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## Nota

# ANIMAL MANURE PHOSPHORUS CHARACTERIZATION BY SEQUENTIAL CHEMICAL FRACTIONATION, RELEASE KINETICS AND $^{31}\text{P}$ -NMR ANALYSIS<sup>(1)</sup>

Tales Tiecher<sup>(2)</sup>, Mohsin Zafar<sup>(3)</sup>, Fábio Joel Kochem Mallmann<sup>(4)</sup>, Edson Campanhola Bortoluzzi<sup>(5)</sup>, Marcos Antonio Bender<sup>(6)</sup>, Lucas Henrique Ciotti<sup>(6)</sup> & Danilo Rheinheimer dos Santos<sup>(7)</sup>

## SUMMARY

Phosphate release kinetics from manures are of global interest because sustainable plant nutrition with phosphate will be a major concern in the future. Although information on the bioavailability and chemical composition of P present in manure used as fertilizer are important to understand its dynamics in the soil, such studies are still scarce. Therefore, P extraction was evaluated in this study by sequential chemical fractionation, desorption with anion-cation exchange resin and  $^{31}\text{P}$  nuclear magnetic resonance ( $^{31}\text{P}$ -NMR) spectroscopy to assess the P forms in three different dry manure types (i.e. poultry, cattle and swine manure). All three methods showed that the P forms in poultry, cattle and swine dry manures are mostly inorganic and highly bioavailable. The estimated P pools showed that organic and recalcitrant P forms were negligible and highly dependent on the Ca:P ratio in manures. The results obtained here showed that the extraction of P with these three different methods allows a better understanding and complete characterization of the P pools present in the manures.

**Index terms:** organic amend, phosphorus bioavailability, phytate, phosphorus fractionation.

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**RESUMO: CARACTERIZAÇÃO DO FÓSFORO EM DEJETOS ANIMAIS POR FRACIONAMENTO QUÍMICO SEQUENCIAL, CINÉTICA DE LIBERAÇÃO E ANÁLISE DE  $^{31}\text{P}$ -RMN**

*Práticas de manejo que visam reduzir a dependência de fertilizantes inorgânicos, como o uso de dejetos animais como fonte de P, podem contribuir para a sustentabilidade da agricultura. Embora informações sobre a biodisponibilidade e composição química do P presente nos dejetos utilizados como fertilizantes são importantes para o entendimento da sua dinâmica no solo, tais estudos ainda são escassos. Dessa forma, objetivou-se com o este trabalho avaliar o uso do fracionamento químico sequencial, da dessorção com resina de troca e da espectroscopia de ressonância magnética nuclear ( $^{31}\text{P}$ -NMR) para caracterizar as formas de P presentes em três diferentes dejetos animais (ave, suíno e bovino). O fracionamento químico sequencial do P, a extração sucessiva com resina e a espectroscopia de  $^{31}\text{P}$ -RMN demonstraram que as formas de P presentes nos dejetos de aves, bovinos e suínos são principalmente inorgânicas e altamente biodisponíveis. O teor de P orgânico e recalcitrante foi desprezível e altamente dependente da relação Ca:P nos dejetos. Os resultados deste trabalho demonstram que o uso dos três diferentes métodos, quando aplicados conjuntamente, permite a caracterização completa do P presente nos dejetos animais.*

*Termos de indexação: adubação orgânica, biodisponibilidade de fósforo, fitato, fracionamento de fósforo.*

## INTRODUCTION

Phosphorus is a critically essential nutrient in natural ecosystems and also regulates global agricultural production (Cordell et al., 2009; Shen et al., 2011), however, soils in most areas of the world are P deficient and limit agricultural yields (King et al., 2012). This deficiency is often met by adding external P to soil in various sources thereby increasing the soil P status. This mode of P application to soil have posed a serious threat to several waterways around the world resulting in an alarming increase in rate of eutrophication (Nash et al., 2007), and nourishing of algal blooms. This boosted algal biomass production has created undesirable impacts on marine biota, livestock and human health (McDowell et al., 2003).

Another growing concern in recent P research and management is P scarcity, which has gained increased attention on the research and policy agenda over recent years, partially due to the 800 % price hike in phosphate rock in 2008 (Cordell et al., 2009; Gilbert, 2009). However, actual estimates of phosphate depletion or peak P vary widely, from the occurrence of a critical point in 30-40 to 300-400 years. Growing consciousness about P scarcity and security, coupled with the environmental impact of P pollution has stimulated research exploring how P is used and lost in the agro-ecosystem (Cordell et al., 2011; Dawson & Hilton, 2011).

