0.80	3.22	4.94
0.36	+	7.53	3.88	3.13	4.34	0.05	0.44	0.34	0.83	3.62	5.23
0.36	+	7.61	4.06	3.43	4.71	0.06	0.83	0.38	0.90	3.63	5.74
0.36	+	8.32	2.77	3.54	4.83	0.05	0.36	0.25	0.69	4.27	5.58

† AvP = the dietary available P percentage.

‡ Ortho P = orthophosphate.

§ Mono P = monoester P.

¶ Pyro P = pyrophosphate.

IP3, 4, 5, 6 = inositol tri-phosphate, tetra-phosphate, penta-phosphate, and hexa-phosphate.

†† TIP = Total inositol phosphates (sum of IP3-IP6).

Table 3. Manure phosphorus characterization with 31P nuclear magnetic resonance spectroscopy (NMR) and high performance liquid chromatography (HPLC) analysis.

Diet properties		Manure	P Characteristics of manure								
			NMR Determination				HPLC Determination				
Phytate†	Phytase	Total P	Ortho P‡	Phytate P	Mono P§	Pyro P¶	IP3#	IP4#	IP5#	IP6#	TIP††
			g kg⁻¹								
0.19	–	9.65	4.91	3.65	4.69	0.04	0.43	0.36	0.51	4.25	5.56
0.19	–	10.12	3.41	5.57	6.65	0.05	0.34	0.28	0.61	5.54	6.77
0.19	–	9.80	2.08	6.30	7.55	0.07	0.22	0.31	0.78	6.21	7.53
0.19	–	9.43	2.23	5.99	6.87	0.15	0.16	0.15	0.56	5.51	6.38
0.07	–	5.30	2.98	1.45	2.31	ND	0.45	0.14	0.12	1.12	1.83
0.07	–	4.80	2.08	2.17	2.67	0.04	0.39	0.09	0.13	1.65	2.26
0.07	–	5.48	2.05	2.79	3.34	0.09	0.14	0.07	0.18	2.12	2.52
0.07	–	5.06	1.72	2.45	3.25	0.08	0.11	0.08	0.19	2.03	2.41
0.28	–	13.23	7.75	4.22	5.42	0.06	0.42	0.45	0.63	4.79	6.30
0.28	–	13.72	5.28	6.42	8.31	0.12	0.36	0.57	1.32	6.87	9.13
0.28	–	13.46	4.06	7.90	9.23	0.01	0.22	0.35	1.37	7.87	9.82
0.28	–	13.01	3.38	7.80	9.45	0.10	0.13	0.32	1.18	7.53	9.16
0.19	+	3.69	2.30	0.65	1.35	0.04	0.41	0.05	0.07	0.51	1.04
0.19	+	2.96	1.20	0.67	1.71	0.04	0.21	0.03	0.05	0.53	0.82
0.19	+	4.23	1.63	1.77	2.51	0.09	0.10	0.07	0.18	1.22	1.58
0.19	+	5.22	1.97	1.52	3.15	0.10	0.09	0.12	0.29	1.52	2.03

† Phytate = the dietary phytate P percentage.

‡ Ortho P = orthophosphate.

§ Mono P = monoester P.

¶ Pyro P = pyrophosphate.

IP3, 4, 5, 6 = inositol tri-phosphate, tetra-phosphate, penta-phosphate, and hexa-phosphate.

†† TIP = Total inositol phosphates (sum of IP3-IP6).

