

**EVLAUATION OF AN EXTANT MODEL FOR THE EXCRETION OF  
PHOSPHORUS AND NITROGEN FROM SWINE FED DIETS WITH AND  
WITHOUT MICROBIAL PHYTASE**

by

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## ABSTRACT

An extant model was evaluated to assess its adequacy for nutrient management planning (NMP) from swine operations in Manitoba that includes predictions for major nutrients of interest, phosphorus (P) and nitrogen (N), and the land base for spreading of manure when standard and phytase supplemented diets are used. Furthermore, the effect of phytase on net greenhouse gas (GHG) emissions from manured dry and wet sandy loam soils was also determined. Data was generated from starter to finisher pigs where 20 pigs (10 per treatment), and sows where 18 (9 per treatment) were used with two dietary treatments. The control diet was formulated to meet the requirement of pigs for nutrients as per the recommendations of NRC (No-phytase) and the second diet was formulated with P level in the No-phytase diet reduced by an average of 0.1 percentage units and diet supplemented with microbial phytase at 500 FTU/kg (Phytase). There was no significant difference in average daily feed intake (ADFI) ( $P > 0.25$ ), average daily gain (ADG) ( $P > 0.173$ ) and feed conversion ratio (FCR) ( $P > 0.084$ ). Model tended to predict the P excretion in all the phases better in the No-phytase than the Phytase diet where in most cases the random variation (ED  $> 50\%$  except in phase IV) contributed the most towards the total error of prediction. However, the model was not satisfactory for predictions when phytase was supplemented to swine diets where error due to regression (ER) was more than 50% in all the phases except the second phase (14-25 kg body weight). Concordance correlation coefficient (CCC) analysis showed that the model was fairly accurate and precise in the starter to finisher phases of production. Evaluation of model adequacy in sow operations showed that most (60% and 50%) of the total error of prediction was associated with ER in the No-phytase diet and ED for the Phytase diets, respectively. The model over-predicted

the excretion of P from sows by 32% in the No-phytase diet ( $\mu = -1.06$ ) and 23.5% in the Phytase diet ( $\mu = -0.783$ ). Furthermore, the model over-predicted the land base required for the spread of manure by 30% in the No-phytase and 21% in the Phytase diets. Generally, the assessment of model adequacy in the growing-finishing pigs showed that the model can be used to predict the excretion of P in the manure of growing-finishing pigs satisfactorily in No-phytase, but not in the Phytase diet. However, the model was not satisfactory for the predictions of both P and N excretion in sows. Application of manure originating from diets supplemented with microbial phytase showed a slight increase in the emissions of net GHG emissions mainly in the form of carbon dioxide ( $\text{CO}_2$ ). Emissions were, however, mainly influence by the moisture content of soils rather than the type of manure where high moisture contents of soils resulted in a significantly higher emissions of both  $\text{CO}_2$  and nitrous oxide ( $\text{N}_2\text{O}$ ).

## DEDICATION

I dedicate this thesis to my late mother, Aster Iyassu, my Grandmother Genet Asghedom, my father Bekele Yitbarek, and my siblings, Robel and Fasil.

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## FOREWORD

This thesis is written in a manuscript format composed of four manuscripts. Manuscript I will be submitted to Canadian Journal of Animal Science, and Manuscripts II and III will be submitted to the Journal of Agricultural Science. Manuscript IV has been submitted to the Journal of Agricultural Science and part of the work was presented at the CSAS in August, 2008. Authors to manuscript IV are A.Yitbarek, M. Tenuta, C. M. Nyachoti, J. France, and E. Kebreab. In the thesis, the four manuscripts were written according to the guidelines for the Journal of Agricultural Science manuscript preparation.

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## LIST OF ABBREVIATIONS

AA	Amino acids	MSPE	Mean square prediction error
ADFI	Average daily feed intake		
ADG	Average daily gain	NMP	Nutrient management planning
ANOVA	Analysis of variance		
BW	Body weight	N	Nitrogen
CCC	Concordance correlation coefficient	NRC	National research council
		NRCS	National Resource Conservation Service
CP	Crude protein		
d	Day	NU	Non unity slope
DM	Dry matter	P	Phosphorus
DOC	Dissolved organic carbon	P: N	P to N ratio
DP	Dressing percentage	R <sub>NM</sub>	Maternal N retention
ECT	Error due to central tendency	RP	Retained phosphorus
		SB	Squared bias
ED	Random variation	SD	Standard deviation
ER	Error due to regression	SL	Sandy loam
FC	Feed consumption	TIN	Total inorganic nitrogen
FCR	Feed conversion ratio	TP	total phosphorus
FFL	Fat free lean	TN	Total nitrogen
FR <sub>FFL</sub>	Fractional fat free lean	VFA	Volatile fatty acids
FTU	Phytase units	WFPS	Water-filled pore space
GHG	Greenhouse gases		
LC	Lack of correlation		
MSD	Mean square deviation		

## 1.0 GENERAL INTRODUCTION

The swine industry is faced with many decisions in the production cycle that includes nutrient supply to animals, cost and type of feed and a range of animal health, welfare and environmental issues that affect the profitability of production operations. Environmental issues from the swine industry perspective include excess nutrient load in soil, mainly phosphorus (P) and nitrogen (N), which affect soil, water and air quality surrounding swine facilities. The main reason behind this threat is that monogastric animal diets are mainly comprised of seeds (cereal grains) or their products (oil seed meal and grain by products) in which about two-thirds of the P is present in the form of phytate which has a low bioavailability due to lack of the enzyme phytase in the gastro-intestinal tract of these animals (Simons *et al.* 1990; Kies *et al.* 2005). Moreover, inorganic P, which has been increasing in its use for the last five decades (Barnett 1994), is supplemented at a higher safety margins to avoid any undesirable effects on swine due to low P availability in feedstuff. Any excess P above the requirement of the animal ends up in the manure. The threat of animal manure to surface waters is also further exacerbated because of the need for adequate water supplies that often results in swine operations being established near easily accessible streams and sources of water (Pip 2005).

In livestock systems, P is removed in the animal products and wastes, and must be replaced if P deficiency is to be avoided in order to maintain a sustainable supply of food for a growing human population. Any excessive P supplied to animals ends up in the manure, which is often applied to a small land base close to animal confinements resulting in a reduced P retention capacity of soils and accelerated loss of runoff-P to watercourses

(Leinweber *et al.* 1999). Swine manure contains significant quantities of P, some of which is in organic forms that are relatively stable and mobile in soils (Gerritse & Vriesema 1984; Chardon *et al.* 1997). Legislations have been adopted in different regions to limit the use of animal manure as fertilizer, as well as the density of swine per area of cultivated land (Jongbloed & Lenis 1998), which in turn influence the economic sustainability of swine operations.

Nutrient management planning (NMP) can assist in the development of a sustainable production system with a maintained profitability and minimized environmental risks from swine operations; and mathematical models are essential tools for understanding and managing the flow of nutrients for NMP. Mathematical models developed for swine production in one region might be adapted for another region. However, these models need to be evaluated using data from the region of interest, and if necessary, modified to take account the unique characteristics of a particular region.

The primary concern surrounding P in Manitoba is the eutrophication of waterways and water bodies, and in particular, Lake Winnipeg (Manitoba Phosphorus Expert Committee, 2006). Manitoba conservation has adopted a mathematical model that predicts the land base requirement for swine manure based on Québec conditions. The difference in ingredients used for swine diets in Manitoba and Québec is one of the main concerns that might result in failure of the model to predict P and N excretion under Manitoba conditions. The model predicts excretion rate for different types of swine (e.g., grower-finisher, sows). To build confidence in the model by producers, farm advisors, feed companies and policy makers in the province, it is necessary to measure key parameters in Manitoban conditions and rigorously test/modify nutrient utilization model.

Therefore, the objectives of these studies were:

1. Collect Manitoba based information on P intake, utilization and excretion from swine fed typical diets and investigate the effect of phytase supplementation on the excretion of P and N from swine (starter to finisher and sows) (Chapter 3).
2. To evaluate the adequacy of an extant empirical model adopted by Manitoba Conservation for the excretion of P and N, and land base requirement for the spread of manure when 'typical' and phytase amended diets are used under Manitoba conditions (Chapter 4 & 5).
3. Application of manure results in GHG emissions. The study aims to quantify GHG emissions from swine manure when standard and phytase amended diets are used (Chapter 6).

## 2.0 LITERATURE REVIEW

### *2.1 Effect of phosphorus on the environment*

Environmental concerns with P are primarily associated with pollution of surface waters (streams, lakes, rivers) (Knowlton *et al.* 2004). Agriculture is considered to be one of the major contributors of P into surface waters along with other point and non-point sources. Within the agriculture sector, intensive livestock production has been identified as a primary source of P in surface waters (Sharpley *et al.* 2003). Changes in the structure of swine production in the last five decades have resulted in a reasonable concern in relation to sustainability of those operations. Manure was historically considered as a scarce and valuable commodity under extensive livestock production. However, the development of large confinement systems based on limited land area has resulted in increased concerns of nutrient loading from manure applications (Jongbloed & Lenis 1998). Manure applications to meet N requirement of crops increases the concentration of soil P. This is because of the difference in the N:P ratio of manure compared to the crop removal, leading to the application of more P than removed by crops (Daniel *et al.* 1994). When the inherent threshold of soils to retain P is exceeded, coupled with the various transport factors in the soil, an increase in runoff P with detrimental effects on surface waters is observed (Sharpley *et al.* 1999). As the P concentration increases, surface water quality is degraded through the process of eutrophication with accompanying compression of the photic zone due to shading, progressive deoxygenation, and increased day-night fluctuations in pH. Increased algae growth, reduced water clarity, low dissolved oxygen or anoxia; and toxins from cyanobacteria (also referred to as blue green algae) are observed,

which can affect aquatic life as well as human and animal health (Smith *et al.* 1999). When algae die and sink to the bottom, extensive amount of dissolved oxygen is used up to decompose the algae resulting in deleterious effects on the fish population in lakes (Smith *et al.* 1999; Pip 2005). Therefore, with a limited farm land base for proper utilization, the application of manure as fertilizer results in P enrichment of surface waters (Fernandez *et al.* 1999a; Sharpley *et al.* 1994).

There are different sources of P that result in overloading of water bodies which include atmospheric, urban point and non-point, and agricultural sources. Atmospheric sources are in dry, wet and phosphine gas deposition forms, where dry deposition is the P bound to dust and other small particulate matter that settles out of the atmosphere, wet deposition being P dissolved in precipitation, in rain and snow, and phosphine a gas generated primarily in the natural environment by wetlands (Flaten *et al.* 2003). Bennet (1999) reported that natural movement of P into the watershed of Lake Mendota, Wisconsin, through dry and wet deposition, makes up only a small percentage (4.7%) of the total inputs in this budget, while human-induced movement of P, through the import of fertilizer and feed supplements, comprises the majority of the budget (95.3%). Urban point and non-point sources could be a significant source of degradation for water bodies. Point sources which are mainly municipal and industrial waste water treatment plant discharges contribute a significantly high soluble P with detrimental effect on the environment. It is difficult to estimate both the point and non-point sources; but their contribution to P in water ways deserves attention. One study by Waschbush *et al.* (1999) reported that the concentration of dissolved P in runoff ranged from 0.12 - 0.39 mg/L from streets and 0.22 - 0.53 mg/L from lawns. Agricultural sources of P include fertilizer

and manure P applications. In 2000, on average, fertilizer P and manure P sources contribute a 9.8 and 1.7 kg P/ha, respectively, in Manitoba soils. The combined effect of fertilizer and manure application resulted in an over application of P in the range of 3.8 - 11.4 kg P/ha in the same year, where localized regions of large livestock populations were the major contributing areas to the excess of P observed (Johnston & Roberts 2001).

One of the obvious solutions to reduce detrimental effect of swine manure on the environment would be to reduce production. However, with reduced agricultural productivity, the maintenance of a stable farm economy, a viable rural economy, and a reliable domestic and global food supply would be seriously threatened.

## 2.2 *Phytic acid*

The availability (release and absorption) of P from feedstuffs affects the requirement for total dietary P in all species, but our assumptions of availability of feed P are based on relatively few studies. Questions remain for all species about appropriate methods and response variables to determine availability of feed P. Measurements of apparent P digestibility are not very useful as estimates of true (or net) availability of feed P because of the effect of endogenous P. Improved P availability from feed would allow the tissue-level needs of the animal to be met with reduced P intake. Dietary P absorbability is a function of release from the feed matrix and the degree to which released phosphate is absorbed at the small intestine. Because P intake and excretion are so tightly linked, the question of whether or not absorbability of feed P can be improved deserves attention (Knowlton *et al.* 2009). Environmental issues come to the forefront in swine operations because diets of swine are composed mainly of seeds or their products in which about

two-thirds of the P is present in the form of phytate-P or phytic acid (Fig. 1) (Simons *et al.* 1990). Even though phytate hydrolyzing enzymes (phytases) are found in different microorganisms, plants, and certain animals, the availability of phytase in mono-gastric animals such as pigs and poultry is minimal with little effect to improve bioavailability of phytate-P (Jongbloed *et al.* 1997; Kornegay 1996). The variability in phytate distribution and endogenous phytase activity in ingredients commonly used in swine feed is another important factor affecting the excretion of phytate bound P and its availability for the animal. For instance, the phytate-P content (g/kg) of wheat and barley which are commonly used in Manitoba diets was reported to be  $2.9 \pm 0.37$  and  $2.6 \pm 0.31$  respectively (Steiner *et al.* 2006); and the phytate-P content (g/kg) of corn commonly used in Québec was reported to be 2.05 (Selle *et al.* 2003). The endogenous phytase activity (FTU/kg) was also reported to be  $2886 \pm 645$  and  $2323 \pm 648$  for wheat and barley respectively (Steiner *et al.* 2006), and less than 50, which is the detection limit, for corn (Selle *et al.* 2003). This variation is one of the factors that determine the extent of P excretion, and any management strategy addressing the excretion of P needs to take in to account the variations in phytate-P and endogenous phytase present in the ingredients.

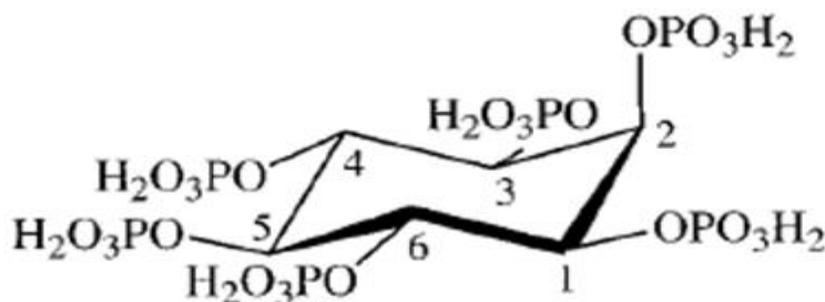


Figure 2.1. Phytic acid (myo-inositol hexakisphosphate) (Wyss *et al.* 1999).

Phytases, enzymes that catalyze the step wise hydrolysis of the phosphate group from phytic acid, thereby improving bioavailability of P and reducing excretion, have been in use for more than a decade in the swine industry (Kornegay 2001, Lassen *et al.* 2001). With the use of microbial phytase, the digestibility of P can be improved by 20% or more, resulting in little or no P supplementation for growing and pregnant sows and a reduction of P excretion by 20 to 30% (Jongbloed & Lenis 1992; Baxter *et al.* 2003). Even in ruminants, the hydrolysis of phytate P was increased by exogenous phytase and total P digestibility tended to increase (Knowlton *et al.* 2009). With the common use of phytase in swine diets, it becomes apparent that all nutrient management strategies need to take in to account the effect of phytase on reduction of nutrient outputs.

### *2.3 Nutrient management planning*

Major inputs to a swine production system include water and feed, and outputs include animal products, manure and gaseous emissions. Manure nutrients can be utilized by the plant after being applied to the soil and subsequently used to produce crops for human and animal consumption (Prince *et al.* 2000). Application of swine manure to meet N requirement of crops results in over-application of P into the soil (Sutton 1994). Prince *et al.* (2000) reported that at least 1.7 times more land is required for P requirement based application than for N, assuming no N losses in manure. However, due to the loss of N through volatilization from slurry and land application systems, applying manure to cropland based on N remaining in the manure to meet a typical corn production yields would result in 3.8 times over-application of P than the corn plant can remove. Developing systems that encourage swine producers to use the nutrients in manure and

reduce the environmental impact of manure management require a thorough understanding of the manure nutrient compositions. In this regard, mathematical models can play a valuable role in determining the amount and composition of slurry in different practical situations (Aarnink *et al.* 1992; Carter *et al.* 2003).

A mathematical model is an equation or a set of equations which describes the behaviour of a system with correspondence between the variables of the model and observable quantities and function for explanation, prediction and decision making purposes (Johnson 2001, Thornley & France 2007). A mass balance approach where animal diet and performance of animals are considered as inputs has proven to be an accurate means of predicting manure nutrient excretion (Carter *et al.* 2003). Excretion of P and N in manure is predicted more accurately with a nutritional based input-output model than the amount collected because obtaining representative samples of stored manure is difficult and sometimes impossible (Powers & Van Horn 2001). Furthermore, the variation among different swine production facilities due to climate, storage and handling practices makes such models advantageous to develop a representative management strategy.

#### *2.4 Predicting phosphorus and nitrogen output from swine operations*

Various models have been developed that describe growth, digestion and nutrient flows in pigs (Moughan *et al.* 1987; Pomar *et al.* 1991; Aarnink *et al.* 1992; Pettigrew 1997; NRC 1998; Dourmad *et al.* 2003; Halas *et al.* 2004). Fernandez *et al.* (1999b) used simple models to predict the P and N flow in weaner to grower pigs and sow production where

total loss was determined as the difference between consumption and retention. The protein digestibility in grower pigs and sows was estimated to be 80% and 85% in nursery pigs. The mean P digestibility for all pigs was assumed to be 45%. Aarnik *et al.* (1992) described retention of P by pigs of body weight range 20-110 kg as follows;

$$P_r = 0.005467W^{0.025}G \quad (2-1)$$

where  $P_r$  is retained P (kg);  $W$  is average body weight of the pig (kg); and  $G$  is average daily weight gain (kg). Nitrogen retention was determined from protein retention.

Barth (1985) and Clanton *et al.* (1988) used empirical approaches to estimate the nutrient content of animal manures. Bridges *et al.* (1995) developed the NCPIG model for the prediction of P and N excretion levels in swine waste for the body weight range of 20-100 kg and compared model prediction with observed data.

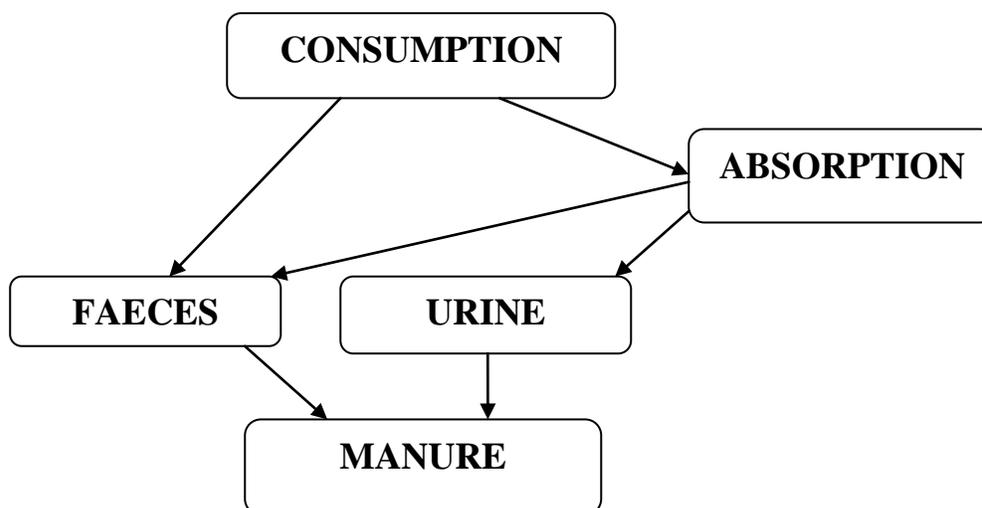


Figure 2.2. A flow diagram of mass balance of nutrients in the body of pigs.