From the statistics of world P fertilizer consumption in 2011, Brazil total phosphate consumption was 3859.5 thousand tons (IFA, 2013). The global forecast of rock phosphate scarcity may lead to shortage of mineral P in the coming decades (Cordell et al., 2009), therefore use of manures as

alternative to mineral P fertilizer is becoming an increasingly essential practice to decrease the demand of mineral P fertilizers for agriculture in Southern Brazil. On the other hand, among various nonpoint P pollution sources by agriculture, the over application of manures relative to typical crop needs particularly in geographical regions where animal production is concentrated and excessive manure quantities are produced is another serious challenge (Toor et al., 2004). Excessive application of manure P to soils in the long term often increases the P transfer to soil and/or surface water (Sharpley et al., 2004). From the last decade, due to increased meat and dairy demands, the Brazilian livestock industry has expanded quickly to around  $1740.7 \times 10^6$  animals in Brazil (IBGE, 2010). This increase in animal population will further increase by 40 % from the current values over the next decade until 2021/22 and  $4106.6 \times 10^3 \text{ Mg yr}^{-1}$  of P is being added to the environment (Table 1).

Most studies carried out on animal manure as fertilizer have focused primarily on the P availability and its forms in soil after manure application (Halajnia et al., 2009; Azeez & Averbek, 2010; Guardini et al., 2012; Shafqat & Pierzynski, 2013). Although information on the bioavailability and chemical composition of P present in manure used as fertilizer are important for understanding its dynamics in the soil, such studies for Brazil are not yet available. In the current fertilization plans, manure application is typically based on total P concentration in the manure because the overall goal is to calculate and equalize the amount of manure P added relative to the crop needs. However, total P quantification provides no information about P chemical forms present in manures and their cycling in soil as well as their subsequent availability to plants.

**Table 1. The estimated increase from 2011/12 to 2021/22 in meat production; livestock and poultry population and phosphorus excretion in Brazil and Rio Grande do Sul**

Variable	Poultry	Cattle	Swine	Total
Meat production predicted in Brazil ( $10^6$ Mg)				
2011/2012	13.0	8.9	3.3	25.1
2021/2022	20.3	11.8	4.1	36.2
Increase predicted in 10-years period (%)	56.1	32.3	22.0	108.3
Number of animals <sup>(1)</sup>				
Brazil ( $10^6$ )	1488.4	211.5	40.8	1740.7
Rio Grande do Sul State ( $10^6$ )	149.3	14.5	5.7	169.5
Rio Grande do Sul State <sup>(2)</sup> (%)	10.0	6.8	13.9	30.5
Phosphorus excretion <sup>(3)</sup> (kg/animal/yr)	0.15	17.77	3.03	-
Brazil ( $10^3$ Mg/yr of P)	223.3	3759.8	123.5	4106.6
Rio Grande do Sul State ( $10^3$ Mg/yr of P)	22.4	257.3	17.2	296.9

<sup>(1)</sup> Data obtained from IBGE (2011). <sup>(2)</sup> Proportion of the national herd (IBGE, 2011). <sup>(3)</sup> Data obtained from Russell et al. (2008).

The P availability from animal manures largely depends on its forms (organic or inorganic), and on the ligand to which they are bound (Leytem et al., 2002). Organic P forms in animal manures are not readily available for plant uptake as they have to be mineralized into inorganic forms in the soil first. However, when animals are fed with supplemental minerals, P organic forms such as phytate can form stable complexes with Ca and other divalent and trivalent cations resulting in reduced hydrolysis of this organic form (Leytem et al., 2008a). Moreover, inorganic P release can be affected by Ca and Mg concentrations in the manure, where Mg concentration could have a detrimental effect on Ca-P stabilization in manure (Nair et al., 2003).