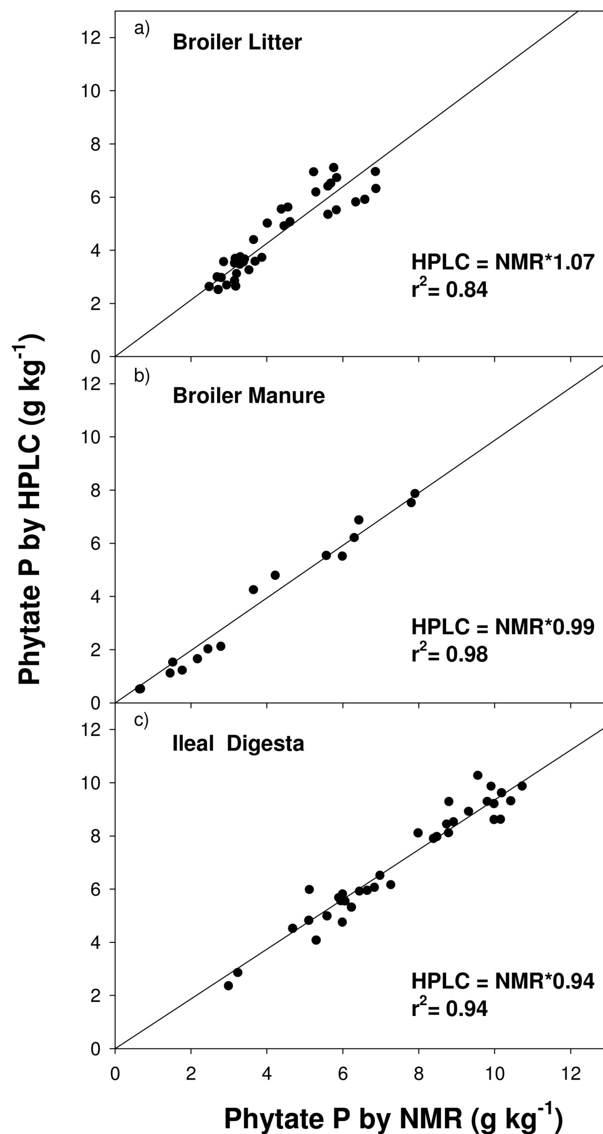

Fig. 3. Comparison of phytate determined in (a) broiler litter, (b) broiler manure, and (c) ileal digesta using ^{31}P nuclear magnetic resonance spectroscopy (NMR) vs. high performance liquid chromatography (HPLC) analysis.

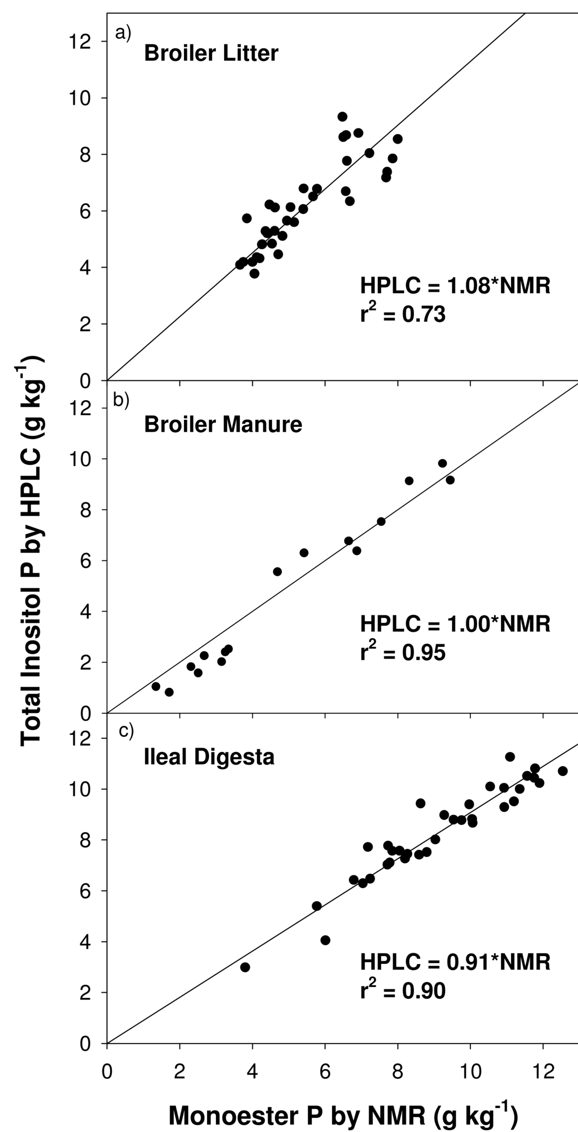

Fig. 4. Comparison of monoester phosphate determined in (a) broiler litter, (b) broiler manure, and (c) ileal digesta using ^{31}P nuclear magnetic resonance spectroscopy (NMR) analysis vs. total inositol phosphate measured in the same samples with high performance liquid chromatography (HPLC) analysis.