Dourmad *et al.* (1992) also determined the N output from different categories of swine as the difference between N intake and retention, and P output was determined as the difference between intake and retention (6 g P/kg of weight gain).

Predictions of P and N flows in sows for the reproductive cycle (pregnancy, lactation) and on an annual basis was also developed by Dourmad *et al.* (1999). Total loss was obtained as the difference between intake and retention, and partitioning of total N and total P in faeces and urine was performed by assuming that protein digestibility is 83% in sows and growing pigs and 85% in weaners; and mean apparent digestibility of P in feeds was assumed to be 45% for sows and growing pigs, and 50% for weaner pigs.

A model was also developed by Dourmad *et al.* (2003) for predicting the volume and composition of effluents produced by pig farms, according to housing, feeding techniques and animal performance. Phosphorus and N excretion was calculated as the difference between intake and retention.

Carter *et al.* (2003) developed a model for the prediction of P, N and DM excretion by swine based on diet chemical composition, feed intake and nutrient retention. For the prediction of N retention in grower to finisher pigs, average daily fat-free lean gain (FFL) was corrected to a fractional FFL ( $FR_{FFL}$ ) gain using the following equation:

$$FR_{FFL} = (0.4767 + (0.02147 \text{ BW}) - (0.0002376 \text{ BW}^2) + (0.000000713 \text{ BW}^3)) \times$$

$$(\text{Avg. FFL (20-120 kg)}) \quad (2-2)$$

For the weaner pigs, extrapolation of the FFL for the grower finisher was used and the following equation was developed to estimate the  $FR_{FFL}$ :

$$= (0.1267 + (0.0347 \text{ BW})) \times (\text{Avg. FFL (20 to 120 kg)}) \quad (2-3)$$

Finally,  $FR_{FFL}$  was converted to whole body protein gain by dividing the result by 2.55. Nitrogen retention was then determined by dividing whole body protein gain by 6.25. Nitrogen excretion was predicted at 14g/d and 39.3 g/d for weanling pigs (5 – 20 kg) and grower-finisher pigs (20 – 120 kg) respectively.

For a gestating sow, N retention was determined by adding the maternal N retention and the N retention in the product of conception.

$$RN_M = (((LTG_M) / 115) \times 23) \times 16 / 10 \quad (2-4)$$

where,  $RN_M$  is the maternal N retention (g/d);  $LTG_M$  is the maternal lean tissue gain (kg).

And,

$$\text{The N retention in the product of conception (g/d)} = \text{No. of pigs} \times 0.34 \text{ g N/d} \quad (2-5)$$

Phosphorus retained by weanling pigs was calculated using the following equation,

$$= ((4.7494 \text{ BW}_F) + 1.754) - ((4.7494 \text{ BW}_I) + 1.754) \quad (2-6)$$

where  $BW_F$  = final body weight and  $BW_I$  = initial body weight.

This is then divided by the number of days in between weights to get the daily P retention. The P retention of growing-finishing pigs was determined using the following equation;

$$= ((4.7635 \text{ BW}_F) + 18.763) - ((4.7635 \text{ BW}_I) + 18.763) \quad (2-7)$$

The prediction for the P retention in the growing to finishing pigs was further corrected for the effect of N gain using the following equation;

$$= (0.2256 N_{\text{weight}}) - (0.000008 N_{\text{weight}}^2) - 0.03 \quad (2-8)$$

$$N_{\text{weight}} (\text{g}) = (\text{BW} (\% \text{ DP}_{120} - ((120\text{kg} - \text{BW}_I) \times 0.05))) \times (\% \text{FFL}_{120\text{kg}} + ((120\text{kg} - \text{BW}_I) 0.07)) / 15.94/10 \quad (2-9)$$

where  $N_{\text{weight}}$  is the N content of the carcass at a given body weight; DP is the dressing percentage and FFL is the fat free lean.

Phosphorus content at a given body weight (g) was determined using the following equation;

$$= (0.2256 \times (\text{Eq. 2-9})) - (0.000008 \times (\text{Eq. 2-9})^2) - 0.03 \quad (2-10)$$

The daily P retention is then determined by subtracting P content at the beginning from P content at the end and dividing by the number of days between weights. Therefore, the predicted P excreted in g/d from the weanling and grower to finisher pigs was 2.3 g/d and 6.7 g/d respectively.

The P retention in the gestating sows was determined by adding the P retained in sow weight gain and that retained in pigs.

$$\text{RP}_{\text{sow}} = ((3.9717 M_{\text{wt}}) + 93.039) / 115 \text{ days} \quad (2-11)$$

$$\text{RP}_{\text{pig}} = (L_{\text{wt}} \times 0.57 \times 10) / 115 \text{ days} \quad (2-12)$$

$$\text{RP}_{\text{placenta}} = (\text{Con.}_{\text{wt}} - L_{\text{wt}}) 0.08 \times 10) / 115 \text{ days} \quad (2-13)$$

where  $RP_{\text{sow}}$  is the retained P in a sow;  $M_{\text{wt}}$  is the maternal weight gain (kg);  $RP_{\text{pig}}$  is the P retained in pigs (g/d);  $L_{\text{wt}}$  is the litter birth weight (kg);  $RP_{\text{placenta}}$  is the retained P in the placenta (g/d) and  $Con_{\text{wt}}$  is the weight gain of conceptus (kg).

Different models have also been used to predict the amount of slurry production and its DM content from swine operations which include the 'O'Callaghan' model (O'Callaghan *et al.* 1971), which determined the daily faecal and urine outputs based on the percentages of feed and water intakes. Williams & Streader (1990) improved the model by adding the effect of rain water on the slurry mass and the following equation was developed to predict the slurry mass and its DM (units were in kg).

$$\text{Slurry} = ((0.602 (\text{feed} + \text{water}) - 8.95)/1000) + \text{rain-water} \quad (2-14)$$

$$\text{Slurry DM} = (9.53 ((0.602 (\text{feed} + \text{water}) - 8.95)/1000 + \text{rain-water})/100) \quad (2-15)$$

Furthermore, Williams & Streader (1990) also developed the 'Digestibility' method to predict the slurry volume and used the following equation with the assumption of 0.6 kg/day weight gain and a moisture content of 38% of that weight, a production of 0.11 kg DM in slurry per kg feed (fresh weight) and density of feed 1400 kg/m<sup>3</sup>.

$$\begin{aligned} \text{Slurry volume, m}^3/\text{day} = & \text{metered water} + \text{rainwater} - (0.6 \times 0.38 \times \text{no. of growers}) \\ & + (77 \times 10^{-6} \times \text{feed consumed, kg}) \end{aligned} \quad (2-16)$$

### 2.5 Manitoba conservation adopted model

The model adopted by Manitoba conservation predicts the excretion of P and N in manure at the time it is excreted by the animal. The model also predicts the land requirement for

spreading swine manure that was based on Québec conditions for different type of swine (e.g., grower-finisher, sows). The following equations are used to predict P, N excretions and the land base requirement, respectively.

$$P = 0.0053 W_G \quad (2-17)$$

$$N = 0.025 W_G \quad (2-18)$$

$$LBR = P_2O_5 \text{ app.} / P_2O_5 \text{ rem.} \quad (2-19)$$

where:  $W_G$  is the daily weight gain (kg); LBR is the land base required for the optimal application of manure (ac/yr/pig);  $P_2O_5 \text{ app.}$  is annual  $P_2O_5$  application (lb), and  $P_2O_5 \text{ rem.}$  is the average crop removal rate for  $P_2O_5$  (lb/ac).

Phosphorus and N retention is the same for all categories of pig production, and it is currently considered that 5.3 grams and 25 grams of P and N are retained for each kilogram of weight gain respectively (CORPEN 2003).

$$P_2O_5 \text{ rem.} = X_1 (X_{1\text{yield}} X_{1\text{rem.}}) + X_2 (X_{2\text{yield}} X_{2\text{rem.}}) + X_n (X_{n\text{yield}} X_{n\text{rem.}}) \quad (2-20)$$

where  $X_1$  is the % of cropland occupied by crop 1;  $X_{1\text{yield}}$  is the 'crop 1' yield in (bu/ac);  $X_{1\text{rem.}}$  is  $P_2O_5$  removal rate by 'crop 1' (lb  $P_2O_5$ /ac);  $X_2$  is the % cropland occupied by crop 2;  $X_{2\text{yield}}$  is the 'crop 2' yield in (bu/ac);  $X_{2\text{rem.}}$  is  $P_2O_5$  removal rate by 'crop 2' (lb  $P_2O_5$ /ac);  $X_n$  is the % of cropland occupied by crop n;  $X_{n\text{yield}}$  is the 'crop n' yield in (bu/ac);  $X_{n\text{rem.}}$  is  $P_2O_5$  removal rate by 'crop n' (lb  $P_2O_5$ /ac).

The difference in the types of ingredients used in swine feed, which is mainly corn in Québec and wheat and barley in Manitoba, as well as the types of crops planted in the fields where manure is spread warrants for an assessment of the adequacy of the model

under Manitoba conditions. The model under Québec conditions assumes that the three major crops for the determination of land base requirement for optimum manure application to be oats (40%), Grain corn (20%) and Canola (40%). However, Manitoba conditions are different where, according to Manitoba agriculture profile (2006), the major crops were spring wheat (39.9%), barley (12.6%), oats (9.8%), canola (20.2%) and alfalfa (17.5%). The model assumes a 58.3, 83.0, 64.3, 109, 90 and 171 lb P<sub>2</sub>O<sub>5</sub>/ha removal rate for spring wheat, barley, oats, grain corn and alfalfa, respectively. This difference needs to be taken in to account for a feasible application of the model in Manitoba.

## 2.6 Model evaluation

Dependability and accuracy of models is critical before they can be used with greater confidence. Such a method also provides the mechanism to evaluate if sensible reductions in dietary inputs can sufficiently balance nutrient budgets when manure nutrients exceed the amounts that can be utilized for crop production on farm (Power & Van Horn 2001). As truthfulness of a model is a difficult task to assess, usefulness of the model for its intended purpose should be the main objective of model evaluation process (Reynolds *et al.* 1981).

Typically, model evaluation is done to assess the predictive accuracy of models, compare several models, understand choices of input parameters, define the range of conditions over which a model is applicable or reliable, and characterize departures between model predicted and observed values for possible model refinement (Gauch *et al.* 2003). McCarl (1984) noted that there are no absolute criteria; evaluation relates to the

potential applications and users of the model, not the model itself, and Law & Kelton (1982) highlighted the necessity for ongoing evaluation as opposed to a one-off exercise before model usage. Use of traditional parametric test statistics requires assumptions concerning the distribution of random variables. If the distribution is assumed normal and identical for simulated and observed data, tests like a paired t-test are possible. If further the variance is independent of the set of input variables, a regression analysis between simulated and observed output can be conducted. Failure to prove a significant difference between real and model data may be due only to insufficient replication or lack of power of the applied statistical test (Mayer & Butler 1993).

This review of model evaluation will focus on linear regression analysis, mean square prediction error (MSPE) and concordance correlation coefficient (CCC) which are commonly used for model evaluation even though some limitations with their extensive use exists.

### *2.6.1 Analysis of linear regression*

A common and simple method to evaluate models is using the simple linear regression of observed vs. predicted values or vice versa. Analla (1998) stated that both observed and predicted values are random; hence, it does not matter which variable to be regressed on the other. However, this technique would result in different slope and intercept values even though the same regression coefficient would be obtained resulting in different conclusions with regard to the accuracy and precision of the model to predict the real system under consideration (Piñeiro *et al.* 2008). A plot of model predicted values on the X-axis and observed values on the Y-axis is generally the most accepted regression

analysis where slope and intercept parameters are compared to the  $Y=X$  (1:1) line (Mayer & Butler 1993; Mayer *et al.* 1994; Tedeschi 2006). Dent & Blackie (1979) proposed testing for these two values simultaneously with an appropriate F statistic. If the model is accurate, the F will be small and the null hypothesis that the slope is one and the intercept is zero will not be rejected. Validity of the F test for evaluating predictive models have been focused on the possible bias in parameter estimates of the slope and intercept (Harrison 1990), or in situations where the errors are auto-correlated (Mayer *et al.* 1994). However, the fact that the F test of zero intercept and unity slope do not account for random error magnitudes during the evaluation of a predictive model especially under the circumstances that a choice needs to be made between two models and the denominator of this test being an expression of the lack of fit of the model being evaluated could create limitations in the use of F test for this purpose. The significance of this test would be inversely proportional to the goodness of fit of the model being evaluated. Under these conditions, the probability of accepting an inefficient model increases (Analla 1998).

Linear regression of observations from the real system on model predictions has been suggested as an inadequate procedure for evaluating predictive models. One of the reasons behind this argument is that in regression analysis, the null hypothesis test is ambiguous because it does not allow distinction between acceptable fit and highly variable data (Harrison 1990; Reckhow *et al.* 1990); i.e., the more scattered the data points, the greater the standard error of the slope. Computed value of the test statistic would also be smaller making the null hypothesis hard to reject, either due to the fact that the slope of the regression line being not significantly different from unity or due to the higher scatter around the line (Mitchell 1997).

Plotting of deviations vs. predicted values can show the uniformity of model performance making it easy to understand the model adequacy where an envelope (e.g. 95% analogous to confidence limits used in statistical analysis experiments) of acceptable precision and the points that must lie within the envelope can greatly assist in declaring a model useful. Such a method provides objectivity in using linear regression as a tool for the assessment of adequacy of mathematical models (Mitchell 1997). In such a case, the residuals are not correlated with the predictions, and the slope of residuals ( $e_i$ ) regressed on predicted values ( $Y_{pi}$ ) is zero if the model is unbiased. A positive or negative slope of  $e_i$  on  $Y_{pi}$  is a test of biased prediction. This is why the residuals should be plotted against the predicted values and not the observed values (St. Pierre 2003). The slope of the regression is an estimate of the linear prediction bias and a t-test is used to assess its significance.

### *2.6.2 Mean Square Prediction Error*

As the concern with model evaluation lies more on the comparison of model predicted and observed values rather than the functional relationship between the two, linear regression has been suggested as an inadequate technique, and the direct comparison of the deviation of the model output from the measurements have been accepted as a more relevant criterion (Kobayashi & Salam 2000). When each pair of data (predicted and observed) are mutually independent, and the model is independent in such a way that the parameters of the model were derived from independent experiments and were not adjusted to the current experiment being predicted, the MSPE estimate is a reliable measure of model accuracy (Kobayashi & Salam 2000; Tedeschi 2006).

$$\text{MSPE} = \sum_{i=1}^n (P_i - O_i)^2 / n \quad (2-21)$$

Where  $P_i$  is predicted value and  $O_i$  is observed value.

$$\text{MSPE} = (P_m - O_m)^2 + (SD_p - SD_o)^2 + 2SD_p SD_o (1 - r) \quad (2-22)$$

where  $P_m$  is the predicted mean,  $O_m$  is observed mean,  $SD_p$  is standard deviation of predicted values,  $SD_o$  is standard deviation of observed values,  $r$  is correlation coefficient.

The first term explains the mean bias and the second term explains the difference in the magnitudes of fluctuation between the observed and predicted values due to the difference in their respective standard deviations, and the third term explaining the lack of positive correlation weighted by the standard deviations.

Finally, MSPE can be expressed as:

$$\text{MSPE} = (P_m - O_m)^2 + (S_p - rS_o)^2 + (1 - r^2) S_o^2 \quad (2-23)$$

The three terms in (2-18) can give more interpretable results than the total error or regression analysis results. They are error in central tendency (ECT) that measures the mean bias, error due to regression (ER) (or mean shift) and random errors (ED) (or disturbance) for the first, second and third terms respectively (Bibby & Toutenburg 1977; Tedeschi 2006; Kebreab *et al.* 2008).

### 2.6.3 Concordance correlation coefficient

The concordance correlation coefficient (CCC), also known as reproducibility index has been suggested as the measure of accuracy and precision of model predictions (Lin 1989; Tedeschi 2006). Accuracy measures how closely model-predicted values are to the true values. Precision measures how closely individual model-predicted values are within each other. In other words, accuracy is the model's ability to predict the right values and precision is the ability of the model to predict similar values consistently. Inaccuracy shows model bias and imprecision shows uncertainty. The following equation is used to describe the CCC.

$$CCC = 2\sigma_{12} / (\sigma_1 + \sigma_2 + (\mu_1 - \mu_2)^2) = r * C_b \quad (2-25)$$

$$C_b = [(v + 1/v + \mu^2) / 2]^{-1}$$

$$v = \sigma_1 / \sigma_2$$

$$u = (\mu_1 - \mu_2) / (\sigma_1\sigma_2)^{-1/2}$$

where  $r$  is a measure of precision, the fraction of the variation about the mean that is explained by a model. The higher the value, ( $0 < r^2 < 1$ ) the better is the prediction by the model;  $C_b$  is bias correction factor that measures how far the best fit line deviates from the line of unity;  $\mu_1$  is mean of observed values;  $\mu_2$  is mean of predicted values;  $v$  is scale shift and  $u$  is location shift relative to scale (i.e. difference of the means relative to the square root of the products of two standard deviations), where positive values of 'u' show under-prediction and negative values show over-prediction of model (Lin 1989; Tedeschi 2006; Kebreab, *et al.* 2008).

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### 3.0 Nutrient flow in swine fed typical and phytase amended diets

#### 3.1 SUMMARY

Two experiments with nursery to finisher pigs and sows were conducted to determine the effect of phytase on the performance of pigs and the excretion of P in faeces and urine. The nursery to finisher pig study used 20 pigs with 10 pigs per treatment, and was divided in to five phases according to requirement of animals for P. The pigs' average initial body weight was 6.5 kg with phase I: 6.5-14 kg BW, phase II: 15-25 kg BW, phase III: 25-50 kg BW, phase IV: 50-80 kg BW and phase V: 80-110 kg BW. In the sow experiment, three groups of six sows (three sows per treatment) with a total of 9 sows per treatment were used. Dietary treatments in all phases were (1) sufficient P level in the diet according to NRC recommendations (No-phytase) and (2) a 0.1 percentage unit P reduced in the No-phytase diet and amended with microbial phytase at 500 FTU/kg (Phytase). Across all the nursery to finisher phases, there was no significant difference in the performance parameters measured with regard to average daily feed intake (ADFI) ( $P > 0.25$ ), average daily gain (ADG) ( $P > 0.173$ ) and feed conversion ratio (FCR) ( $P > 0.084$ ), between the No-phytase and Phytase treatment diets. Faecal P was significantly reduced with the use of phytase in all nursery to finisher pigs ( $P < 0.045$ ). Mixed results were observed with regard to urine P where phytase supplementation non significant reduction of P (phase II:  $P = 0.131$ ) and a significant increase in urinary P excretion (phase I:  $P = 0.003$ ; phase III:  $P = 0.032$  and phase V:  $P < 0.0001$ ). Total P excretion was significantly lowered with the addition of phytase to the diet in all the phases except phase IV ( $P = 0.124$ ). Phytase

amendment in sows did not result in a significantly different P excretion in both the faeces ( $P=0.363$ ) and urine ( $P=0.866$ ). Phytase amendment did not affect the N excretion in any phase or type of pigs. In conclusion, phytase amended diet showed a positive effect on the mitigation of P excretion, without a significant difference on performance of pigs between the two treatments in all categories.

### 3.2 INTRODUCTION

Swine diets are composed mainly of seeds (cereal grains) or their products (oil seed meal and grain by products) (Simons *et al.* 1990). Although these types of diets contain an important proportion of P, most of this P is in the form of phytate-P which is almost indigestible to the pig resulting in a higher proportion being excreted. The indigestibility of phytate-P forces feed manufacturers and farmers to add inorganic P to the diet in order to meet the requirements of the animal. Microbial phytase which catalyzes the hydrolysis of phytic acid in the upper digestive tract, liberating free orthophosphates and inositol, has been shown to improve the digestibility of phytate-P (Joengbloed 1998). As a result, more of the P from cereal grains is available to the animal and less is excreted. Moreover, the necessity for the addition of inorganic P is minimized resulting in a reduced cost of diet. With the use of microbial phytase, the digestibility of P can be improved by 20% or more resulting in little or no supplementation for growing and pregnant sows' diet with inorganic P (Baxter *et al.* 2003). The same level of microbial phytase also reduced the total P excreted by 33% when phytase was added to a low P diet (Kornegay & Verstegen 2001). Other studies showed that supplemental phytase resulted in a 25-30% increase in P utilization and 40% decrease in P excretion (Cromwell *et al.* 1995). Hahn *et al.* (1997)

showed that overall average daily gains of pigs fed a basal diet supplemented with microbial phytase, cereal phytase or inorganic P were similar, and were approximately 33% greater than that of the pigs fed only the control diet. Therefore, the objectives of the experiment were to (1) investigate the nutrient flow in different types of pigs fed 'typical' and P reduced, phytase amended diets and (2) use the data collected to evaluate an extant model that predicts P excretion and land requirement for spreading swine manure.