Analytical methods are available to characterize P forms in manures, but most of them are based on some type of chemical fractionation, such as sequential extraction with various acids or bases (Dou et al., 2000; Sharpley & Moyer, 2000). However, these methods only separate manure total P into fractions with different solubility, but cannot identify the exact organic or inorganic P species it contains. There has been a growing interest in the use of new analytical methods to characterize the organic and inorganic P species in manures and manure amended soils. The solution  $^{31}\text{P}$  nuclear magnetic resonance ( $^{31}\text{P}$ -NMR) spectroscopy has been successively used to characterize organic P in manures to provide an insight into the dynamics and availability of manure organic P forms (Toor et al., 2003). However, because to the complexity, sophisticated instrumentation and relative high cost of the analysis, these techniques are not usually available in laboratories thus limiting their use on a large scale.

In this background, such kind of studies are needed to generate reliable results underlying the design and implementation of management strategies for suitable manure use in agro-ecosystems. Therefore,

the methods of Chang and Jackson sequential P fractionation,  $^{31}\text{P}$ -NMR spectroscopy and successive desorption with anion exchange resin membrane were applied to characterize the different P forms in cattle, poultry and swine manures, to provide information on the bioavailability and chemical composition of P in these manures.

## MATERIAL AND METHODS

### Manure collection

Manures samples were collected from the region of Restinga Seca, in Rio Grande do Sul, in Southern Brazil. Cattle manure (CM) and swine manure (SM) were collected in dunghills while poultry manure (PM) was collected from poultry houses. Each manure sample was composed of 20 samples collected from different points in the dunghills and in the poultry house. As the aim of this study was to evaluate methods to characterize different P species in manure types, all the subsamples of each manure type were mixed and a homogenized representative sample was composed and dried at  $50^\circ\text{C}$ . After drying each sample was sieved ( $<2$  mm), and three replications were used for further analysis.

### Chemical characterization

Total N, P, K, Ca, and Mg in all three manure samples were estimated by acid digestion at  $350^\circ\text{C}$  for 2 h according to Tedesco et al. (1995). Total P was determined by Olsen & Sommer (1982) and readings were carried out later with the molybdate-antimony-ascorbic acid method of Murphy & Riley (1962). Total organic P was estimated by an ignition method, based on the difference between the P amounts extracted with  $0.5\text{ mol L}^{-1}\text{ H}_2\text{SO}_4$  on ignited ( $550^\circ\text{C}$ , 2 h) and non-ignited manure samples (Olsen & Sommers,

1982). Total Ca and Mg were determined with an atomic absorption spectrophotometer, total K with a flame photometer and total N by the micro-Kjeldahl method (Tedesco et al., 1995).

### Phosphorus fractionation

The fractionation was performed to selectively extract discrete P pools with chemical extractants. The manure P forms were extracted by a modified fractionation scheme described by Chang & Jackson (1957). Briefly, in each step, 1.0 g of manure sample and 50 mL of extractant (1:50 sample to extractant ratio) was filled in 60 mL centrifuge tubes with five replications. The tubes were shaken on an end-over-end shaker at 25 °C for varying periods, according to the extractant type. Sequentially, the extracting solutions used were: 1.0 mol L<sup>-1</sup> NH<sub>4</sub>Cl for 30 min; 0.5 mol L<sup>-1</sup> NH<sub>4</sub>F at pH 8.2 for 16 h; 0.1 mol L<sup>-1</sup> NaOH for 16 h; citrate bicarbonate dithionate (CBD) for 16 h; and 0.25 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> for 16 h. After the extractions, the residual material was dried at 50 °C and the content of residual P was estimated by digestion of 0.5 g residue with H<sub>2</sub>SO<sub>4</sub> + H<sub>2</sub>O<sub>2</sub> at 200 °C for 2 h (Olsen & Sommers, 1982). In the alkali extract of NaOH, total P was estimated by digestion with H<sub>2</sub>SO<sub>4</sub> and ammonium persulphate in an autoclave at 121 °C (USEPA, 1971), and subsequent P determination, according to Murphy & Riley (1962). In the other extracts, the content of P was directly determined by following the Murphy & Riley (1962) method.