Discussion

The phytate concentrations in all of the samples analyzed with ^{31}P NMR and HPLC analysis were very consistent, even though the extraction conditions were quite different. There was greater variation in phytate concentrations observed between the two methods for the litter samples, compared to ileal and manure samples. This is not surprising and is likely a result of the reduced homogeneity of the litter samples. Since litter contains manure, woodchips, feathers, spilled feed, and possibly other components, obtaining truly homogeneous subsamples can be difficult. The nutrient composition of broiler litters has been found to vary significantly within poultry houses due to the proximity of feeders and waterers to the sampling point, and obtaining homogeneous samples can be difficult (Singh et al., 2004; Tasistro et al., 2004). Therefore, it was not surprising that the litter data showed greater variability in phytate determination between the two methods than the ileal and manure samples. The smaller sample size of the ileal digesta and manure allowed better homogenization of the samples and therefore more representative subsamples could be obtained for analysis. This improvement in homogeneity of the samples is evidenced by an improvement in the relationship of phytate determination between the two methods, with the r^2 improving from 0.84 with the litter samples to 0.94 and 0.98 for the ileal and manure samples, respectively.

Based on both the ^{31}P NMR and HPLC analysis, it was apparent that the majority of organic P in fresh samples of ileal digesta, litter, and manure were in the form of IP esters, rather than other organic P compounds such as phospholipids, sugar phosphates, or microbially derived organic P. This is consistent

with other published research using ^{31}P NMR to characterize broiler litter (Maguire et al., 2004; Turner, 2004; Turner and Leytem, 2004; McGrath et al., 2005). This may not be the case where litters and manures are stored for long periods of time in moist and warm conditions. The monoester P concentrations determined by ^{31}P NMR analysis closely approximated the total inositol P content of ileal digesta, litter, and manures. As with the phytate determination, there was a better relationship between monoester P and the total inositol phosphate concentration in the ileal and manure sample, which again we would attribute to improved sample homogeneity.

The determination of individual inositol phosphates is easier with the HPLC method as poor resolution of the phosphate monoester region of the ^{31}P NMR spectra require further spectral deconvolution to quantify each of the lower esters. Deconvolution software can be used to resolve overlapping signals in the NMR spectrum into their component peaks. This requires a degree of operator input, in particular to determine the number and characteristics of the deconvoluted peaks, but it more accurately quantifies signals from individual compounds where these can be identified using authentic standards. Turner et al. (2003b) has demonstrated that ^{31}P NMR spectroscopy and spectral deconvolution can be used to determine inositol phosphates in alkaline soil extracts having poor spectral resolution in the monoester P region, and this technique should be applicable to ileal, manure, and litter samples as well.

Conclusions

There was very good agreement between the two methods for phytate quantification even though the extraction and analytical techniques were quite different. Results of our evaluation provided assurance that determination of phytate in ileal digesta, manure, or litter using ^{31}P NMR analysis is accurate, even though the extraction conditions are quite different than traditional HPLC methods. An improvement in sample analysis is seen with decreased sample size and more through homogenization, suggesting that greater replication of analysis may be needed when it is difficult to homogenize samples to obtain representative subsamples. The use of HPLC analysis for determination of phytate in ileal, manure, and litter samples is economical and reliable. As an alternative, ^{31}P NMR analysis for phytate determination is a valid technique and is preferred if you are interested in obtaining information on the inorganic and other organic P compounds in the sample.

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