### 3.3 MATERIALS AND METHODS

#### 3.3.1 *Experimental diets*

Starter-finisher pigs were divided in to five phases and fed according to their nutrient requirements (NRC 1998). Two diets, a positive control (No-phytase) and a negative control (Phytase), based on barley-corn-wheat-soybean meal were formulated (Table 1). The No-phytase diet was formulated to contain P level according to NRC recommendations, and the Phytase diet was formulated with the P in the No-phytase diet reduced by 0.1percentage units and diet supplemented with the enzyme phytase at 500 FTU/kg. Calcium level was lowered in the Phytase diet to minimize its antagonistic effect on the efficiency of phytase where calcium is believed to form an aggregate with the enzyme (Qian *et al.* 1996, Liu *et al.* 1998). All other nutrients were formulated to meet the requirements recommended by NRC (1998). Ferric oxide was used as a dietary marker to identify the start and end of a total faecal and urine collection.

### 3.3.2 *Animal experiment and sample collection*

Twenty piglets (average starting and finishing weight of 6.5 kg and 110 kg respectively) were housed in metabolism cages for ease of faeces and urine collection, and were randomly assigned to the two treatment diets at the T. K. Cheung Centre for Animal Science Research, University of Manitoba with ten pigs per dietary treatment. Pigs were given ad libitum access to feed and water. Daily feed consumption, water consumption and weekly body weight were monitored for the entire period for individual pigs. The experimental period consisted of ten days of adaptation followed by five days collection of faeces and urine from individual cages. Urine was collected using a jug via a funnel below the metabolism cage. Ten mL of 6N HCl was added to capture the possible loss of N in the form of ammonia gas. Faeces were collected in plastic bags. Phosphorus and N content in manure of individual pigs were determined by summing nutrient contents in both the faeces and urine. Samples of manure were taken and frozen for further analysis and soil incubation trials. Pigs were moved to floor pens for exercise when the collection period was over.

In the sow trial, six non-pregnant sows were blocked to two treatment diets with three sows per treatment. The experiment was divided into three periods. Sows were randomly assigned to the two diets that were formulated in a similar way to nursery-finisher pigs (Table 3.2). The animals were fed for eight days of adaptation period before the collection period started. After the eighth day, pigs were fitted with urinary catheters for a total urine collection of five days. During collection days, feed consumption, total faecal excretion, water consumption, water spillage and room temperature were recorded. After taking samples of urine and faeces for laboratory analysis of P and N, the remaining

samples were pooled along with the water spillage to make manure in a drum container for the determination of manure volume. Phosphorus in faeces and urine were summed to determine the total output of the nutrients in the manure from each animal. The same was done for N to determine total output in the manure of each animal. Body weight of sows was measured at start and end of collection days.

All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Protocol Management and Review Committee, and pigs were handled according to the guidelines described by the Canadian Council on Animal Care (CCAC 1993).

### *3.3.3 Sample preparation and chemical analysis*

Faecal and feed samples were oven dried and ground to pass through a 1 mm screen and thoroughly mixed and sent to Central Testing Laboratory Ltd. (Winnipeg, MB. Canada) along with urine samples for the determination of total P (AOAC 923.03) and N (Leco Version 2.2). Dry matter was determined according to AOAC 934.01 (AOAC 1990). Nutrient content in the manure was obtained by adding the nutrient content in the faeces and urine of individual pigs.

### *3.3.4 Statistical analysis*

Data was subjected to ANOVA using the MIXED procedure of SAS (SAS, 2002). All tests were considered significant at  $P < 0.05$ . Shapiro-Wilk's W (Shapiro & Wilk 1965) test was used to ensure the normal distribution of data.

Table 3.1: *Composition of diets (% , as-fed basis) of nursery to finisher pigs used in the study*<sup>1</sup>

Ingredient	Phase I		Phase II		Phase III		Phase IV		Phase V	
	No-phytase	Phytase								
Barley	22.5	23.0	24.8	24.9	20.5	22.0	23.8	24.1	10.5	11.5
Corn	12.1	12.2	11.5	11.6	19.5	20.0	25.5	25.4	59.8	60.0
Wheat HRW	21.5	24.1	24.8	24.8	20.7	20.4	23.9	23.9	10.0	10.1
Canola meal	11.0	6.10	10.0	10.0	5.00	5.00	4.00	4.00	4.30	2.00
SBM 48%	20.4	23.1	22.2	22.7	16.6	16.3	10.5	10.7	8.00	9.40
SDPP	3.40	3.40	-	-	-	-	-	-	-	-
Vegetable oil	5.40	5.00	3.20	3.00	2.80	2.60	2.70	2.50	1.00	1.00
Limestone	1.20	0.90	0.84	0.82	0.81	0.78	0.53	0.62	0.61	0.55
Biophose	0.47	0.09	0.88	0.39	0.60	0.10	0.49	-	0.42	-
Iodized Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vit-Min Premix	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lys-HCl	0.42	0.43	0.26	0.25	0.20	0.22	0.17	0.17	0.12	0.12
DL-Methionine	0.10	0.11	0.07	0.07	0.05	0.05	0.05	0.05	-	-
Threonine	0.27	0.27	0.18	0.17	0.15	0.15	0.15	0.15	0.04	0.05
Peas	-	-	-	-	11.9	11.1	7.00	7.30	-	-
Oats	-	-	-	-	-	-	-	-	4.00	4.00
Phytase, FTU/kg	-	500	-	500	-	500	-	500	-	500
Calculated composition of diet*										
ME, kcal/kg	3271	3265	3265	3271	3271	3270	3269	3271	3276	3288
CP, %	22.6	22.5	21.0	21.3	18.9	18.8	15.8	16.0	13.2	13.2
Ca, %	0.75	0.57	0.70	0.61	0.60	0.51	0.45	0.40	0.45	0.35
tP, %	0.63	0.53	0.60	0.50	0.50	0.40	0.45	0.35	0.40	0.30
Av. P, %	0.22	0.14	0.31	0.21	0.22	0.12	0.20	0.10	0.16	0.07
Lysine, %	1.50	1.50	1.30	1.30	1.20	1.20	0.91	0.92	0.69	0.69
Tryptophan, %	0.25	0.25	0.28	0.29	0.23	0.24	0.19	0.19	0.14	0.16
Determined composition of diet										
tP, %	0.65	0.49	0.65	0.55	0.44	0.41	0.47	0.41	0.48	0.39
CP, %	23.9	22.2	23.2	22.0	19.1	19.0	19.6	18.8	15.1	13.9

FTU= phytase units

\*ME = metabolizable energy; tP = total phosphorus; Av. P = available phosphorus; CP = crude protein.

<sup>1</sup>No-phytase diet was formulated to meet the pigs' P requirement and the Phytase diet had reduced dietary P and supplemented with microbial phytase.

Table 3.2: *Composition of sow experimental diets used in the study*<sup>1</sup>

Ingredients	No-phytase	Phytase
Barley	15.1	16.3
Wheat HRW	44.9	44.9
Canola meal	15.1	15.1
SBM 48%	14.1	14.1
Veg. oil	4.80	4.40
Limestone	0.98	0.69
Biophose	0.80	0.30
Iodized Salt	0.25	0.25
Vit-Min Premix	3.50	3.50
Lys-HCl	0.20	0.20
Phytase, FTU/kg	-	500
Calculated nutrient content in the diet*		
ME, kcal/kg	3265	3265
CP, %	19.7	19.8
Ca, %	0.75	0.56
tP, %	0.60	0.50
Av. P, %	0.32	0.22
Lysine, %	1.20	1.20
Determined nutrient content of diet:		
tP, %	0.60	0.51
CP, %	19.9	20.9

FTU= phytase units

\*ME = metabolizable energy; tP = total phosphorus; Av. P = available phosphorus; CP = crude protein.

<sup>1</sup>No-phytase diet was formulated to meet the pigs' P requirement and the Phytase diet had reduced dietary P and supplemented with microbial phytase.

### 3.4 RESULTS

Results of performance showed that the Phytase diet was not significantly different from the No-phytase diet in regard to the average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) in all the phases of nursery to finisher pigs (Table 3.3). The P content per kg of body weight calculated as the retention of P to the ADG was variable across the phases, with a range of 4.21 to 7.49 g/kg (Table 3.3). However, the average for the starter to finisher phases of production was 5.77 g/kg, which is close to the 5.30 g/kg estimated for the model adopted by Manitoba Conservation. Performance parameter values were not significantly different between the two treatments; therefore, a detailed description of the results is not given.

There was a significantly reduced P intake in the Phytase treatment in all phases ( $P < 0.05$ ) except in phase III ( $P = 0.318$ ) (Table 3.4). A mixed result was observed with regards to the retention of P for the treatments in all the phases. There was no significant difference in retention of P in all the phases ( $P > 0.05$ ) except in phase IV, where a 21.8% ( $P = 0.009$ ) reduction was observed instead. In all the phases No-phytase diet resulted in a reduction of P in faeces. In the nursery stage of production, a 30% significant reduction in the amount of P in faeces was observed ( $P = 0.001$ ). However, a significant 400% increase in the urinary P was observed when phytase was added to the P-deficient diet ( $P = 0.003$ ) (Table 3.4). A mixed result was observed in the grower stage. In phase II, a 42% reduction in the urinary P was observed ( $P = 0.131$ ) and in phase III a 140% increase in the urine P was observed ( $P = 0.032$ ) (Table 3.4). A 25-29% decrease in the faecal P was observed in the grower phase ( $P = 0.045$  and  $0.018$  in phases II and III, respectively). In the case of finisher pigs, phytase resulted in a 24-31% decrease in faecal P ( $P = 0.028$  and

0.001 in phases IV and V, respectively). Phytase supplementation to finisher pigs resulted in a 43% ( $P = 0.143$ ) increase and a 68% ( $P < 0.0001$ ) decrease in urine P in phases IV and V, respectively, compared to the No-phytase diet. There was a 24% and 30% reduction in the fecal P in phases IV and V, respectively (Table 3.4). There was a significant reduction in total P in all phases of the starter to finisher phases with a 21, 30, 22, 17 and 40% reduction in the total P in the phases I, II, III, IV and V, respectively, as a result of amendment of phytase to the P deficient diet (Table 3.4).

In sows, there was no significant difference observed in feed intake and N intake ( $P = 0.685$  and  $0.460$ , respectively). However, a significant difference in P intake was observed ( $P = 0.013$ ). Phytase amendment to a P reduced diet did not show a significant reduction in P excreted in the faeces ( $P = 0.363$ ) and urine ( $P = 0.866$ ). Total P output was 66.7% and 69.1% of the total P intake in the No-phytase and Phytase diets, respectively ; and showed no significant difference between the two dietary treatments ( $P = 0.566$ ). These values were 71.6% and 61.3% for the excretion of N. Furthermore, there was no significantly reduced N excretion as result of phytase amendment to a P reduced diet ( $P = 0.420$ ) (Table 3.5).

In all the performance trials with starter to finisher pigs and sows, phytase supplementation did not result in a significant reduction/increase in the excretion of N in the faeces and urine, even though in all the faecal excretions numerical reduction was observed with Phytase diet.

Table 3.3. Performance of started to finisher pigs fed standard and phytase amended diets

Phase	Treatment	ADFI <sup>1</sup>	ADG <sup>2</sup>	Feed: Gain	P
		----- kg/d -----			g/kg <sup>3</sup>
I (6.5-14 kg)	No-phytase	0.53	0.38	1.39	6.56
	Phytase	0.50	0.33	1.51	5.74
	SED <sup>4</sup>	0.024	0.023	0.026	0.492
	<i>P</i> (No-phytase vs. Phytase)	0.546	0.190	0.084	0.675
II (15-25 kg)	No-phytase	0.79	0.55	1.44	4.21
	Phytase	0.65	0.56	1.16	4.38
	SED	0.023	0.026	0.063	0.417
	<i>P</i> (No-phytase vs. Phytase)	0.773	0.817	0.743	0.876
III (25-50 kg)	No-phytase	1.65	0.73	2.28	5.13
	Phytase	1.47	0.80	2.13	5.07
	SED	0.074	0.036	0.116	0.307
	<i>P</i> (No-phytase vs. Phytase)	0.917	0.173	0.266	0.958
IV (50-80 kg)	No-phytase	2.25	0.69	2.95	7.45
	Phytase	1.96	0.82	2.69	4.91
	SED	0.087	0.086	0.035	0.789
	<i>P</i> (No-phytase vs. Phytase)	0.257	0.309	0.44	0.102
V (80-110 kg)	No-phytase	2.6	0.74	3.52	6.77
	Phytase	2.25	0.72	3.52	7.49
	SED	0.087	0.082	0.049	0.745
	<i>P</i> (No-phytase vs. Phytase)	0.519	0.906	0.812	0.337

<sup>1</sup>ADFI = Average daily feed intake; <sup>2</sup>ADG = Average daily gain; <sup>3</sup>g/kg = g P/kg body

weight; <sup>4</sup>SED = Standard error of the difference of the two means

Table 3.4. Phosphorus and nitrogen intake, retention and fecal, urine and manure excretion from starter to finisher pigs

Phase	Treatment	Intake		Retained		Phosphorus		Nitrogen		Total excretion	
		P	N	P	N	Faecal	Urine	Faecal	Urine	P	N
----- g/d -----											
I (6.5-14 kg)	No-phytase	3.46	20.4	2.47	14.7	0.97	0.02	2.83	2.91	0.99	5.74
	Phytase	2.68	19.7	1.90	13.8	0.68	0.10	2.66	3.21	0.78	5.87
	SED <sup>1</sup>	0.139	0.886	0.102	0.585	0.051	0.006	0.217	0.422	0.028	0.493
	<i>P</i> (No-phytase vs. Phytase)	0.001	0.551	0.001	0.189	0.001	0.003	0.589	0.624	0.019	0.854
II (15-25 kg)	No-phytase	5.12	29.5	2.32	15.3	2.60	0.19	6.54	7.66	2.79	14.2
	Phytase	4.42	28.3	2.45	14.3	1.85	0.11	5.03	8.25	1.96	14.0
	SED	0.143	1.248	0.212	0.675	0.029	0.012	0.644	1.142	0.051	1.685
	<i>P</i> (No-phytase vs. Phytase)	0.003	0.167	0.684	0.814	0.045	0.131	0.115	0.718	0.032	0.866
III (25-50 kg)	No-phytase	7.20	50.7	3.72	25.1	3.42	0.05	8.18	17.3	3.47	25.5
	Phytase	6.75	50.6	4.05	22.9	2.58	0.12	7.08	20.7	2.70	27.8
	SED	0.312	2.248	0.151	0.906	0.229	0.008	0.345	1.66	0.234	1.911
	<i>P</i> (No-phytase vs. Phytase)	0.318	0.173	0.139	0.018	0.018	0.032	0.038	0.168	0.031	0.409
IV (50-80 kg)	No-phytase	10.6	70.6	5.13	22.2	4.90	0.56	11.3	37.1	5.46	48.4
	Phytase	8.55	63.6	4.01	16.8	3.73	0.80	9.54	37.3	4.53	46.8
	SED	0.386	2.674	0.269	2.155	0.345	0.028	0.835	2.248	0.030	2.609
	<i>P</i> (No-phytase vs. Phytase)	0.002	0.084	0.009	0.028	0.028	0.143	0.167	0.956	0.124	0.684
V (80-110 kg)	No-phytase	12.3	63.0	4.98	23.8	5.52	1.82	9.00	30.2	7.34	39.2
	Phytase	9.81	56.3	5.41	22.0	3.83	0.58	8.11	26.2	4.41	34.3
	SED	0.368	2.005	0.363	2.071	0.316	0.033	0.61	2.485	0.025	2.719
	<i>P</i> (No-phytase vs. Phytase)	0.000	0.029	0.418	0.904	0.001	<0.001	0.316	0.263	<0.001	0.215

<sup>1</sup>SED = Standard error of the difference of the two means

Table 3.5: *Excretion of P and N in the faeces and urine of sows from diets supplemented with microbial phytase*

Phase	Treatment	Intake			FCR <sup>1</sup>	Retention		Excretion					
		Feed	P	N		P	N	P			N		
		Kg/d	--- (g/d) ---			--- (g/d) ---		----- (g/d) -----			----- (g/d) -----		
								Faecal	Urine	Total	Faecal	Urine	Total
Sows	No-phytase	2.79	16.4	87.1	3.33	1.98	9.33	8.76	2.17	10.9	9.46	52.9	60.8
	Phytase	2.88	14.8	92.9	6.04	2.19	10.3	7.86	2.36	10.2	9.36	47.6	55.5
SED <sup>2</sup>		0.084	0.182	6.404	0.262	0.153	0.153	1.163	0.197	0.849	1.287	6.891	4.519
<i>P</i> (No-phytase vs. Phytase)		0.685	0.013	0.460	0.188	0.183	0.186	0.522	0.444	0.566	0.944	0.526	0.420

<sup>1</sup>FCR = Feed conversion ratio

<sup>2</sup>SED = Standard error of the difference of the two means

### 3.5 DISCUSSION

The effect of phytase on the digestibility of P via hydrolysis of phytate-P and hence a reduced concentration in the manure of swine is well documented. Addition of phytase was able to ameliorate the potential negative effect of a reduced P formulated in the diet in this experiment. Variable retention of P in g/kg of body weight was observed in the range of 4.21 to 7.49 g/kg. Different studies have reported variable rates of retention per kg of weight gain. For instance, Han *et al.* (1997) reported a 7.02 g P/kg for finisher pigs; O'Quinn *et al.* (1997) reported a 4.16 g P/kg for starter pigs and Harper *et al.* (1997) reported a 4.25 g P/kg for grower pigs. The average retention of P was 5.77 g/kg for a pig from starter to the finisher production, which was not different from the model assumption of 5.3 g/kg. This variability in g P/kg body weight shows the requirement of phase specific nutritional management for the excretion of P.

Phytase resulted in a reduced faecal P being excreted in the nursery to finisher pigs. These observations were consistent with other studies where phytase had a similar effect on the faecal P (Jongbloed *et al.* 1992; Lei *et al.* 1993). Similar observations were reported in the study of Beaulieu *et al.* (2007), where addition of phytase at 500 FTU/kg resulted in similar ADG, ADFI and FCR with the positive control where inorganic P was added to meet the requirement of the nursery pigs. In the same study, phytase resulted in a significantly reduced P output in both the faeces and urine in nursery pigs which is in agreement with the current study. In another study, when phytase was added to a diet at 500 FTU/kg, there was no significant difference observed in the bone ash of pigs receiving sufficient P and deficient P with supplemental phytase (Braña *et al.* 2006), suggesting that phytase prevented the negative impact that would have resulted from a

deficiency of P in the diet for nursery pigs. Ketaren *et al.* (1993) reported that phytase supplementation had no effect on feed intake but increased live weight gain, decreased FCR and increased protein retention, energy retention and daily protein deposition.