### Phosphorus release pattern

The P and Ca desorption capacity was evaluated by successive extractions with anion-cation exchange resin (ACER), 0.1 g of each manure sample with five replications into 60 mL centrifuge tubes with 50 mL of distilled water and one sheet of bicarbonate-saturated ACER. The suspension was shaken for 16 h in an end-over-end shaker at ±25 °C. The resin sheets were then removed and washed with distilled water to remove the solid fractions, and washed again in 10 mL of 0.5 mol L<sup>-1</sup> HCl to transfer the P and Ca retained on the resin into the acid solution. The extraction was repeated 18 times consecutively, when the amount of extracted P and Ca became constant and near zero. The P content extracted each time was determined by the method of Murphy & Riley (1962). Calcium was determined with an atomic absorption spectrophotometer. The cumulative values of desorbed P and Ca were calculated, using the first-order kinetics equation proposed by McKean & Warren (1996) as follows:

$$P \text{ or } Ca_{\text{desorbed}} = \beta - (\beta - \alpha)e^{-\lambda t} \quad (1)$$

where  $\beta$  is the maximum amount of P or Ca desorbed;  $\alpha$  is the amount of P or Ca desorbed in the first extraction (readily bioavailable portion);  $\lambda$  is the desorption rate constant; and  $t$  is the extraction time.

### <sup>31</sup>P-NMR analysis

The <sup>31</sup>P-NMR analysis was performed with five 0.5 g subsamples, of dried manure material in 15 mL centrifuge tubes with 10 mL of 0.25 mol L<sup>-1</sup> NaOH + 50 mmol L<sup>-1</sup> EDTA (Turner, 2008). The tubes were shaken for 4 h on an end-over-end shaker at 25 °C. After centrifugation at 2510 RCF (relative centrifugal force) for 15 min, the extract of one subsample was used to estimate total P, Ca, Mg, Fe, and Al concentration. The extracts of the other four manure subsamples were combined and transferred into 100 mL snap cap tubes. The extracts were frozen and lyophilized to complete dryness. Subsequently, the lyophilized extract was dissolved in 2.7 mL of 0.25 mol L<sup>-1</sup> NaOH + 50 mmol L<sup>-1</sup> EDTA. An amount of 0.3 mL D<sub>2</sub>O was added and the mixture vortex-stirred for 5 min. After 120 min rest, the supernatant was separated by centrifugation (2510g for 15 min), filtered (<0.45 µm) and transferred to 5 mm NMR tubes (Cade-Menun, 2005). The P spectra were obtained in a Bruker Advance DPX 400 spectrometer at a frequency of 162 MHz with proton decoupling. A pulse angle of 90° was used at 20 °C an acquisition time of 0.2 and 15 s relaxation time (McDowell et al., 2006). The number of scans was 12,000. The peak area was determined by electronic integration at a baseline of 1 Hz.

The chemical shifts were obtained in relation to 85 % orthophosphoric acid and interpreted, according to Doolette et al. (2009), as follows: orthophosphate (6.2 ppm), pyrophosphate (-4.3 ppm), *myo*-inositol hexakisphosphate - *myo*-IHP (5.9, 5.0, 4.7 and 4.6 ppm), other monoesters (4.2 to 5.9 ppm) and diesters (-0.16 ppm). Software MestRe-C v 2.3a was used for peak area integration.

### Statistical analysis

For the comparison of the P proportion extracted in each fraction of Chang and Jackson fractionation, and the P and Ca desorption kinetic parameters obtained by fitting the McKean and Warren equation, the means of each manure type were compared by the Kruskal-Wallis *H* test (non-parametric test).

## RESULTS AND DISCUSSION

Total manure P contents (11.4, 58.1 and 28.0 g kg<sup>-1</sup> in PM, SM and CM, respectively) are presented in table 2. In contrast to the total P contents, PM contained most organic P (4.1 g kg<sup>-1</sup>) whereas both SM and CM contained less than 0.1 g kg<sup>-1</sup>. Total Ca and Mg contents were also highest in SM. Moreover, higher total Ca and lower total Mg was determined in PM than CM. The ratio of Ca for P and Mg varied significantly and Ca:P were 1.3, 0.3 and 0.7 whereas Ca:Mg were 0.5, 2.2 and 0.9 for PM, CM and SM, respectively. Averaged across all manure types, the



total Mg:P was  $0.65 \pm 0.05$ . Total K and N did not vary greatly, and their mean contents were 21.5 and 23.3 g kg<sup>-1</sup>, respectively.

Many researchers documented that manure Ca and Mg content control P release. Nair et al. (2003) stated that P release is influenced primarily by Ca and Mg concentrations in the manure. The Mg concentration in the manure could have a detrimental effect on Ca-P stabilization in manure. Our results show that to minimize the environmental impact of

manure applications, it is necessary to reduce the manure Mg contents through dietary control or by manure pre-treatment prior to soil application.

The results of chemical fractionation showed the highest proportion of P extracted with NH<sub>4</sub>Cl in PM followed by CM and then SM (Table 2). The two first extractants from this fractionation (1.0 mol L<sup>-1</sup> NH<sub>4</sub>Cl and 0.5 mol L<sup>-1</sup> NH<sub>4</sub>F pH 8.2 extracted an approximate mean of 90 % of the total P for all manure types. On average, the proportion of P extracted by 0.25 mol L<sup>-1</sup>

**Table 2. Selected chemical characteristics, sequential chemical P fractionation, P and Ca desorption kinetic parameters and organic P molecular species determined by <sup>31</sup>P nuclear magnetic resonance spectroscopy in three manure types**

Variable	Manure					
	Poultry		Cattle		Swine	
Total element concentration	g kg <sup>-1</sup>		g kg <sup>-1</sup>		g kg <sup>-1</sup>	
P	11.4 ± 0.3		28.0 ± 1.1		58.1 ± 2.9	
Ca	14.9 ± 1.4		8.9 ± 0.5		39.3 ± 0.9	
Mg	7.0 ± 0.3		19.9 ± 0.7		36.1 ± 0.5	
K	24.2 ± 0.4		18.2 ± 0.4		22.1 ± 0.7	
N	18.7 ± 0.5		29.3 ± 0.8		21.9 ± 1.3	
Organic P	4.1 ± 0.8		<0.1		<0.1	
P chemical fractionation	mg kg <sup>-1</sup>	%	mg kg <sup>-1</sup>	%	mg kg <sup>-1</sup>	%
1.0 mol L <sup>-1</sup> NH <sub>4</sub> Cl	8976 ± 438	78.0 a	19184 ± 464	64.1 b	31610 ± 852	57.8 c
0.5 mol L <sup>-1</sup> NH <sub>4</sub> F pH 8.2	1217 ± 184	10.6 b	8930 ± 339	29.8 a	18138 ± 696	33.2 a
0.1 mol L <sup>-1</sup> NaOH	293 ± 25	2.5 b	1250 ± 51	4.1 a	410 ± 85	0.7 c
CBD	20 ± 11	0.2 a	39 ± 22	0.1 a	111 ± 11	0.2 a
0.25 mol L <sup>-1</sup> H <sub>2</sub> SO <sub>4</sub>	915 ± 223	7.9 a	227 ± 22	0.7 b	4282 ± 569	7.9 a
Residual	139 ± 26	1.2 a	290 ± 37	0.9 a	128 ± 6	0.2 b
P desorption parameter	g kg <sup>-1</sup>	%	g kg <sup>-1</sup>	%	g kg <sup>-1</sup>	%
α-P	3.78 ± 0.8	33.1 a	9.2 ± 1.6	32.8 a	11.4 ± 1.2	19.6 b
β-P	10.1 ± 0.7	88.6 b	27.1 ± 1.6	96.9 a	52.1 ± 3.2	89.6 b
λ-P*	0.033 ± 0.003 a	-	0.033 ± 0.003 a	-	0.016 ± 0.001 b	-
Ca desorption parameter	g kg <sup>-1</sup>	%	g kg <sup>-1</sup>	%	g kg <sup>-1</sup>	%
α-Ca	3.2 ± 0.7	24 a	2.2 ± 0.3	21 a	1.7 ± 0.2	5 b
β-Ca	13.5 ± 1.0	100 a	10.9 ± 1.0	103 a	37.3 ± 0.8	112 a
λ-Ca*	0.015 ± 0.001 a	-	0.012 ± 0.002 a	-	0.007 ± 0.001 b	-
NaOH+EDTA extraction	mg kg <sup>-1</sup>	%	mg kg <sup>-1</sup>	%	mg kg <sup>-1</sup>	%
P	11872 ± 587	104	31520 ± 371	113	59533 ± 399	102
Ca	339 ± 12	2.3	178 ± 15	2.0	960 ± 32	2.4
Mg	106 ± 5	1.5	247 ± 11	1.2	323 ± 3	0.9
Fe	603 ± 24	-	259 ± 24	-	120 ± 11	-
Al	142 ± 15	-	0	-	0	-
P speciation by <sup>31</sup> P-NMR	mg kg <sup>-1</sup>	%	mg kg <sup>-1</sup>	%	mg kg <sup>-1</sup>	%
Orthophosphate	11102	93.3	31815	99.4	62523	99.1
Pyrophosphate	52	0.4	56	0.2	181	0.3
Total inorganic P	11154	93.7	31871	99.6	62705	99.4
Myo-IHP	539	4.5	0.0	0.0	0	0.0
Others monoesters	185	1.6	129	0.4	395	0.6
Diesters	22	0.2	0.0	0.0	0	0.0
Total organic P	746	6.3	129	0.4	395	0.6