Phytase resulted in a reduced P in the faeces and higher P content in the urine of grower pigs. Other studies have also shown similar results. In the study of Braña *et al.* (2006), addition of phytase at 500 FTU/kg to grower pigs resulted in improved ADG and bone ash compared to a negative control where P was deficient, but was not significantly different to the positive control where P was supplied at a requirement level. Other reports have also shown similar results with regard to the effect of phytase on the performance of grower pigs where phytase was able to restore ADG and ADFI to the levels of the positive control (Harper *et al.* 1997). A variable average g P/kg body weight was estimated in this experiment where a range of 4.21 g P/kg body weight (grower phase) to a 7.49 g P/kg body weight (finisher phase) was observed. Similar results have been reported in other studies; Han *et al.* (1997) reported a 7.02 g P/kg for finisher pigs; O'Quinn *et al.* (1997) reported a 4.16 g P/kg for starter pigs and Harper *et al.* (1997) reported a 4.25 g P/kg for grower pigs. Emiola *et al.* (2009) showed that supplementation of phytase at 500 FTU/kg to grower pigs resulted in a 27% reduction in faecal P excretion. The reduction in faecal P was 44% when phytase was supplemented at a 1000 FTU/kg level in the same study. However, mixed results are reported on the effect of phytase on urinary P excretion. Some studies showed that addition of phytase to grower diets resulted in a reduced urine P ranging from a 40 to 94% reduction (Brady *et al.* 2002; Emiola *et al.* 2009), while others showed that addition of phytase resulted in increased urine P excretion (Han *et al.* 1997; Pomar *et al.* 2008). Harper *et al.* (1997) also observed a 22-27% reduction of P excreted

when dietary P level was reduced and the diet supplemented with phytase. However, contribution of urinary P to the total P output was marginal.

As with the growing pigs, phytase had a similar effect on the performance as well as on the excretion of P in the faeces and urine of finisher pigs. In this phase, there was no significant difference in the performance of animals among the two treatment diets. Faecal P was reduced and urine P increased as a result of phytase supplementation. Other studies have also shown similar results where addition of phytase to a diet with a deficient P level had a similar effect with a diet formulated to contain a sufficient P level as per the requirement of the finisher pigs (For e.g. Cromwell *et al.* 1993). O'Quinn *et al.* (1997) reported that addition of phytase to P deficient diet resulted in a 30% reduction in faecal P, and a 460% increase in urine P in finisher pigs. This observation is consistent with the current study where faecal P was reduced and urine P increased as a result of addition of phytase to P-deficient diet. The same study showed that performance of pigs fed with 500 FTU amended diet was not significantly difference from pigs receiving sufficient P level in the diets. In all the phases, addition of phytase resulted in a reduced total P excreted from the pigs confirming the results that were reported in other studies showing that phytase helps in preventing P loading in the environment.

There was no phytase effect on the excretion of N observed with all the phases in this study. The effect of phytase on digestibility of CP is inconsistent. The observations of this study are in agreement with other studies in relation to the effect of phytase on N excretion. Some studies reported that phytase supplementation had no effect on the digestibility of CP and amino acids (e.g., Fan *et al.* 2005; Liao *et al.* 2005a; Liao *et al.*

2005b; Kies *et al.* 2005). Ketaren *et al.* (1993) observed that the faecal digestibility of CP was unaffected by supplementing a semi-purified SBM diet even at 1,000 FTU/kg of diet.

However, some studies have reported a positive effect of phytase on the retention of N and hence its excretion. Mroz *et al.* (1992) for instance reported that addition of phytase improved the retention of N, Ca, and P with a daily diminished excretion of 5.5, 2.2 and 1.9 g/d respectively. The mode of action of phytase on CP digestibility has been suggested to be the prevention of the formation of protein-phytate complexes mainly under low pH conditions in the gut resulting in improved protein digestibility (Kies *et al.* 2006). In the current study, diets were formulated to contain sufficient amount of CP, and more studies with sub-optimal levels of CP and phytase supplementation are required for more assessment of the effect of phytase on N utilization.

In sows, phytase did not show any effect on the reduction of P excreted both in the faeces and urine. Other studies, however, reported the opposite where total P content in the faeces of sows fed the phytase diet decreased by 27.2% compared to those fed a standard diet (Baidoo *et al.* 2003) suggesting an improved digestibility of P, even though the study was conducted on lactating sows. Kemme *et al.* (1997) observed that the efficacy of phytase in improving digestibility of P decreased in the order of lactating sows, growing-finishing pigs, sows at the end of pregnancy, piglets, and sows at mid pregnancy. As the sows used in this study were neither pregnant nor lactating, observations of Kemme *et al.* (1997) might explain the absence of any effect of phytase in reducing excretion of P in the manure of sows in this trial.

### 3.6 CONCLUSION

Phytase supplementation to starter – finisher pigs' diet resulted in a reduced total P excretion in the manure. Faecal P which is the major component of manure from these categories of animals was significantly reduced. However, urine P was increased in some cases as a result of phytase supplementation. Supplementation of a P deficient diet with microbial phytase did not result in significantly different performance of animals, i.e., ADFI, ADG and FCR, in all phases of production. In the sow study, dietary phytase supplementation did not result in a reduced faecal and urine P excretion. Phytase did not have any effect on the excretion of N in the manure of starter-finisher pigs as well as sows.

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## 4.0 Evaluation of an extant model for the excretion of phosphorus and nitrogen from starter to finisher pigs

### 4.1 SUMMARY

Mathematical models can be useful tools in nutrient management planning (NMP) for swine operations to assess nutrient outputs and design on farm mitigation options. Data from twenty pigs (average starting weight of 6.5 kg and finishing weight of 110 kg) was used to assess the adequacy of an extant model for the excretion of P and N from pigs in the starter to finisher phases of production. Two dietary treatments were used with ten pigs per treatment. Dietary treatments were a control diet (No-phytase) with P level in the diet formulated to meet the requirement of pigs and a phytase diet (Phytase) where the level of P in No-phytase diet was reduced by an average of 0.1 percentage units and diet supplemented with microbial phytase. Model predictions were assessed using different statistical methods which included regression analysis of observed on predicted, partitioning of mean square prediction error (MSPE) into error due to central tendency (ECT), error due to regression (ER) and error due to deviation (ED), and concordance correlation coefficient (CCC). Model prediction for the excretion of P in the starter phase was more satisfactory for the No-phytase ( $r^2=0.48$ ,  $P < 0.05$ ) than the Phytase ( $r^2=0.20$ ;  $P > 0.05$ ) diet. The random variation or ED (82.8%) was the major contributor towards the total MSPE in the No-phytase diet, and the ER (80.4%) in the Phytase diet. CCC analysis showed that the model was fairly accurate and precise in the starter phase. The model tended to under-predict the N excretion in the manure by 21.0% and 15.8% in the No-

phytase and Phytase diets, respectively ( $\mu = 0.88$  and  $0.64$  respectively). Most of the errors associated with the MSPE in the grower phase were due to ED in the No-phytase (58% and 67.4% in phases II and III, respectively) and Phytase diets (51.5% in phase II). However, ER was the highest in the Phytase diet of phase III (ER =64%). Predictions were precise but not accurate in phase II for both diets, and were highly precise and accurate in phase III for both treatments. The ECT, ER and ED were 23.6%, 23.6% and 52.8%, respectively for the No-phytase diet, and 49.7%, 45.1% and 5.20%, respectively for Phytase diet in the finisher phase of production. The model predicted well the land base requirement for the spreading of manure in all phases and both dietary treatments. Generally, the assessment of model adequacy showed that the model can be used to predict the excretion of P in manure of starter to finisher pigs satisfactorily, but not the N excretion when standard diets are used. The model failed to predict excretion of both P and N when phytase was supplemented to diet. Furthermore, the model was able to show the amount of reduced land base requirement for the spread of manure as a result of supplementation of phytase to diets of swine in all categories.

## 4.2 INTRODUCTION

Environmental issues are considered to be of great concern for the sustainability of swine operations as public concern about environmental pollution from intensive swine production is increasing. Major concerns of pressure on the environment from swine operations include the load of excess nutrients, mainly P and N, which affect soil, water and air surrounding swine facilities (Fernández *et al.* 1999b, Nahm 2002). Various

nutritional strategies have been shown to have a positive mitigation effect on the excretion of nutrients from swine which include the supplementation of microbial phytase to monogastric diets to improve bioavailability of P thereby reducing excretion (Joengbloed 1998). Understanding outputs of P and N from swine operations is important in implementing management strategies for the mitigation of pressures on the environment. Since obtaining representative samples of stored manure is difficult and sometimes impossible, mathematical models can be reliable tools in predicting the amount of manure nutrients such as P and N excreted from swine (Powers & Van Horn 2001), provided that accurate manure analysis in a particular facility is not feasible. Furthermore, the variation among different swine production facilities with climate, storage and handling practices makes such models advantageous to develop a widely applicable management strategy. However, dependability and validity of models needs to be assessed critically before they can be used with greater confidence for their intended purposes (Reynolds *et al.* 1981). Model evaluation also provides the mechanism to assess if sensible reductions in dietary inputs and various mitigation options such as addition of enzymes can sufficiently balance nutrient budgets when manure nutrients exceed the amounts that can be utilized for crop production on farm (Powers & Van Horn 2001). Different evaluation techniques can be used to evaluate prediction models. These methods include and are not limited to regression analysis (St. Pierre *et al.* 2003) where residuals are assessed with the predicted values as the independent variables, assessment of the errors of prediction more commonly known as MSPE and its partitioning in to three components, ECT, ER and ED, which allow the direct comparison of the deviation of the model output from the measurements (Kobayashi & Salam 2000), and CCC which can assess the accuracy,

precision, and over- or under-prediction of models (Lin 1998; Tedeschi 2006). Therefore, the objective of this experiment was to evaluate an extant model for the prediction of P and N excretion, and the land base requirement for the spread of manure from starter to finisher pigs, and further assess the predictions when microbial phytase is supplemented to diets to mitigate the excretion of P.

### 4.3 MATERIALS AND METHODS

#### *4.3.1 Animal trials*

This section has been covered in the ‘Materials and methods’ section of Chapter 3.

#### *4.3.2 Model description*

The model evaluated was a biologically empirical model with feed intake, body weight and number of animals as inputs. The model predicted P and N in the manure at the moment it is produced by the animal using the difference between the quantity consumed and retained in the body of pigs.

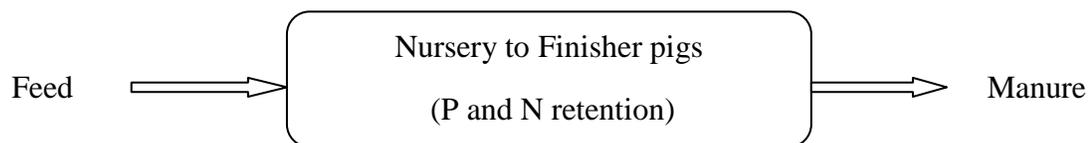


Figure 4.1 Schematic representation of the determination of mineral P and N in the manure of pigs.

$$\text{Intake P (kg)} = P_f (\%) \text{FI (kg)} \quad (4-1)$$

where:  $P_f$  is the percentage of P in feed; FI is the total daily feed intake.

The model assumes total retained nutrients to be 5.3 g of P and 25 g of N per kg of body weight gain in pigs in all phases of production;

$$P = 0.0053 W_G \quad (4-2)$$

$$N = 0.025 W_G \quad (4-3)$$

where:  $W_G$  is the daily weight gain (kg).

The experimentally observed and model predicted land base requirements (ac./yr/pig), based on inputs of observed and predicted total P excretions, respectively, were both calculated using the following equation;

$$\text{LBR} = P_2O_5 \text{ app.} / P_2O_5 \text{ rem.} \quad (4-4)$$

where: LBR is the land base required for the optimal application of manure (ac./yr/pig);  $P_2O_5$  app. is annual  $P_2O_5$  application (lb), and  $P_2O_5$  rem. is the average crop removal rate for  $P_2O_5$  (lb/ac).

$$P_2O_5 \text{ rem.} = X_1 (X_{1\text{yield}} X_{1\text{rem.}}) + X_2 (X_{2\text{yield}} X_{2\text{rem.}}) + X_n (X_{n\text{yield}} X_{n\text{rem.}}) \quad (4-5)$$

where  $X_1$  is the % of cropland occupied by crop 1;  $X_{1\text{yield}}$  is the 'crop 1' yield in (bu/ac);  $X_{1\text{rem.}}$  is  $P_2O_5$  removal rate by 'crop 1' (lb  $P_2O_5$ /ac);  $X_2$  is the % cropland occupied by crop 2;  $X_{2\text{yield}}$  is the 'crop 2' yield in (bu/ac);  $X_{2\text{rem.}}$  is  $P_2O_5$  removal rate by 'crop 2' (lb  $P_2O_5$ /ac);  $X_n$  is the % of cropland occupied by crop n;  $X_{n\text{yield}}$  is the 'crop n' yield in (bu/ac);  $X_{n\text{rem.}}$  is  $P_2O_5$  removal rate by 'crop n' (lb  $P_2O_5$ /ac).

The land base requirement values determine the optimal spread of manure to agricultural lands without a significant negative impact on the environment and reduced runoff in to surface waters, thereby minimizing the hazardous effects of nutrients on aquatic systems. As Eq. (4-4) is used to calculate the land base requirement based on model calculation for P crop removal rates based on crop removal rates of Québec conditions, results from the current study on land base requirement need further assessment with actual field crop P removal rate studies under Manitoba conditions and model adjusted accordingly for a successful mitigation strategy.

The model predicted values were compared to the experimentally observed values for P and N to assess the adequacy of the mathematical model for its intended purpose of P and N management as a NMP strategy for swine production in Manitoba. Furthermore, the land base requirements were also compared for both scenarios.

#### *4.3.3 Model evaluation*

The model was evaluated using different statistical tools which included regression analysis, partitioning of MSPE into three components namely, the ECT, ER and ED, and CCC, which was discussed in detail in Chapter 2.

## 4.4 RESULTS

Model evaluation results using regression analysis, MSPE, and CCC for all phases are given in Tables 4.1-4.4.

### *4.4.1 Model evaluation of P and N excretion for starter pigs*

Regression analysis of observed vs. predicted P in the manure from starter pigs showed a better prediction for the No-phytase ( $r^2 = 0.48$ ,  $P < 0.05$ ) than the Phytase ( $r^2 = 0.20$ ,  $P > 0.05$ ) diet. Visual assessment using regression of residuals on predicted values showed less scatter of observation and prediction values for both treatment diets (Fig. 4.2 a. and 4.3 a.). However, the intercept and slope for the regression analysis of the No-phytase diet were significantly different from zero ( $P = 0.044$ ) and one ( $P = 0.0256$ ) respectively, but not for the Phytase diet ( $P = 0.680$  and  $P = 0.200$ , respectively). Results from comparison of model predictions and observed values showed that most of the errors associated with the predictions of P in manure were due to random variation (ED = 87.8%) for the No-phytase diet. The ECT and ER were relatively small contributing 10.6% and 1.60%, respectively towards the total MSPE. For the Phytase diet, the main error partition contributing toward the total MSPE was the ER with 80.4%, and the ECT and the ED were 7.7% and 11.9% of the total MSPE, respectively. The CCC analysis demonstrated that the model was fairly precise ( $r = 0.65$ ,  $P < 0.05$  and  $0.40$ ,  $P > 0.05$  for the No-phytase and Phytase diets, respectively) and accurate ( $C_b = 0.78$  and  $0.60$  for the No-phytase and Phytase diets, respectively). The model was overpredicting by an average of 20% for the No-phytase ( $\mu = -0.420$ ) and by 18% for the Phytase ( $\mu = -0.478$ ) diets.

Table 4.1: *Evaluation of Manitoba Conservation model for predicted P excretion in the manure of nursery to finisher pigs*

Descriptive Statistics	Phase I (6.5 – 14 kg)		Phase II (15 – 25 kg)		Phase III (26 – 50 kg)		Phase IV (51 – 80 kg)		Phase V (80 – 110 kg)	
	No-phytase	Phytase	No-phytase	Phytase	No-phytase	Phytase	No-phytase	Phytase	No-phytase	Phytase
Diet	No-phytase	Phytase	No-phytase	Phytase	No-phytase	Phytase	No-phytase	Phytase	No-phytase	Phytase
MSE	0.57	0.28	3.69	2.34	0.53	1.14	7.28	1.44	5.12	5.78
RMSE	0.75	0.53	1.92	1.53	0.73	1.07	2.70	1.20	2.26	2.40
$r^2$	0.48	0.20	0.13	0.51	0.62	0.41	0.00	0.23	0.18	0.02
MSPE	0.53	0.25	3.18	1.99	0.57	1.10	6.22	1.34	4.68	4.85
RMSPE	0.73	0.50	1.78	1.41	0.75	1.05	2.49	1.16	2.16	2.20
Partitioning of MSPE (%)										
ECT	10.6	7.70	27.4	76.6	4.10	5.57	34.1	9.00	23.6	49.7
ER	1.60	80.4	3.14	2.27	28.5	56.0	30.1	68.6	23.6	45.1
ED	87.8	11.9	69.5	21.2	67.4	38.5	35.8	22.4	52.8	5.20
Concordance correlation coefficient										
$C_b$	0.78	0.60	0.42	0.31	0.98	0.82	0.64	0.75	0.84	0.31
$r$	0.65*	0.40	0.62*	0.74*	0.79*	0.71*	---	0.65*	0.62*	---
$\mu$	-0.42	-0.48	1.32	1.50	0.18	0.31	-1.05	0.41	-0.62	-1.84

MSE = Mean square error; RMSE = Root mean square error; MSPE = Mean square prediction error; RMSPE = Root mean

square prediction error; ECT = Error due to central tendency; ER = Error due to regression; ED = Error due to random variation.

Table 4.2: *Statistical summary of results from regression of predicted on observed P excretion in nursery to finisher pigs.*

Descriptive statistics	Phase I (6.5 – 14 kg)		Phase II (15 – 25 kg)		Phase III (26 – 50 kg)		Phase IV (51 – 80 kg)		Phase V (80 – 110 kg)	
	No-phytase	Phytase	No-phytase	Phytase	No-phytase	Phytase	No-phytase	Phytase	No-phytase	Phytase
Diet										
Intake (g/d)	3.46	2.68	5.12	4.20	7.2	6.68	10.6	8.55	12.3	9.81
Excreted (g/d)										
Observed mean	0.99	0.78	2.79	2.21	3.47	2.70	5.46	4.53	7.34	4.41
Predicted mean	1.45	0.92	2.17	1.43	3.33	2.42	6.92	4.19	8.40	5.95
Observed std. dev.	0.21	0.17	1.42	1.21	0.80	0.66	1.49	0.61	1.70	0.50
Predicted std. dev.	0.41	0.47	0.53	0.31	0.90	1.17	1.30	1.22	1.71	1.41
Linear regression										
Intercept (H0: $\beta=0$ )	P >0.044	P >0.680	P >0.320	P >0.002	P >0.136	P >0.729	P >0.003	P >0.970	P >0.061	P >0.110
Intercept	0.04 ( $\pm 0.002$ )	0.00 ( $\pm 0.003$ )	0.01 ( $\pm 0.002$ )	0.00 ( $\pm 0.002$ )	0.01 ( $\pm 0.003$ )	0.00 ( $\pm 0.006$ )	0.04 ( $\pm 0.009$ )	0.00 ( $\pm 0.014$ )	0.03 ( $\pm 0.012$ )	0.04 ( $\pm 0.022$ )
Slope	0.31 ( $\pm 0.114$ )	0.49 ( $\pm 0.351$ )	0.06 ( $\pm 0.058$ )	0.24 ( $\pm 0.089$ )	0.27 ( $\pm 0.076$ )	0.43 ( $\pm 0.181$ )	-0.05 ( $\pm 0.307$ )	0.95 ( $\pm 0.626$ )	0.43 ( $\pm 0.321$ )	-0.42 ( $\pm 0.978$ )

MSE = Mean square error; RMSE = Root mean square error; MSPE = Mean square prediction error; RMSPE = Root mean square

prediction error; ECT = Error due to central tendency; ER = Error due to regression; ED = Error due to random variation

Model prediction for manure N in the starter phase was not satisfactory because there was a high ECT proportion of the total MSPE with 50.9% in the No-phytase diet and 57.4% in the Phytase diet. Regression analysis of observed vs. predicted P excretions showed a better relationship from swine fed Phytase ( $r^2 = 0.40$ ,  $P < 0.05$ ) diet compared to the No-phytase ( $r^2 = 0.35$ ,  $P > 0.05$ ) diet. However, the intercept and slope of the regression line were significantly different from zero and one respectively in the No-phytase ( $P = 0.019$ ,  $P = 0.037$ , respectively) and Phytase ( $P = 0.024$ ,  $P = 0.017$ , respectively) diets. The CCC analysis showed a more precise prediction for the Phytase than the No-phytase diet ( $r = 0.66$ ,  $P < 0.05$  and  $0.43$ ,  $P > 0.05$ , respectively), and a similar accuracy ( $C_b = 0.56$  and  $0.53$  respectively) in both treatment diets. Moreover, the model tended to overpredict N excretion in manure by 36.8% ( $\mu = -1.32$ ) in the No-phytase diet and by 38.2% in the Phytase diet ( $\mu = -1.24$ ).