Means followed by the same letter in a row comparing different manure types are not different by the Kruskal-Wallis H test at  $p < 0.05$ .

\* Values are in g kg<sup>-1</sup> h<sup>-1</sup>.

H<sub>2</sub>SO<sub>4</sub> was 7.9 % in PM and SM, while only 0.7 % in CM. The other extractants of the Chang and Jackson method extracted very low amounts of P.

The readily bioavailable proportions of P and Ca ( $\alpha$ -P and  $\alpha$ -Ca), determined with successive anion-cation exchange resin release kinetics were 33.0 % higher in PM and 22.5 % in CM, respectively) than in SM which was 19.6 and 5.0 %, respectively (Table 2). However, potentially available P ( $\beta$ -P) was higher in CM (96.9 %) than in PM and SM (89.1 %). Thus,  $\alpha$ -P represents only 37.4, 33.9 and 21.9 % of  $\beta$ -P in PM, CM and SM, respectively. Desorption constants of P ( $\lambda$ -P) and Ca ( $\lambda$ -Ca) in SM was 0.016 and 0.007 mg kg<sup>-1</sup> h<sup>-1</sup>, respectively. This concentration is approximately half of that in PM and CM (0.033 and 0.0135 mg kg<sup>-1</sup> h<sup>-1</sup>, respectively). The desorption proportions of PM and CM were similar for P and Ca until the third extraction, However, from then on until the end, PM was close to SM proportions (Figure 1).

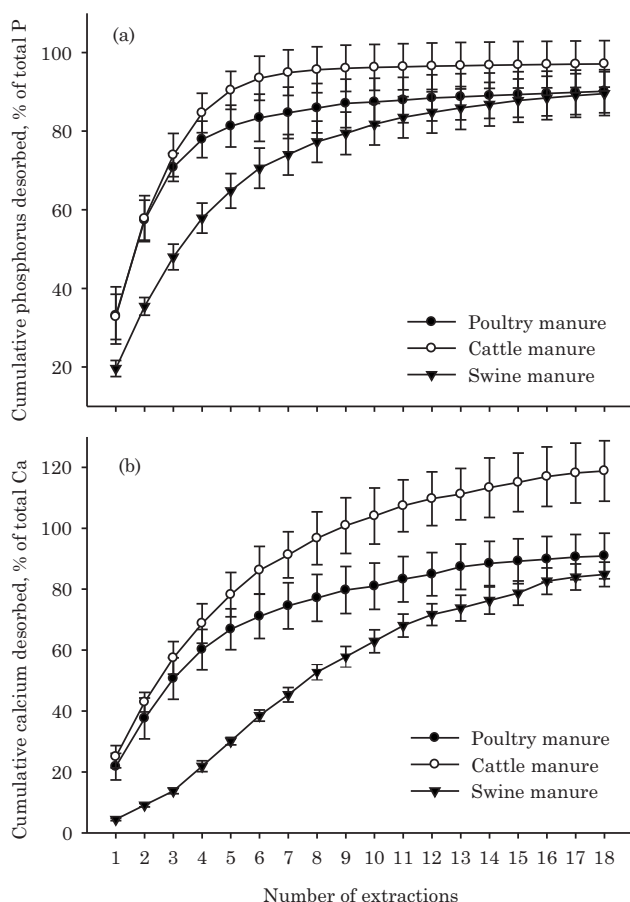
The results for CM indicated that the higher proportion of P labile forms (sum of NH<sub>4</sub>Cl and NH<sub>4</sub>F), and potentially bioavailable P ( $\beta$ -P), compared to the other manure types typically show higher total Mg:Ca

and lower total Ca:P ratios. In addition, the lowest total Ca:P ratio in CM also explains the lowest proportion of P extracted by H<sub>2</sub>SO<sub>4</sub>, which is considered as P bonded to Ca in the Chang and Jackson fractionation. On the other hand, in PM and SM there was a higher proportion of P bound to Ca than in CM, as evidenced both by the higher total Ca:P ratio and the higher proportion of P extracted by H<sub>2</sub>SO<sub>4</sub>. Furthermore, the P proportion not desorbed from all manure types after the successive extractions with resins (total P minus  $\beta$ -P) was very close to the P amount bonded to Ca estimated by the extraction with H<sub>2</sub>SO<sub>4</sub>. Therefore, this indicates that P-Ca linkage decreases P availability. In this regard, when the total Ca:P ratio is higher, there is a net transformation of more soluble (dicalcium phosphate) to less soluble P compounds (hydroxylapatite) as reported by Toor et al. (2005), which decreases the potential of P transfer to water.

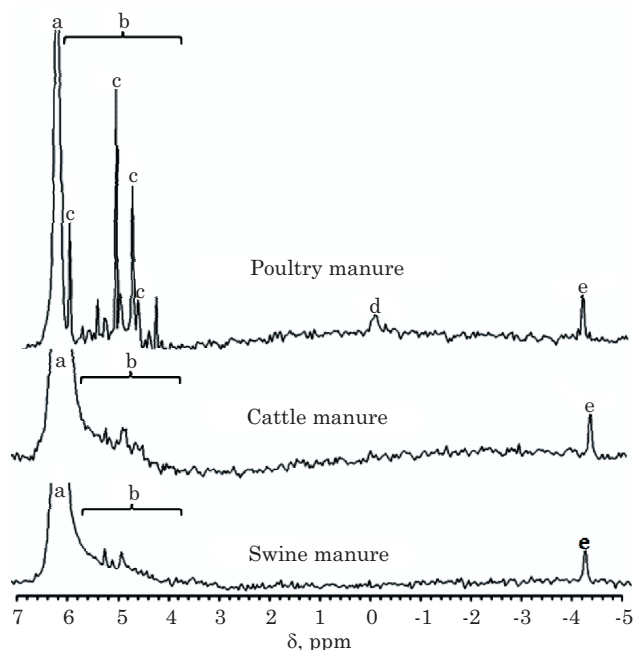
The extraction with NaOH + EDTA for <sup>31</sup>P-NMR spectroscopy analysis was sufficient to extract almost all P from the manures (Table 2). The NaOH + EDTA extracts contained low concentration of paramagnetic metals (Ca, Mg, Fe, and Al) that can interfere with the quality of signal acquisition of the nuclear magnetic resonance spectra. This indicates that the spectra obtained are true representatives of P forms existing in each manure without interference of paramagnetic ions (Cade-Menun, 2005).

In all manure types, the principal P form detected by <sup>31</sup>P-NMR was inorganic P, almost entirely in the orthophosphate form at  $\delta$  = 6.2 ppm (on average 99.7 % of the inorganic fraction) and the rest in the pyrophosphate form at  $\delta$  = -4.3 ppm (Table 2). The organic P in CM and SM was 0.4 and 0.6 %, respectively mainly in the form of phosphate monoester ( $\delta$  = 5.9 to  $\delta$  = 4.2 ppm). However, in PM the proportion of organic P was 6.3 % and only 0.2 % in the diester form of DNA ( $\delta$  = -0.16 ppm) and the rest in monoester form. In this analysis, no spike was used in the samples with known organic P compounds as recommended by Doolette et al. (2009). However, in the monoester organic P region, it was possible to clearly identify *myo*-inositol hexakisphosphate in PM (*Myo*-IHP) by the chemical shifts at  $\delta$  = 5.9, 5.0, 4.7, and 4.6 (Figure 2). Phytate (*Myo*-IHP) was the main organic form found in PM, reaching 72 % of the organic P.