#### 4.4.2 Model evaluation of P and N excretion for grower pigs

Model predictions of P and N in the grower pigs were assessed for two phases of production, phases II and III. Comparison of observed and predicted P excretion in the manure in phase II showed that for the No-phytase diet 69.5% of the errors of MSPE were due to ED, 3.14% due to ER and 27.4% ECT. There was a similar proportion of the errors with the Phytase diet where 76.6%, 2.27% and 21.2% of the total MSPE was because of ECT, ER and ED, respectively. Furthermore, predictions of P in the manure were fairly precise for both No-phytase ( $r = 0.62$ ,  $P < 0.05$ ) and Phytase ( $r = 0.74$ ,  $P < 0.01$ ) diets. However, the model tended to be more accurate in the No-phytase ( $C_b = 0.42$ ) than the Phytase diet ( $C_b = 0.31$ ), even though these values are not significantly different. The

model underpredicted P excretion in the manure by 35% in both the No-phytase ( $\mu = 1.318$ ) and Phytase ( $\mu = 1.501$ ) diets.

Visual assessment of regression of residuals on predictions showed more scatter in both treatment diets (Fig 4.2 b, c & Fig 4.3 b, c), and regression analysis showed that the slope was significantly different from one for the No-phytase ( $P = 0.0019$ ) and the Phytase ( $P = 0.0297$ ) diets and the intercept was not significantly different from zero for the No-phytase diet ( $P > 0.3196$ ) but was significantly different from zero for the Phytase diet ( $P > 0.002$ ).

With regard to the prediction for N in the manure in grower pigs in phase II, the model performed better for the No-phytase than the Phytase diet, where a higher proportion of MSPE was due to ED in the former (76.1%) than the latter (41.3%). Most of the errors in the Phytase diet in this phase were associated with ECT (54.6%). Similarly, the model did not predict N excretion accurately ( $C_b = 0.48$  for No-phytase and 0.34 for Phytase diets). However, there seemed to be a precise prediction for both treatment diets ( $r = 0.63$ ,  $P < 0.05$  and  $0.77$ ,  $P < 0.01$  for the No-phytase and Phytase diets, respectively). The model under predicted the excretion of N in the manure by 21% in the No-phytase diet ( $\mu = 0.88$ ) and by 27% in the phytase diet ( $\mu = 1.60$ ).

In phase III, the model seemed to perform well in the prediction of P in the manure for the No-phytase diet where most of the MSPE was related with the unexplained variation (ECT = 4.10%, ER = 28.5% and ED = 67.4 %). However, for Phytase diet, there was a higher proportion of the MSPE due to systemic variation or ER (ECT = 5.57%. ER = 56.0% and ED = 38.5%). Regression analysis results suggested a rather good agreement

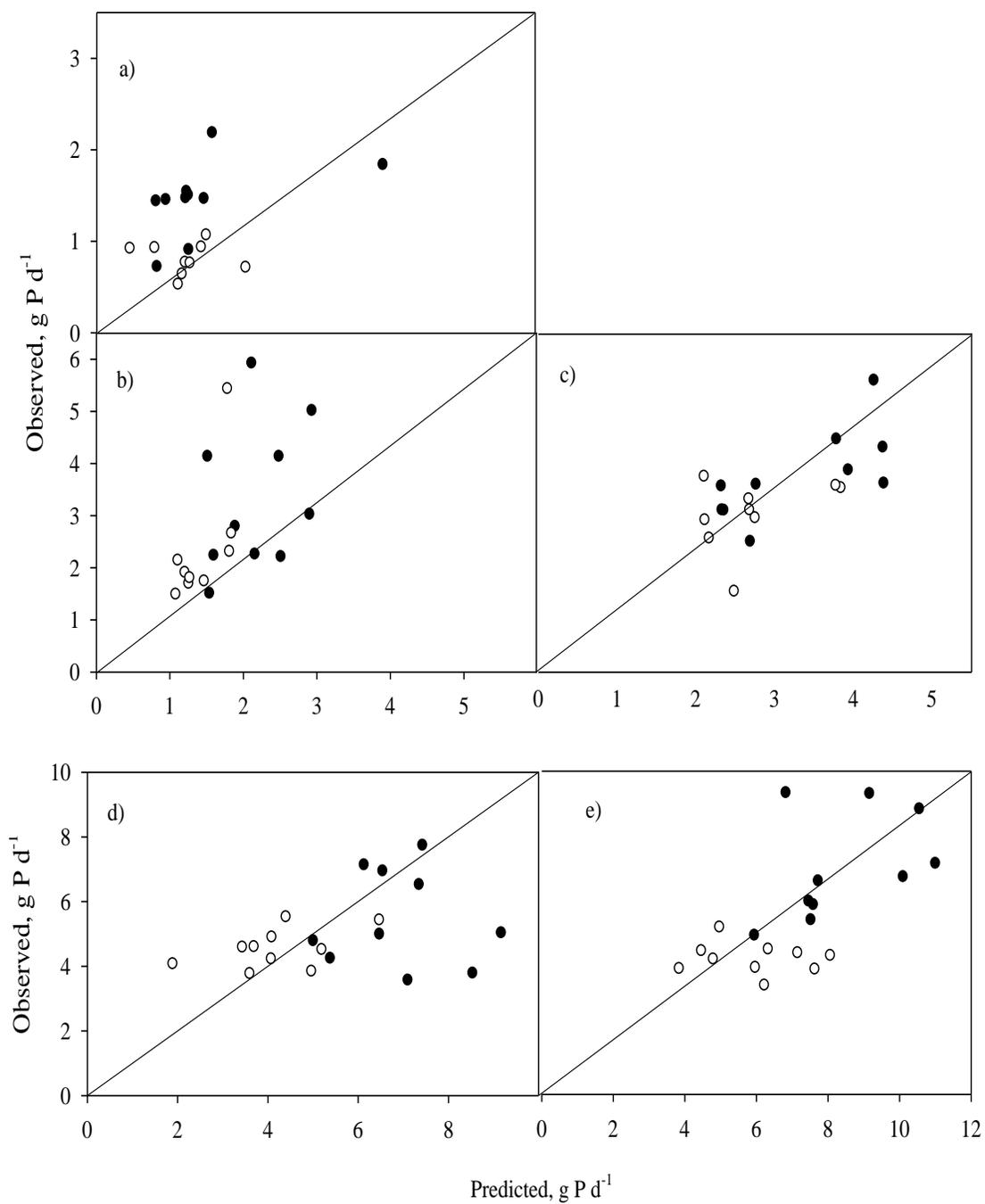


Figure 4.2. Plots of observed vs. predicted manure P. a) represents a plot of finisher pigs, phase IV ( $r^2 = 0.002$  and  $0.23$  for No-phytase and Phytase diets, respectively), b)

represents a plot of finisher pigs, phase V ( $r^2 = 0.18$  and  $0.02$  for control and phytase diets, respectively), c) represents a plot of grower pigs, phase III ( $r^2 = 0.62$  and  $0.41$  for No-phytase and Phytase diets, respectively), d) represents a plot of finisher pigs, phase IV ( $r^2 = 0.002$  and  $0.23$  for No-phytase and phytase diets, respectively), e) represents a plot of finisher pigs, phase V ( $r^2 = 0.18$  and  $0.02$  for control and phytase diets respectively). Solid symbol = No-phytase and Open symbol = Phytase.

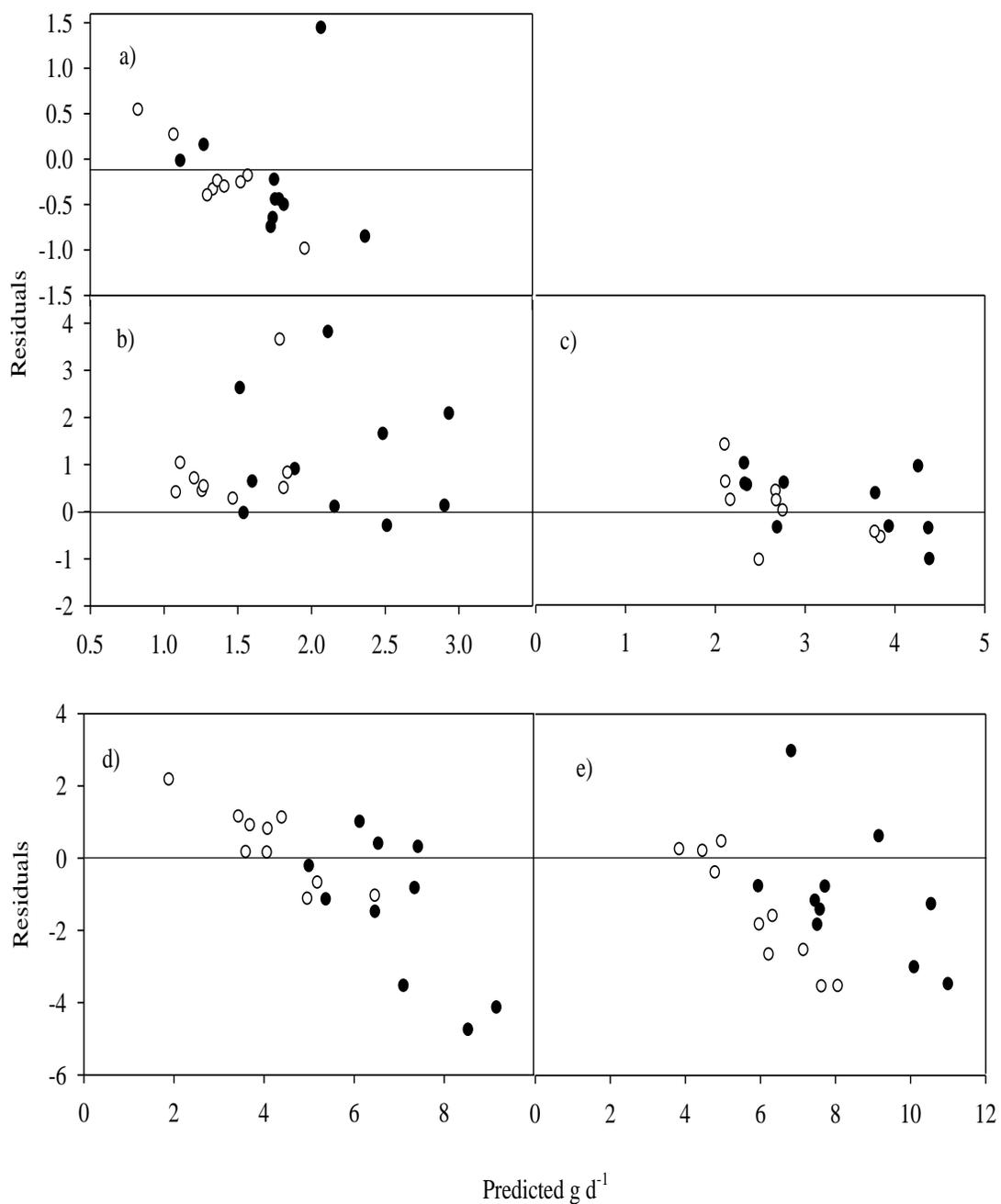


Figure 4.3. Plots of residuals vs. predicted manure P. a) represents starter phase, b) grower phase II, C) Grower phase III, d) Finisher phase IV and e) Finisher phase V. Solid symbol = No-phytase diet and open symbol = Phytase diet.

between predicted and observed values for P excretion for the No-phytase ( $r^2 = 0.62$ ,  $P = 0.01$ ) and Phytase ( $r^2 = 0.41$ ,  $P < 0.05$ ) diets. The intercept was significantly different from zero in both the No-phytase ( $P = 0.14$ ) and Phytase ( $P = 0.73$ ) diets. However, the slope in the No-phytase ( $P = 0.007$ ) and Phytase ( $P = 0.046$ ) diets was not significantly different from one.

The CCC analysis showed that the accuracy and precision of the model to predict the P excretion in the manure was good in both the No-phytase ( $C_b = 0.89$ ;  $r = 0.75$ ,  $P < 0.01$ ) and Phytase diets ( $C_b = 0.63$ ;  $r = 0.76$ ,  $P < 0.01$ ). Model underprediction for P was observed in both the No-phytase ( $\mu = 0.47$ ) and Phytase ( $\mu = 1.03$ ) diets. There was a 4.32 and a 10.4% underprediction for the No-phytase and Phytase diets respectively.

For the prediction of N in the manure in phase III, regression of observed vs. predicted values showed that the model predicted the excretion of N in the manure adequately in both the No-phytase and Phytase diets reasonably well ( $r^2 = 0.58$ ,  $P < 0.01$  and  $0.43$ ,  $P < 0.05$  respectively). Furthermore, the slope and intercept test in the regression analysis showed that the intercept was significantly different from zero in both No-phytase ( $P = 0.180$ ) and Phytase ( $P = 0.523$ ), and the slope was not significantly different from one in both No-phytase ( $P = 0.0111$ ) and Phytase ( $P = 0.037$ ) diets. The MSPE analysis showed that about 72% of the error was associated with the ED in the No-phytase and approximately 52.6% with the ECT in the Phytase diets. There was a small systemic error in both treatment diet predictions ( $ER = 7.67$  and  $1.04$  for No-phytase and Phytase diets, respectively). The model was fairly accurate and precise ( $C_b = 0.89$  and  $0.63$ , and  $r = 0.75$ ,  $P < 0.01$  and  $0.76$ ,  $P < 0.01$  for No-phytase and Phytase diets, respectively).

The model under-predicted the N excretion by 9.42% and 22.3% in the No-phytase ( $\mu = 0.47$ ) and Phytase ( $\mu = 1.03$ ) diets respectively.

#### 4.4.3 Model evaluation of P and N excretion for finisher pigs

The finisher pig category was also divided into two phases and the model predictions assessed accordingly. In the fourth phase of production, the model predictions for the excretion of P in the manure were ambiguous, with equal partitioning of the total MSPE among the three sources of errors (ECT = 34.1%, ER = 30.1% and ED = 35.8%) in the No-phytase diet. However, for Phytase diet, there was a clear shift in the errors, with ER contributing 68.6% of the errors associated with MSPE, and 9 % and 22.4% of ECT and ED respectively. The CCC analysis showed a good accuracy for both the No-phytase and Phytase diets ( $C_b = 0.64$  and  $0.75$  respectively). Even though, the precision was high for the Phytase diet, assessment of precision for the No-phytase diet was not possible.

The model overpredicted P excretion by 27% in the No-phytase diet ( $\mu = -1.047$ ) and underpredicted P excretion in the Phytase diet by 7.5% ( $\mu = 0.406$ ). Regression analysis results showed a weak relationship between observed and model predicted values for the No-phytase ( $r^2 = 0.0028$ ,  $P > 0.05$ ) than the Phytase diet ( $r^2 = 0.2239$ ,  $P > 0.05$ ) and the slope was not significantly different from one in both the No-phytase ( $P = 0.8853$ ) and Phytase ( $P = 0.1672$ ). However, the intercept was significantly different from zero in the No-phytase diet ( $P = 0.0033$ ), but was not significantly different from zero in the Phytase diet ( $P = 0.9662$ ).

Comparison of model predicted and observed N excretions in the manure of finisher pigs showed no relationship in the case of the No-phytase diet ( $r^2 = 0.02$ ). However, there was a high correlation value ( $r^2 = 0.82$ ) in the case of Phytase diet. The slope of regression line was not significantly different from one in the No-phytase diet ( $P = 0.800$ ), but was significantly different from one in the Phytase diet ( $P = 0.0003$ ). The intercept was significantly different from zero in the No-phytase ( $P = 0.0059$ ), but not in the Phytase diet ( $P = 0.147$ ). About 55% of the errors in the No-phytase and 90% of the errors in the Phytase observed were mainly as a result of ECT. ED values were 34.4% for the former and 10.4% for the later. The CCC analysis showed that the model prediction for the excretion of N in the Phytase diet seemed to be reasonably precise ( $r = 0.86$ ,  $P < 0.001$ ) but less accurate ( $C_b = 0.23$ ). However, model predictions in the No-phytase diet were neither accurate ( $C_b = 0.44$ ) nor precise ( $r = 0.33$ ,  $P > 0.05$ ). The model underpredicted the N excretion in both the No-phytase and Phytase diets by 22 ( $\mu = 1.54$ ) and 34% ( $\mu = 2.50$ ), respectively.

Model evaluation using MSPE in finisher pigs of phase V for P excretion showed that ECT, ER and ED contribution toward the total error was 23.6%, 23.6% and 52.8% respectively for the No-phytase diet and 49.7%, 45.1% and 5.20% respectively for Phytase diet. Model prediction for P excretion in the manure was relatively accurate for the No-phytase ( $C_b = 0.84$ ) than the Phytase ( $C_b = 0.31$ ) diet. Precision of P excretion was good for the No-phytase diet ( $r = 0.62$ ,  $P < 0.05$ ) and could not be determined for the Phytase diet. Regression analysis of observed vs. predicted P excretion in the manure showed that there was a weak relationship between the two in both treatment diets. The slope and intercept for both the No-phytase and Phytase diets were not significantly

Table 4.3: *Evaluation of model for nursery to finisher pigs for predicted N excretion in the manure*

Descriptive Statistic	Phase I (6.5 – 14 kg)		Phase II (15 – 25 kg)		Phase III (26 – 50 kg)		Phase IV (51 – 80 kg)		Phase V (80 – 110 kg)	
	No-phytase	Phytase	No-phytase	Phytase	No-phytase	Phytase	No-phytase	Phytase	No-phytase	Phytase
Diet										
MSE	10.3	8.87	44.0	31.4	29.1	74.5	232	348	202	85.4
RMSE	3.21	2.98	6.63	5.60	5.39	8.63	15.2	18.7	14.2	9.24
$r^2$	0.35	0.40	0.18	0.74	0.58	0.43	0.02	0.82	0.21	0.34
MSPE	8.74	8.42	39.2	26.5	23.2	59.6	185	279	161	68.3
RMSPE	2.96	2.90	6.26	5.15	4.82	7.72	13.6	16.7	12.7	8.26
Partitioning of MSPE (%)										
ECT	50.9	57.4	23.3	54.6	19.9	52.6	54.9	89.4	30.9	66.7
ER	26.3	11.5	0.60	4.09	7.67	1.04	10.7	0.20	0.57	2.98
ED	22.8	31.1	76.1	41.3	72.4	46.4	34.3	10.4	68.5	30.3
Concordance correlation coefficient										
$C_b$	0.53	0.56	0.48	0.34	0.89	0.63	0.44	0.23	0.60	0.57
$r$	0.43	0.66*	0.63*	0.77*	0.75*	0.76*	0.33	0.86*	0.63*	0.79*
$\mu$	-1.32	-1.24	0.88	1.60	0.47	1.03	1.54	2.50	0.88	1.23

MSE = Mean square error; RMSE = Root mean square error; MSPE = Mean square prediction error; RMSPE = Root mean square prediction error; ECT = Error due to central tendency; ER = Error due to regression; ED = Error due to random variation

Table 4.4: Statistical summary of results from regression of predicted on observed N excretion in nursery to finisher pigs

Descriptive statistics	Phase I (6.5 – 14 kg)		Phase II (15 – 25 kg)		Phase III (26 – 50 kg)		Phase IV (51 – 80 kg)		Phase V (80 – 110 kg)	
	No-phytase	Phytase	No-phytase	Phytase	No-phytase	Phytase	No-phytase	Phytase	No-phytase	Phytase
Diet										
Intake (g/d)	20.4	19.7	29.5	27.9	50.6	50.2	70.6	63.6	63.0	56.3
Excreted (g/d)										
Observed mean	5.74	5.87	14.2	14.0	25.5	27.8	48.4	46.8	39.2	34.3
Predicted mean	7.85	8.11	11.2	10.2	23.1	21.6	38.0	30.8	31.8	27.2
Observed std. dev.	1.44	1.82	5.98	4.10	5.49	6.83	8.23	8.03	12.1	6.14
Predicted std. dev.	1.78	1.78	1.97	1.38	4.57	5.63	5.49	5.14	5.88	5.36
Linear regression										
Intercept (H0: $\beta=0$ )	P >0.020	P >0.017	P >0.001	P >0.014	P >0.180	P >0.520	P >0.006	P >0.147	P >0.011	P >0.403
Intercept	0.02 ( $\pm 0.007$ )	0.02 ( $\pm 0.007$ )	0.05 ( $\pm 0.008$ )	0.02 ( $\pm 0.007$ )	0.03 ( $\pm 0.025$ )	0.02 ( $\pm 0.035$ )	0.21 ( $\pm 0.056$ )	0.04 ( $\pm 0.025$ )	0.1068 ( $\pm 0.032$ )	0.04 ( $\pm 0.047$ )
Slope	0.36 ( $\pm 0.142$ )	0.39 ( $\pm 0.142$ )	0.09 ( $\pm 0.068$ )	0.30 ( $\pm 0.067$ )	0.43 ( $\pm 0.131$ )	0.435 ( $\pm 0.18$ )	0.05 ( $\pm 0.172$ )	0.47 ( $\pm 0.079$ )	0.202 ( $\pm 0.121$ )	0.41 ( $\pm 0.201$ )

MSE = Mean square error; RMSE = Root mean square error; MSPE = Mean square prediction error; RMSPE = Root mean square prediction error; ECT = Error due to central tendency; ER = Error due to regression; ED = Error due to random variation.

different from one and zero, respectively, (slope:  $P = 0.218$  and  $0.110$ ; intercept:  $P = 0.061$  and  $0.681$  for No-phytase and Phytase diets, respectively).

Regression analysis of observed on predicted values showed a weak relationship between the observed and predicted N in the manure in both No-phytase and Phytase diets ( $r^2 = 0.21$  and  $r^2 = 0.34$ , respectively,  $P > 0.05$ ). The intercept was not significantly different from zero for the No-phytase ( $P = 0.011$ ) but not for the Phytase diet ( $P = 0.403$ ), and the slope was significantly different from one for both the No-phytase ( $P = 0.134$ ) and Phytase ( $P = 0.079$ ) diets.

Partitioning of the MSPE showed ED being the major contributor towards the total error in the No-phytase (68.5%) diet than the Phytase (30.3%) diet in the prediction of N in manure. Most of the errors in the Phytase diet were caused due to ECT (66.7%). This value was 30.9% for the No-phytase diet. In both the No-phytase and Phytase diets, ER was relatively small (0.57% and 2.98%, respectively). There was an acceptable prediction precision by the model for both the No-phytase and Phytase diets ( $r = 0.63$  and  $0.72$  respectively;  $P < 0.05$ ). Similarly, model predictions were fairly accurate for both the No-phytase ( $C_b = 0.60$ ) and Phytase ( $C_b = 0.57$ ) diets. Furthermore, the model underpredicted N excretion in No-phytase and Phytase diets by 19% ( $\mu = 0.88$ ) and 21% ( $\mu = 1.23$ ) respectively.

#### *4.4.4 Land base requirement determination using observed and predicted P outputs*

A higher land base is required when a shift is made from N-based to a P-based application rates to agricultural lands. However using phytase in a reduced P diet could result in a lower land base requirement for the spread of manure compared to standard diets with no addition of the enzyme. In the assessment of the land base requirement in this study, the observed and model predicted land base requirements showed mixed results. In the starter phase of production, the Phytase diet resulted in a 24% reduction in the land base required. The reduction was predicted to be 39% with the model evaluated ( $P < 0.05$ ) (Table 4.5). There was a 29 and a 23% reduction in the land base required in ha/pig/year with addition of phytase to the grower diet ( $P < 0.05$ ). The model predicted this reduction to be 33 and 25% ( $P < 0.05$ ). In the case of finisher pigs, addition of phytase resulted in a reduced land base requirement for the spreading of manure by 17 and 40% ( $P < 0.05$ ) in phases IV and V, respectively. The model was able to predict a statistically significant reduced land base requirement of 40 and 30% for the spreading of manure when phytase was added to the diet of finisher pigs ( $P < 0.05$ ) in phase IV and V respectively. Because the volume of manure produced by the grower and finisher pigs is the highest in any swine operation, the results suggest that the use of phytase could significantly help in reducing P excretion and consequently environmental pollution.

Table 4.5: *Land base requirements for the spread of manure from two dietary treatments as for observed and model predicted values*

Phase (BW)	Diet	Observed (ha/pig/year)	Predicted (ha/pig/year)
I (6.5 – 14 kg)	No-phytase	0.021 <sup>b</sup>	0.031 <sup>a</sup>
	Phytase	0.016 <sup>b</sup>	0.019 <sup>b</sup>
II (15 – 25kg)	No-phytase	0.058 <sup>a</sup>	0.045 <sup>ab</sup>
	Phytase	0.041 <sup>b</sup>	0.030 <sup>c</sup>
III (25 – 50 kg)	No-phytase	0.073 <sup>a</sup>	0.069 <sup>ab</sup>
	Phytase	0.056 <sup>ab</sup>	0.052 <sup>b</sup>
IV (50 – 80 kg)	No-phytase	0.114 <sup>b</sup>	0.144 <sup>a</sup>
	Phytase	0.095 <sup>bc</sup>	0.087 <sup>c</sup>
V (80 –110 kg)	No-phytase	0.153 <sup>a</sup>	0.175 <sup>a</sup>
	Phytase	0.092 <sup>c</sup>	0.124 <sup>b</sup>

<sup>a,b,c</sup> Means followed by the same letter in a phase are not significantly different ( $P < 0.05$ ).

#### 4.5 DISCUSSION

The model adopted by Manitoba Conservation has not been evaluated in Manitoba. Models need to be evaluated thoroughly before they could be applied for their intended purposes. In the starter phase of production, the model can be described as adequate based on high proportion of the MSPE due to ED in the No-phytase diet. Regression assessment and CCC also showed the same trend for the prediction of P in the manure in the starter phase for both diets. The model did not predict manure P content from the phytase amended diet adequately, possibly due to systemic changes in the bioavailability of P as it

was affected by supplementation of phytase. This was clearly observed as the ER was much higher in the Phytase diet. The model also struggled to predict N excretion in the starter phase and showed a higher ECT. A general over-prediction of N excretion was observed. Model prediction for the excretion of P in the grower phases was adequate where the assessment of model using the regression analysis, MSPE and the CCC showed an acceptable agreement between the observed values and model predicted values. Therefore, this model can be a useful tool for the prediction of P in the grower phase. The model also showed a good agreement with the observed values for N excretion in manure of grower pigs even though further assessment in this regard needs to be carried out to build more confidence.

Model predictions for the excretion of P in the slurry of grower and finisher pigs were in close agreement with the study of Dourmad *et al.* (2003) where the model was able to predict the reduced P excretion by 25 and 35% in the manure of grower and finisher pigs fed a standard diet. Model predictions for the Phytase diet showed more deviations, possibly because the model does not take into account the effect of phytase on the utilization of P and N as well as effect of phytase on growth. It is difficult to explain the effect of phytase on the growth response which possibly occurs due to the increase in available P, or its action on other nutrients to release either more energy or more amino acids (AA) (Ketaren *et al.* 1993). Model prediction results for P, but not N excretion in the finisher pigs in this study were close to simulation results reported by Bridges *et al.* (1995) where simulations were carried out for the excretion of P and N under actual commercial facility. Bridges *et al.* (1995) observed a 7.42 g/d and 5.26 g/d simulated P excretion for a standard and P deficient diet supplemented with phytase. However, the

simulated N excretion for the finisher pigs in the same study was not in agreement with the current study's observations where a 20.34 g/d and 13.9 g/d excretion was observed for the standard and phytase supplementation diets respectively. The model of Bridges *et al.* (1995) was a dynamic model which takes into account the reduction of CP and supplementation of diet with synthetic AA. This particular difference with the current model which is an empirical model might be the major cause for the difference in the prediction of N excretions. Furthermore, model predicted values for P and N outputs in the current study are not agreement with the study of Dourmad *et al.* (1999) where the estimated excretion of P was 3.19 g/d and 8.39 g/d for starter and finisher pigs, respectively. In the current study, these values were 2.0 g/d and 6.22 g/d, respectively. Furthermore, the N output prediction was not in agreement for all the phases. Carter *et al.* (2003) reported P excretion to be 2.3 g/d/pig and 6.7 g/d/pig for the starter and grower-finisher, respectively. The model of Carter *et al.* (2003) included more elaborate processes involved in the retentions of both P and N in the body. Therefore, the ability of the current model to predict values close to that of Carter *et al.* (2003) can be seen as a beneficial characteristic of the current model for use in NMP.

The use of phytase resulted in a reduced landbase that would be required for the spreading of manure to agricultural field in all the phases of production. The model predicted that the required landbase would be reduced by 21-43% as a result of reduction in P intake. This was mainly because the excretion of P in the manure was significantly reduced. Possible errors in the experimental and model assessment procedures may have accumulated over to the final assessment in the optimal landbase requirement.

#### 4.6 CONCLUSION

The observed results suggest that the model can be used for the prediction of P in the manure of pigs when standard diets are fed. However, when phytase was supplemented to swine diets and dietary P supplemented was reduced, the current model was not satisfactory as more systemic variation was observed in model predictions. With a supplementation of phytase to P deficient swine diets, excretion of P can be reduced and hence the landbase requirement with minimal effect on the economic sustainability and viability of swine operations in Manitoba. The model was found to be useful for estimating the landbase requirement for the spread of manure. However, the model was found to be inadequate for the prediction of N in the manure of starter to finisher pigs. Potential sources of errors between the observed and simulated data include potential differences between the genetics of the pigs used to develop the model and those used to evaluate the model and any errors associated with experimental data collection.

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## 5.0 Model evaluation for the excretion of phosphorus and nitrogen from dry sows

### 5.1 SUMMARY

An experiment was conducted to evaluate an extant model for the excretion of phosphorus (P) and nitrogen (N) from sows. A mass balance approach that calculated nutrient excretion as the difference between nutrient intake and retention was used. Three periods of three sows per treatment diet (total of 18 sows) were used. Treatment diets were formulated to be sufficient in P according to NRC recommendations containing 6.0 g total P/kg diet (No-phytase) and No-phytase diet with P level reduced to 5.1 g total P/kg diet and supplemented with microbial phytase at 500 FTU/kg (Phytase). Dry sows were housed in gestation stalls and total collection of faeces and urine was carried out for five collection days after seven days of acclimatization. Ferric oxide was used to identify the start and end of collection days, and total urine was collected using urinary catheters. Total P and N in manure were determined by summing the contents in faeces and urine. Observed nutrients (P & N) in manure were compared to model predicted values with feed intake, body weight and number of animals being used as inputs. Furthermore, the landbase requirement for the optimal application of manure was determined and compared for the observed and predicted values. Regression analysis of observed vs. Predicted P excretion showed that the intercept was not significantly different from zero in both the No-phytase ( $P > 0.674$ ) and Phytase ( $P > 0.399$ ) diets. Similarly, the slopes of the regression were not significantly different from one for both the No-phytase ( $P = 0.099$ )

and Phytase diets ( $P = 0.2516$ ). Model evaluation using mean square prediction error (MSPE) showed that 60 and 50% of the total error of prediction was from variation from regression line (ER) in the No-phytase diet and random error (ED) for the Phytase diet, respectively. There was a systematic error with the model predictions mainly in the No-phytase diet. The same trend was observed with the prediction of N by the model where ER (60.1%) and ED (85.3%) contributed most towards total MSPE for the No-phytase and Phytase diets respectively. The model overpredicted the excretion of P from sows by 32% in the No-phytase diet ( $\mu = -1.06$ ) and 23.5% in the Phytase diet ( $\mu = -0.783$ ). Model prediction for the excretion of N was not satisfactory for the No-phytase diet where the ER contributed the most (60.1%) towards the total MSPE. However ED was the highest (85.3%) in the Phytase treatment predictions. Model underpredicted excretion of N by 11.1% in the No-phytase diet and overpredicted N excretion by 3.16% in the Phytase diet. Furthermore, the model overpredicted the landbase required for the spread of manure by 30% in the No-phytase and 21% in the Phytase diet. The observations show inconclusive results for the prediction of P and N excretions by sows, and the landbase requirement prediction was not close to the observed values. Therefore, the current study showed that the model need to be refined if it was to be used as a tool in nutrient management planning (NMP) of sow operations.

## 5.2 INTRODUCTION

Sustainable production is one of the most pressing challenges facing the swine industry. Major concerns of pressure on the environment from swine operations include excess

nutrient load, mainly P and N, which affect soil, water and air surrounding swine facilities (Fernández *et al.* 1999a; Nahm 2002). Sows contribute the highest proportion of both P and N with 26 and 22% of the total outputs, respectively from swine industry after growing-finishing pigs (Fernández *et al.* 1999b). Sows consume diets that are comprised mainly of seeds (cereal grains) or their products (oil seed meal and grain by products) that contain about two-thirds of the P in the form of phytate-P which has a low bioavailability due to lack of the enzyme phytase in the gastro-intestinal tract of these animals (Simons *et al.* 1990; Kies *et al.* 2005). This results in a higher excretion of P in manure increasing the risk of P loss from land surrounding swine facilities. Manure applications to meet N requirement of crops increase the concentration of soil P. This is because the N:P ratio of manure is lower than that for crop removal leading to the application of more P than removed by crops (Daniel *et al.* 1994). When the inherent threshold of soils to retain P is exceeded, coupled with the various transport factors in the soil, an increase in runoff P with detrimental effects on surface waters is observed (Sharpley *et al.* 1999), with eutrophication of surface water being the major consequence (Smith *et al.* 1999). Various mitigation strategies have been studied extensively that can assist the swine industry to achieve a sustainable production practice while maintaining profitability. Among those mitigation strategies, the use of microbial phytase has shown a promising opportunity by improving the digestibility of phytate-P in sows and reducing output to the environment (Jongbloed *et al.* 2004; Liesegang *et al.* 2005). A comprehensive nutrient management planning (NMP) where the amount of imported and exported nutrients can be quantified on the farm can provide an essential tool for understanding the flow of nutrients in the life cycle of animals. A rapid and accurate on-farm determination of swine slurry nutrient

concentrations can minimize potential environmental damage to water bodies while providing crops with nutrients for optimal yields (Higgins *et al.* 2004).

Mathematical models have been used for understanding and predicting the flow of nutrients in various categories of pigs including sows (e.g. Aarnink *et al.* 1992; Dourmad *et al.* 1992; Carter *et al.* 2003), and can be valuable tools because obtaining representative samples of stored manure for analysis of nutrients are difficult and sometimes impossible (Powers & Van Horn 2001). Furthermore, with the common use of phytase to optimize bioavailability of P in swine, NMP strategies should consider nutrient outputs in farms where microbial phytase use is a common practice. Therefore, the objective of this study was to evaluate an extant model for the use in NMP of nutrients especially P and N under conventional and phytase supplemented diets, and further assess the prediction of land base requirement for the optimal manure application for the two dietary treatments.

### 5.3 MATERIALS AND METHODS

#### *5.3.1 Animal trial*

Six sows were blocked into two treatment diets with three sows per treatment and repeated over three periods with different sows. Sows were randomly assigned to the two diets; one control diet where the level of P in the diet was formulated to meet the requirement of the animals according to NRC (1998) (No-phytase) and the second diet with the P level in the diet reduced by 0.1 percentage units (Phytase). The animals were fed for seven days of adaptation period before the actual collection periods were started.

After seventh day, pigs were fit with urinary catheters for a total urine collection of five days, and total faecal collection was also performed. During these five days of collection, feed consumption, water consumption, water spillage and room temperature were recorded. Phosphorus and N in faeces and urine were added to determine total output of the nutrients in the manure from individual animal. Body weight of sows was measured at start and end of collection days.

All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Protocol Management and Review Committee, and pigs were handled according to the guidelines described by the Canadian Council on Animal Care (CCAC, 1993).

### *5.3.2 Model description*

The model evaluated was empirical and the inputs were feed intake, body weight and number of animals. The model predicted P and N in the manure at the moment it is produced by the animal using the difference between the quantity consumed and retained in the body of pigs.

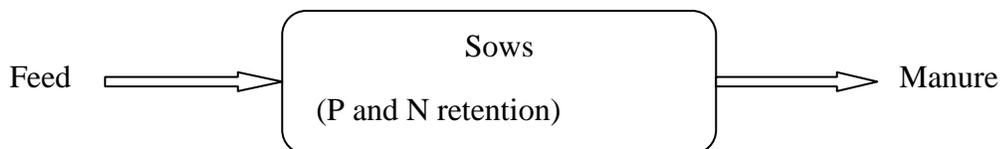


Figure 5.1. Schematic representation of the determination of P and N in the manure of sows.

$$\text{Intake P (kg)} = P_f (\%) \times \text{FC (kg)} \quad (5-1)$$

Where:  $P_f$  is the percentage of P in feed; FC is the total daily feed consumption.

The model assumes a total retained nutrient of 5.3 g of P and 25 g of N for every kg of body weight gain in pigs in all phases of production:

$$P = 0.0053 W_G \quad (5-2)$$

$$N = 0.025 W_G \quad (5-3)$$

where:  $W_G$  is the daily weight gain (kg).

The body composition of sows on a g/kg basis was similar to the study of May & Rozeboom (2008) where they found the average P and N content of sows to be 5.63 and 24.9 g/kg.

The model predicted the land base requirement (ac/yr/pig) using the following formula:

$$\text{LBR} = P_2O_5 \text{ app.} / P_2O_5 \text{ rem.} \quad (5-4)$$

Where: LBR is the land base required for the optimal application of manure (ac/yr/pig);  $P_2O_5$  app. is annual  $P_2O_5$  application (lb), and  $P_2O_5$  rem. is the average crop removal rate for  $P_2O_5$  (lb/ac).

$$\begin{aligned} P_2O_5 \text{ rem.} = & X_1 (X_{1\text{yield}} - X_{1\text{rem.}}) + X_2 (X_{2\text{yield}} - X_{2\text{rem.}}) + \\ & + X_n (X_{n\text{yield}} - X_{n\text{rem.}}) \end{aligned} \quad (5-5)$$

where:  $X_1$  is the % of cropland occupied by crop 1;  $X_{1\text{yield}}$  is the ‘crop 1’ yield (bu/ac);  $X_{1\text{rem}}$  is  $\text{P}_2\text{O}_5$  removal rate by ‘crop 1’ (lb  $\text{P}_2\text{O}_5/\text{ac}$ );  $X_2$  is the % cropland occupied by crop 2;  $X_{2\text{yield}}$  is the ‘crop 2’ yield (bu./ac.);  $X_{2\text{rem}}$  is  $\text{P}_2\text{O}_5$  removal rate by ‘crop 2’ (lb  $\text{P}_2\text{O}_5/\text{ac}$ );  $X_n$  is the % of cropland occupied by crop n;  $X_{n\text{yield}}$  is the ‘crop n’ yield (bu/ac);  $X_{n\text{rem}}$  is  $\text{P}_2\text{O}_5$  removal rate by ‘crop n’ (lb  $\text{P}_2\text{O}_5/\text{ac}$ ).

The land base requirement values determine the optimal spread of manure to a land base without a significant accumulation of nutrients, mainly P and N, and reduced runoffs in to surface waters thereby minimizing the hazardous effects of nutrients on aquatic systems.

The model predicted values were compared to observed values for P, N and land base requirements to assess the adequacy of the mathematical model for its intended purpose of P and N management as a nutrient management plan strategy for swine production in Manitoba. The land base requirement was calculated based on model calculations with inputs of observed and predicted P outputs.

### *5.3.3 Statistical analysis*

Statistical analysis was performed using the MIXED procedure of SAS (SAS, 2002). The period effect was analyzed for its significance. All observations within a treatment diet were pooled for the assessment of the adequacy of the model. If the ANOVA was significant for a given class variable, then 1-df contrasts were conducted to compare specific treatment means. All tests were considered significant at  $P < 0.05$ . Shapiro-Wilk’s

W (Shapiro & Wilk 1965) test was used to ensure the normal distribution of both the observed and predicted data.