Our results are in agreement with those of He et al. (2007) and Leytem et al. (2008b), who characterized P compounds in poultry and dairy manures by solution <sup>31</sup>P-NMR in NaOH + EDTA extracts. They found that in dairy manure, inorganic P was the major P fraction (64-73 %), followed by 6 % phytic acid, 14 to 22 % other monoesters, and 7 % phosphodiester, whereas in the poultry manure, results showed that orthophosphate accounted for 51 to 63 %, phytic acid 24 to 33 %, other phosphomonoesters 6 to 12 %, and phospholipids and DNA 2 % each. McDowell & Stewart (2005) also separated manure P forms in fresh and



**Figure 1. Cumulative phosphorus (a) and calcium (b) desorbed by successive anion-cation exchange resin extractions in poultry, cattle, and swine manure.**



**Figure 2. Solution  $^{31}\text{P}$  nuclear magnetic resonance spectra of poultry, cattle, and swine manure. The letters indicate the P form as follow: a = orthophosphate, b = monoester phosphates, c = *Myo*-inositol hexakisphosphate (phytate), d = diester phosphate (DNA), and e = pyrophosphate.**

air-dried dung of animals (dairy cattle, deer, sheep) by  $^{31}\text{P}$ -NMR. The  $^{31}\text{P}$ -NMR data showed that changes in organic P concentration with drying can be attributed to degradation of diesters. This may explain the low concentration of organic P assessed by  $^{31}\text{P}$ -NMR in our manure samples, especially in the diester fraction. However, our organic P results obtained with  $^{31}\text{P}$ -NMR spectroscopy were consistent with those estimated by the ignition method. Although CM and SM represented minor amounts of organic P, PM contained a considerable proportion, mainly in the form of phytate P (PP).

According to Tamim et al. (2004) and Leytem et al. (2008a), supplemental minerals, such as Ca and other divalent and trivalent cations can form stable complexes with phytate and result in reduced PP hydrolysis. The ability to hydrolyze PP by phytase may be reduced at greater concentrations of Ca because of increased pH in the intestine and an increase in Ca-PP salt formation (Manangi & Coon, 2008). The Ca-PP complexes are only soluble at low pH values or in the presence of strong complexing agents such as EDTA (Seaman et al., 2003; Turner, 2004). Thus, based on the combined analysis of the proportion of P extracted with  $\text{H}_2\text{SO}_4$  by the Chang and Jackson fractionation and the higher proportion of PP (*Myo*-IHP) obtained by  $^{31}\text{P}$ -NMR spectroscopy, we can infer that most of the P bonded to Ca in PM was possibly Ca chelated by phytate, or an organic mineral complex with Ca-PP (Table 2).

Aside from this low availability fraction, the lability of the remainder P present in PM was high, similar to CM as indicated by the high proportion of  $\alpha$ -P,  $\alpha$ -Ca and the higher  $\lambda$ -P and  $\lambda$ -Ca, and the higher proportion of P extracted by  $\text{NH}_4\text{Cl}$  in both PM and CM. On the other hand, SM showed no PP and although its Ca:P ratio was approximately half that of PM, most of the Ca in this manure probably formed low solubility inorganic complexes with P e.g., hydroxylapatite (Toor et al., 2005). This also explains the lower proportion of  $\alpha$ -P and  $\alpha$ -Ca in SM, lower  $\lambda$ -P and  $\lambda$ -Ca and also the lower P proportion extracted by  $\text{NH}_4\text{Cl}$ .

The discussion and the interactions pointed out above show that the results obtained by sequential chemical fractionation of P, successive extraction with resins and  $^{31}\text{P}$ -NMR spectroscopy allowed a complete characterization of the manure P and further indicated that the inorganic P fraction is predominant in all three manure types and is highly bioavailable (Table 2). Consequently, manure surface application under NT may increase the potential for soluble inorganic P loss in surface runoff at increased rates if the manure contribution to the available P pool is not taken into account for crops already fertilized with manure, as recommended by the CQFSRS/SC (2004). In this way, successive and long-term manure applications need to be controlled to avoid excessive amendments and consequent environmental problems, especially those related with P leaching to surface and groundwater.

## CONCLUSIONS

1. The sequential chemical fractionation of P, the successive extraction with resins and the  $^{31}\text{P}$ -NMR spectroscopy methods showed that the P forms in poultry, cattle and swine dry manures are mostly inorganic and highly bioavailable.
2. The organic and recalcitrant P forms were negligible and are highly dependent on the Ca:P ratio in manures.
3. The results obtained here show that the three different methods, when applied together, allow a complete characterization of the P present in manures.

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