#### 5.3.4 Model evaluation

The model was evaluated using different statistical tools which included regression analysis, partitioning of MSPE in to three components namely, the error due to central tendency (ECT), ER and ED, and concordance correlation coefficient (CCC), which was discussed in detail in Chapter 2.

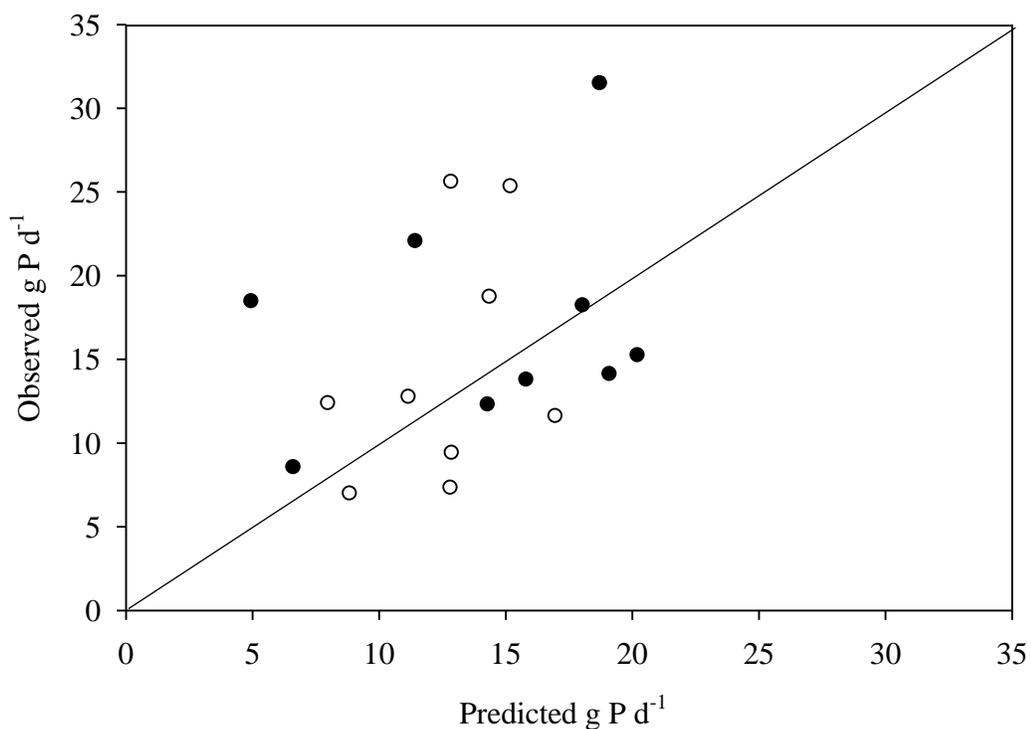


Figure 5.2 Plot of observed vs. Predicted values of manure P in sows. ( $r^2 = 0.32$  for No-phytase diet and  $r^2 = 0.18$  for Phytase diet). Solid symbol = No-phytase diet and open symbol = Phytase diet.

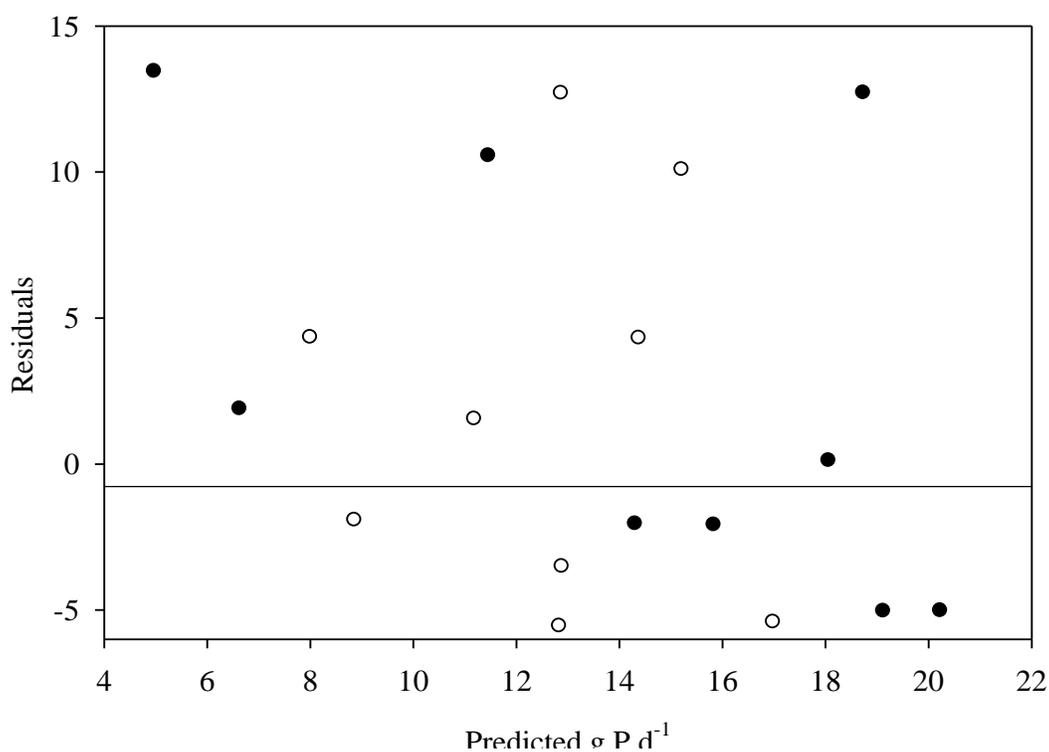


Figure 5.3 Plot of residuals vs. Predicted P output in manure of sows. Solid symbol = No-phytase diet and open symbol = Phytase diet.

#### 5.4 RESULTS

Regression analysis of observed vs. Predicted showed a weak relationship between the two for the P excretion in the manure of sows in both the No-phytase ( $r^2 = 0.25$ ) and Phytase ( $r^2 = 0.18$ ) diets. Visual assessment of residuals also showed similar observations as more scatter in data points was observed for both No-phytase and Phytase diets (Fig. 5.2 & 5.3). However, the intercept was not significantly different from zero in both the No-phytase ( $P > 0.869$ ) and Phytase ( $P > 0.421$ ) diets (Table 5.1). Similarly, the slopes of

the regression were not significantly different from one for both the No-phytase ( $P = 0.114$ ) and Phytase diets ( $P = 0.282$ ).

Table 5.1. *Statistical summary of results from regression of predicted on observed P and N excretion from sows*

Descriptive statistics	P		N	
	No-phytase	Phytase	No-phytase	Phytase
Intake (g/d)	16.4	14.8	87.0	92.9
Excreted (g/d)				
Observed mean	10.9	10.2	62.3	57.0
Predicted mean	14.4	12.6	55.4	58.8
Observed std. Dev.	3.03	3.82	13.9	15.4
Predicted std. dev.	5.57	2.88	19.5	10.1
Linear regression				
Intercept ( $H_0: \beta=0$ )	$P > 0.869$	$P > 0.421$	$P > 0.651$	$P > 0.531$
Intercept	1.27 ( $\pm 7.43$ )	5.05 ( $\pm 5.91$ )	15.0 ( $\pm 31.2$ )	19.7 ( $\pm 29.9$ )
Slope	1.04 ( $\pm 0.57$ )	0.54 ( $\pm 0.46$ )	0.65 ( $\pm 0.50$ )	0.61 ( $\pm 0.50$ )

Further assessment of model predictions using partitioning of MSPE revealed that most (60%) of the total error of prediction was associated with ER in the No-phytase diet and ED (50%) in the Phytase diet. Prediction assessment using CCC showed that model

predictions were similarly precise for the No-phytase ( $r = 0.61$ ,  $P < 0.05$ ) and the Phytase diet ( $r = 0.60$ ,  $P < 0.05$ ). However, predictions were more accurate for the latter ( $C_b = 0.76$ ) than the former ( $C_b = 0.45$ ). There was a 32 and 24% overprediction of P excretion in the No-phytase ( $\mu = -0.42$ ) and Phytase diets ( $\mu = -0.24$ ) respectively.

Nitrogen is another nutrient of concern in swine operations with respect to environmental pressure. Hence adequate understanding of N flow in sow operations is crucial for development of economically and environmentally sustainable swine operations. Therefore, the model was also assessed for N prediction in sows. Regression analysis of observed on predicted values showed that the intercept was not significantly different from zero in both treatment diets ( $P > 0.651$  for the No-phytase and  $P > 0.531$  for the Phytase diets) and the slope was not significantly different from one in both diets ( $P = 0.238$  for the No-phytase diet and  $P = 0.265$  for the Phytase diet). Partitioning of the MSPE showed that model predictions for N performed well for the Phytase than the No-phytase diet. The ECT contributed less than 5% towards the total MSPE in both treatment diets. The ER was however different in both diets contributing the most towards the total error in the No-phytase (60.1%) than in the Phytase diet (9.76%). Random error contribution towards the total error was higher in the Phytase diet (85.3%) than in the No-phytase diet (39.8%). Furthermore, CCC assessment showed that model predictions were accurate and precise in both the No-phytase and Phytase diets ( $C_b = 0.92$  and  $0.74$  respectively). However, model predictions were more precise in the No-phytase ( $r = 0.71$ ,  $P < 0.05$ ) than the Phytase diet ( $r = 0.59$ ,  $P > 0.05$ ). Model underpredicted N excretion in the manure of sows by 11% in the No-phytase diet ( $\mu = 0.04$ ) and over-predicted N

excretion in the manure of sows by 3.2% when phytase was supplemented to the diet ( $\mu = -0.8$ ).

Supplementation of phytase to sow diet resulted in 6.67% ( $P > 0.05$ ) and 12.8% ( $P > 0.05$ ) reduction of land base requirement for the optimal manure application for the experimentally observed and model predicted values respectively (Table 5.3). The model prediction for optimal spread of manure was not satisfactorily predicted where an overprediction of 30 and 21% was observed in the No-phytase and Phytase treatment diets respectively, even though this overprediction was not significantly different ( $P > 0.139$ ).

## 5.5 DISCUSSION

The observations of the current study are in agreement with the study of Carter *et al.* (2003) on the excretion of P but not N. Carter *et al.* (2003) developed a more descriptive model for prediction of nutrient excretion based on differing nutritional scheme for gestation and lactation sows. The amount of P excretion predicted by the current model is also in agreement with the recommendation of the National Resource Conservation Service (NRCS) of the United States Department of Agriculture (USDA) where an excretion of P for gestation sows was estimated to be 10.0 g/d/sow. However the prediction for N excretion was not in agreement with that of NRCS. Model prediction of the excretion of P was not in agreement with the estimates by Fernández *et al.* (1999b) where P excretion was estimated at 16.2 g/d/sow for the standard diet. However, the N excretion prediction was close to that of Fernández *et al.* (1999b) where excretions were estimated at 61.6 g/d/sow for the standard diet. The land base requirement observed in this

study were similar to that reported by May & Rozeboom (2008) where the annual  $P_2O_5$  output was reported to be 29,750 kg for a 2,400 sow breeding herd, and the total land base required for the optimal spread manure was 524 ha.

Table 5.2. *Evaluation of model predicted P and N excretion in the manure of sows*

Statistic	P		N	
	No-phytase	Phytase	No-phytase	Phytase
MSE	2.02	19.2	387	238
RMSE	1.42	4.38	19.7	15.4
$r^2$	0.25	0.18	0.14	0.17
MSPE	1.99	14.9	300	185
RMSPE	1.41	3.87	17.3	13.6
<u>Partitioning of MSPE (%)</u>				
ECT	11.4	32.9	0.11	4.97
ER	62.4	16.7	60.1	9.76
ED	26.2	50.4	39.8	85.3
<u>Concordance correlation coefficient</u>				
$C_b$	0.78	0.94	0.92	0.74
r	0.61*	0.60*	0.71*	0.59
$\mu$	-0.42	-0.24	0.04	-0.80

MSE = Mean square error; RMSE = Root mean square error; MSPE = Mean square prediction error; RMSPE = Root mean square prediction error; ECT = Error due to central tendency; ER = Error due to regression; ED = Error due to random variation

Table 5.3. *Land base requirement for manure spreading based on the P content of manure from sows fed standard and phytase diets*

Phase	Diet	Observed ----- ha/sow/year -----	Predicted
Sows	No-phytase	0.24 <sup>a</sup>	0.32 <sup>a</sup>
	Phytase	0.23 <sup>a</sup>	0.28 <sup>a</sup>

<sup>a,b,c</sup> Means followed by the same letter in a phase are not significantly different ( $P < 0.05$ ).

To the author's knowledge, this is the only study that evaluated the model adopted by Manitoba Conservation using local data. However, it is recommended that a further assessment of this model be carried out to build the required confidence in the model for its intended purposes. This is important because the data used in this study showed variability in the observations for the outputs of total P and N in sows. Mitchell (1997) suggested that with more scatter in data point for evaluation of predictive models, the computed value of the test statistic would be smaller making the null hypothesis hard to reject, either due to the fact that the slope of the regression line being not significantly different from unity or due to the higher scatter around the line. Therefore, the observation of the current study for the prediction of nutrient excretions in sow operations needs further assessment.

## 5.6 CONCLUSION

Model evaluation showed that the model adopted by Manitoba Conservation to predict the excretion of P and N in sows was not satisfactory when a standard diet was used.

This model performed better when phytase was supplemented to a P-deficient diet. However, due to the scatter of the data, conclusions based on the current study are not sufficient enough to report the model as useful for both treatment diets.

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## 6.0 Effect of phytase supplementation on greenhouse gas emissions from swine manure application

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## 6.1 SUMMARY

An experiment was conducted to examine the effect of diet on manure composition and subsequent impact on net greenhouse gas (GHG) emissions from soil. Manure was collected from pigs fed two diets supplemented with or without phytase. A factorial design with three manure treatments, two soils and two moisture levels was used for the soil greenhouse gases study. The three manure addition treatments were no manure (Control), manure from unsupplemented (No-phytase) diet and manure from phytase supplemented (Phytase) diet. The phosphorus (P) concentration in the negative control diet (Phytase) was reduced by 0.14 percentage units, and supplemented with 500 FTU/kg phytase. Two sandy loam (SL) black chernozem (CB) soils from Southern Manitoba, Canada namely Carman (SL-1) and Gunton (SL-2) were used. Two soil moisture levels simulated 'normal' and 'wet' conditions with 0.50 and 0.80 water-filled pore space (WFPS), respectively. Manure was added to microcosms to 20,000 L/ha equivalent to simulate manure application based on normal crop P requirement and to 60,000 L/ha to meet crop N requirement. The 0.80 WFPS treatments produced more GHG than the 0.50 WFPS treatments with carbon dioxide contributing the most towards the total emission. There was a two way interaction for soil types and manure, soil types and moisture levels as well as manure and moisture levels for emissions of GHG. Manure addition increased nitrous oxide emissions from each soil and moisture level, and the increase in emissions was higher for the 0.8 WFPS than 0.5 WFPS treatment. When the SL-1 and SL-2 soils were treated with No-phytase manure, there was an increase in nitrous oxide emissions of 34.6 and 38.4  $\mu\text{g}/\text{kg}/\text{day}$ , respectively. The emission was 23.0 and 42.5  $\mu\text{g}/\text{kg}/\text{day}$  with Phytase manure addition, respectively. The SL-2 soil was a sink for methane gas in the No-phytase manure addition. Regardless of the soil and moisture level, the Phytase manure resulted in

lower concentrations of inorganic N remaining at the end of the experiment. Plant available-P concentrations were lower with Phytase manure in the SL-2 soil, probably due to lower total P added with the manure. Phytase supplementation increased acetate in manure which may have had implications for soil methane emissions. The results indicate that for a successful dietary management strategy in the mitigation of GHG, a good understanding of the soil properties that influence emissions becomes necessary. Supplementation of Phytase to swine diets might contribute to a slight increase in GHG emissions, particularly during wet season application.

## 6.2 INTRODUCTION

As the world population increases, demand for food and thus agricultural production increases. A doubling of global food demand is expected over the next 50 years, which is going to put pressure on local and global environments (Komatsuzaki & Ohta 2007). A shift toward intensive livestock production to meet the food demand is expected to lead to an increase in total amount of nutrient output in manure from animal operations, most of which is applied to small land areas close to animal confinements resulting in environmental degradation. Although application of manure can improve soil chemical and physical properties; there is a concern regarding environmental quality due to manure nutrients such as phosphorus (P) building up in soil. Increased greenhouse gas (GHG) emissions from manure-applied soils are also a major threat to the environment (Komatsuzaki & Ohta 2007). Higher emissions of GHG are associated with intensively managed animals where high quality diets are provided compared to extensively managed animals (Mosier et al. 2004). The main GHGs of concern are carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) with global warming

potentials of 1, 23 and 296, respectively, (IPCC 2006). Appropriate manure management strategies help mitigate GHG emissions from swine production units, and dietary manipulation that does not affect the performance of animals is a promising management strategy (Clemens & Ahlgrimm 2001, Kebreab 2006, Cardenas *et al.* 2007).

The effect of phytase, an enzyme added to monogastric diets to improve bioavailability of P and mitigate its impact on the environment is very well documented (Harper *et al.* 1997, Selle & Ravindran 2007, Veum & Ellersieck 2008). At pH values of less than 6, the normal acidity in the stomach of pigs, phytate appears as an anion with three to five negative charges which binds different minerals as well as proteins. In addition, at low pH and low cation concentration, phytate-protein complexes are formed due to direct electrostatic interaction. These complexes appear to be responsible for the decreased bioavailability of the complexed minerals and are also more resistant to proteolytic digestion at low pH (Bebot-Brigaud *et al.* 1999, Chernay 1980). Phytase supplementation prevents the formation of precipitates of protein-phytate complexes (Kies *et al.* 2006). Therefore, supplementing phytase to swine diet might reduce the excretion of different forms of nitrogen (N) to the environment. However, we are unaware of reported studies on the effect of manure from phytase supplemented diets on net CO<sub>2</sub>, N<sub>2</sub>O, and CH<sub>4</sub> emissions when applied to soil. As phytase supplementation becomes common practice, reliable estimation are necessary to design and assess policies for mitigating GHG emissions.

The objective of this experiment was to determine the effect of phytase supplementation to swine diets on manure characteristics, net GHG emissions from manure-treated soils and plant-available P concentrations in soil.

## 6.3 MATERIALS AND METHODS

### 6.3.1 *Animals and diets*

Twenty piglets (average body weight 10.6 kg) were housed in metabolism cages and were randomly assigned to two treatment diets at the T. K. Cheung Centre for Animal Science Research, University of Manitoba. Dietary treatments were an adequate-P diet (No-phytase) and a P-deficient diet supplemented with phytase at 500 FTU/kg (Phytase). Chemical composition of the diets is given in Table 6.1. Calcium concentration in the Phytase diet was lowered to minimize its antagonistic effect on the efficiency of phytase where calcium is believed to form a complex with the enzyme (Qian *et al.* 1996, Liu *et al.* 1998). All other nutrients were formulated to meet the requirements recommended by NRC (1998). Pigs were given *ad libitum* access to feed and water. Daily feed consumption, water consumption, water spillage and weekly body weight were monitored for the entire experimental period. The experimental period consisted of ten days for adaptation followed by five days collection of faeces and urine from individual pigs. Urine was collected using a jug via a funnel below the metabolism cage. Ten mL of 6 N HCl was added to capture the possible loss of N in the form of ammonia gas. Faeces was collected in plastic bags.

All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Protocol Management and Review Committee, and pigs were handled according to the guidelines described by the Canadian Council on Animal Care (CCAC, 1993).

Table 6.1. *Calculated chemical analysis of treatment diets*<sup>1</sup>

Nutrients (g/kg)	No-phytase	Phytase
Ca	7.54	5.73
Total P	6.30	5.30
Available P	2.24	1.42
Crude Protein	226	225
Lysine	14.5	14.5
Methionine	4.20	4.18
ME, kcal/kg	3,271	3,266
Phytase, FTU/kg	—	500

<sup>1</sup>No-phytase diet was formulated to meet the pigs' P requirement and the Phytase diet had reduced dietary P and supplemented with microbial phytase.

### 6.3.2 Soil incubation trial

A 3×2×2 factorial combination of three manure types (No manure, No-phytase and Phytase), two sandy loam soils SL-1 (from Carman, MB, Canada) and SL-2 (from Gunton, MB, Canada) and two soil moisture levels 0.50 and 0.80 water-filled pore space (WFPS) were used. Initial soil physico-chemical properties are given in Table 2. The bulk density for the SL-1 and SL-2 soils was 1.10 and 1.20 g/cm<sup>3</sup>, respectively. Manure applications equivalent to accommodate P requirements of crops (20,000 L/ha) and N requirement of crops (60,000 L/ha) were used. Soils were homogenized with a third of the total manure application equivalent to 1% of the dried soil mass to meet the P requirement of crops and were packed into aluminium or PVC rings. After the

13<sup>th</sup> day of first application, the second manure application to accommodate the N requirement of crops equivalent to 3% of the dried soil mass was packed in to rings. Moisture was topped up to the initial level with distilled water. High moisture levels were applied to simulate a wet period with high potential for denitrification and low moisture levels to a dry period with high potential for mineralization and nitrification. Rings were placed in 1.5 L wide-mouth sealer jars and covered with perforated parafilm. Jars were placed at 20°C and in the dark. The jars were moved within the incubator each day to avoid preferential drying and the moistures were topped up twice a week.

Headspace gas was sampled on five consecutive days each week. The Parafilm was removed and a lid containing a serum septum was placed on each jar. Two hours later, after the gases had accumulated, a 10 mL sample of gas was withdrawn using a syringe and placed in a 6 mL evacuated Exetainer vial (Labco, UK). Carbon dioxide, CH<sub>4</sub> and N<sub>2</sub>O concentration in sample gas was determined by using a gas chromatograph equipped with an electron capture detector, flame ionization detector, and thermal conductivity (CP3800, Varian, Middelburg, The Netherlands) and a CombiPal robotic sample introduction system (CombiPal, Zwingen, Switzerland). Gas concentrations were tracked to determine an appropriate time for the second manure application, being, when emissions declined to that of the Control treatment.

Table 6.2. *Some physico-chemical properties of soils before manure application*

Soil	Sand	Silt	Clay	Bulk density	Organic matter	pH	EC	TIN	eDOC
	----- g/kg-----			(g/cm <sup>3</sup> )	g/kg		mS/cm	mg/kg	mg/L
SL-1	566	240	194	1.1	36	5.8	0.29	52.6	12.7
SL-2	586	324	90	1.2	60	7.5	0.62	101.8	29.7

Once the GHG emission rates decreased following the second manure addition, the soil and rings were dismantled. A wet 0.5 M K<sub>2</sub>SO<sub>4</sub> extraction (1(w):5(v) soil:extract) was performed on the soils at setup and upon dismantling to analyze for NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>/NO<sub>2</sub><sup>-</sup> and extractable dissolved organic carbon (eDOC) (Tenuta *et al.* 2000). Gravimetric moisture content was determined on a 15 g subsample of soil and remaining soil dried and ground for 0.1 M Na<sub>2</sub>CO<sub>3</sub> extraction and analysis of P by colorimetry using a spectrophotometer (Ultrospec II-4050, Cambridge, UK) and color development with citric acid. Manure samples were sent to Bodycote-Norwest Laboratories (Winnipeg, MB, Canada) for analysis of P, N, pH and electrical conductivity (EC). A portion was retained to analyze for eDOC by colorimetric analysis using a Technicon AAII Autoanalyzer (Pulse Instruments, Saskatoon, SK, Canada) and phenolphthalein colour development. Ion suppression chromatography analysis (ICS-1000 Ion Chromatograph, Oakville, ON) was used to determine volatile fatty acids (VFA) according to Tenuta *et al.* (2002).

### 6.3.3 Statistical analysis

Data were analyzed using the Proc Mixed procedure of SAS (SAS Institute, Cary, NC, USA). Analysis of variance was performed for emissions data of the two manure applications and their cumulative fluxes, and means are reported for the cumulative GHG emissions. When effects were not significant at  $\alpha \leq 0.05$ , lower significant interactions were considered for comparison of means analysis. Data on GHG emissions were log transformed ( $\log_{10}+1$ ), except for methane emissions, to minimize the confounding effects of differences in variance of the observations. Back transformed values (geometric mean) are reported.

## 6.4 RESULTS

### 6.4.1 Pig performance

The mean P concentration in both the No-phytase and Phytase diets was close to the formulated value a 0.14 percentage units less P in the latter compared to the former (Table 6.1). Daily P intake was reduced by 0.6 g in the phytase diet without a significant impact on the feed conversion ratio of pigs (FCR;  $P < 0.08$ ). Faecal P excretion from pigs fed the phytase supplemented diet was significantly lower compared to the unsupplemented diet ( $P < 0.03$ ) (Table 6.3). However, there was significantly greater excretion of P in urine as a proportion of intake in the phytase supplemented diet ( $P = 0.02$ ) (Table 6.3).

Table 6.3. *Effect of phytase supplementation on performance and phosphorus excretion in starter pigs*

Diet:	No-Phytase	Phytase	S.E.D (D.F. = 9) <sup>1</sup>	<i>P&gt;F</i>
P intake (g/d)	3.00	2.40	0.179	0.02
FCR <sup>2</sup>	2.10	1.60	0.212	0.08
<u>Faecal P:</u>				
g/d	1.20	0.68	0.081	0.003
g/kg of intake	4.03	2.86		
<u>Urine P:</u>				
g/d	0.03	0.10	0.030	0.0003
g/kg of intake	0.10	0.42		

<sup>1</sup>S.E.D. = standard error of difference; D.F. = degrees of freedom.

<sup>2</sup>FCR = feed conversion ratio.

#### 6.4.2 Manure composition

Manure collected from pigs fed the phytase supplemented diet had lower pH, total VFA and total ammonia N compared to manure from the unsupplemented diet. Total N was reduced by 10 mg/g, total VFA by 12 mM/L and P by 2 mg/g in Phytase manure. Phytase supplementation increased the concentration of acetate, which is a precursor of CH<sub>4</sub> production, by 4.1 mM/L (Table 6.4).

Table 6.4. *Chemical composition of No-Phytase and Phytase manures. Results are composite analysis given based on fresh weight of manure*

Manure		
	No-Phytase	Phytase
Moisture (%)	95.1	95.9
pH	6.40	6.20
Total P (mg/g)	8.00	6.00
Total N (mg/g)	50.0	40.0
Volatile fatty acids (VFA), mM/L		
Acetate	78.9	83.0
Propionate	47.0	35.5
Isobutyrate	0.90	1.00
n-Butyrate	12.1	10.1
Isovalerate	3.50	1.80
Caproate	5.50	4.80
Total VFA	148	136

### 6.4.3 Greenhouse gas emissions

Emission of CO<sub>2</sub> and N<sub>2</sub>O were influenced greatly by a change in the moisture content of the soils in both manure treated and untreated conditions ( $P < 0.0001$ ). This was clearly observed due to the interaction of moisture level with both the manure and soil type treatments where the 2/3 manure application coupled with the high moisture resulted in increased emissions of CO<sub>2</sub> and N<sub>2</sub>O in both soils (Figures 6.1 & 6.2). The effect of treatments and their interaction on GHG emissions followed the same trend in

both the 1/3 and 2/3 manure applications (Table 6.5). There was no significant interaction effect of manure by soil type ( $P = 0.28$ ) on cumulative CO<sub>2</sub> emission (Table 6.6). Emissions of cumulative CH<sub>4</sub> in soils treated with both types of manure increased significantly compared to untreated soils. However, there was no significant difference in emissions among the manure treatments ( $P = 0.51$ ) and moisture content did not significantly affect CH<sub>4</sub> emissions ( $P = 0.54$ ) (Table 6.6). The result does not seem to be consistent with the higher acetate concentration in Phytase manure.

Nitrous oxide emission was significantly increased ( $P < 0.001$ ) as a result of application of manure from either diet treatment to each of the soils (Tables 6.5 & 6.6). There was no significant difference in cumulative emissions of CO<sub>2</sub> between the No-phytase and Phytase manure applications under dry soil moisture conditions ( $P = 1.0$ ), while a significant difference in emissions was observed in wet conditions ( $P = 0.037$ ). The untreated SL-1 soil resulted in a significantly higher emission of CO<sub>2</sub> ( $P < 0.0001$ ) and N<sub>2</sub>O ( $P < 0.0001$ ) even though no significant difference in emissions of both gases was observed when the soils were treated with manure from the diet treatments. There was a 2-fold and 2.5-fold increase in emissions of CO<sub>2</sub> as a result of increased moisture level in the SL-1 and SL-2 soils, respectively, and the later acted as a net sink for the CH<sub>4</sub> produced as a result of application of manure ( $P = 0.001$ ) (Table 6.7). Net emission of N<sub>2</sub>O was influenced greatly by the application of manure. When the SL-1 and SL-2 soils were treated with No-phytase manure, there was an increase in N<sub>2</sub>O emissions of 34.6 µg/kg soil/day and 38.4 µg/kg soil/day, respectively. These values were 23.0 µg/kg/day and 42.5 µg/kg/day, respectively, when Phytase manure was used (Table 6.7). When moisture level in the soils was increased from a dry to a wet condition, a 22.0 and a 6.75-fold increase in emissions of N<sub>2</sub>O was observed for SL-1 and SL-2 soils, respectively (Table 6.8).

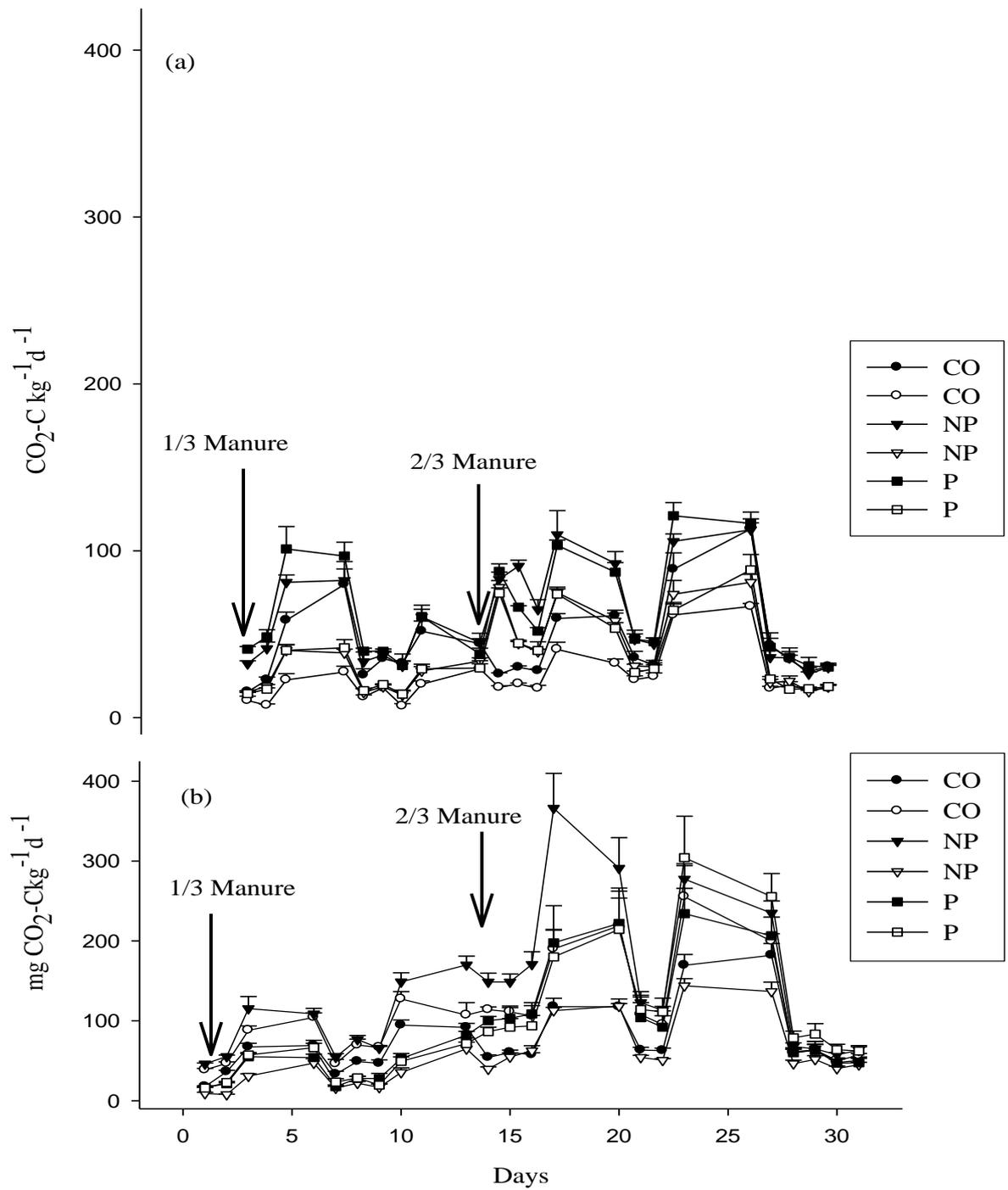


Figure 6.1: CO<sub>2</sub> emissions for 0.50 (a) and 0.80 (b) WFPS following application of swine manure. Solid and open symbols represent SL-1 and SL-2 soils, respectively; CO=No manure, NP =No-phytase and P = Phytase.

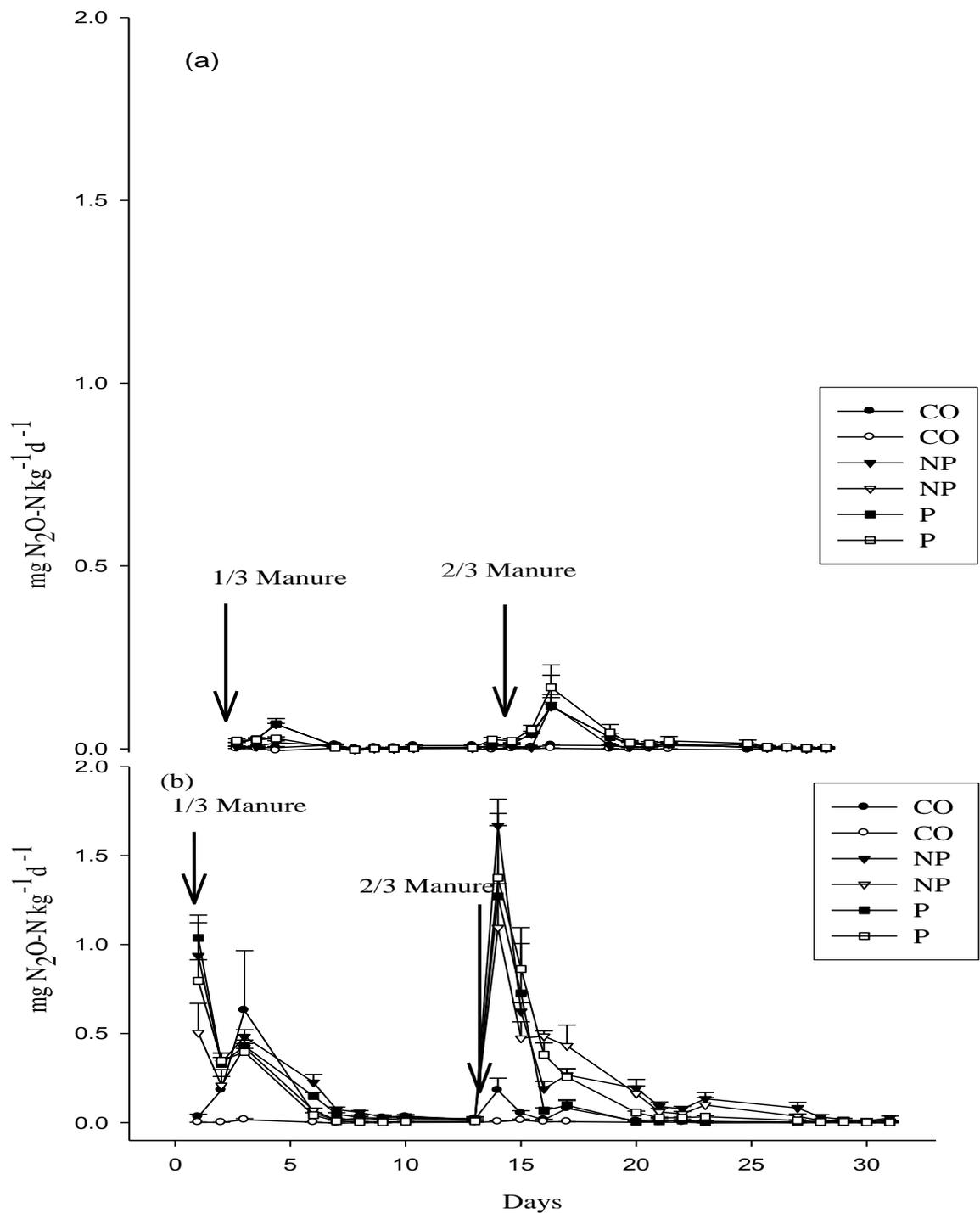


Figure 6.2:  $\text{N}_2\text{O}$  emissions for 0.5 (a) and 0.8 (b) WFPS following application of swine manure. Solid and open symbols represent SL-1 and SL-2 soils, respectively; CO=No manure, NP =No-phytase and P =Phytase.

#### 6.4.4 Soil Nutrients

##### 6.4.4.1 Plant available phosphorus

The Olsen P test showed that adding manure increased plant available P significantly ( $P < 0.05$ ). No-phytase and Phytase manure treatments resulted in a 245% ( $P = 0.032$ ) and 513% ( $P < 0.001$ ) increases in Olsen P compared to the Control, and Phytase manure resulted in a higher Olsen P (78% higher,  $P = 0.017$ ) at the end of the incubation period compared to No-phytase manure. There was a 51% difference between the two soil types with higher values observed for the SL-2 soil than the SL-1. High moisture content resulted in a 56% reduction in Olsen P at the end of the trial (Table 6.9). No three way interaction of manure, soil and moisture was observed on the recovery of Olsen P ( $P = 0.1329$ ).

##### 6.4.4.2 Inorganic nitrogen

The  $K_2SO_4$  extraction results showed that untreated SL-2 soil had more initial total inorganic N (TIN) than SL-1 (101.8 mg N/kg soil vs. 52.6 mg N/kg soil, respectively). In both soil types, initial N was primarily in the form of  $NO_3^-$ . After incubation, the recovery of TIN was significantly affected by the two way interactions of manure, soil and moisture. All of the low moisture treatments contained more TIN after the incubation than the high moisture treatments. The No-phytase SL-1 treatment showed a significantly higher TIN recovery compared to the others which were not significantly different from the Control manure and soil treatment combinations. Less recovery of TIN was observed in the SL-2 than the SL-1 soil and high moisture resulted in a significantly lower recovery in both soils (Table 6.9). The Phytase

Table 6.5. Analysis of variance for manure, soil and moisture effects and their interactions expressed as *P* values on GHG emissions for accommodating *P* (1/3 Manure) and *N* (2/3 Manure) requirements of crops

Treatment Factors	1/3 Manure application				2/3 Manure application			
	CO <sub>2</sub> -C	N <sub>2</sub> O-N	CH <sub>4</sub> -C	CO <sub>2</sub> eq.	CO <sub>2</sub> -C	N <sub>2</sub> O-N	CH <sub>4</sub> -C	CO <sub>2</sub> eq.
	----- <i>P</i> > F -----							
Manure	<0.0001	<0.0001	0.1203	<0.0001	<0.0001	<0.0001	0.9987	<0.0001
Soil	<0.0001	0.0018	0.0003	<0.0001	<0.0001	0.1062	<0.0001	<0.0001
Moisture	<0.0001	<0.0001	0.2924	<0.0001	<0.0001	<0.0001	0.8411	<0.0001
Manure*Soil	0.8309	0.0002	0.0372	0.8304	0.1103	<0.0001	0.0506	0.1102
Manure*Moisture	0.6732	0.0428	0.8122	0.6739	0.0309	0.1227	0.6060	0.0308
Soil*Moisture	0.0238	0.1355	0.1712	0.0237	<0.0001	0.1546	0.7006	<0.0001

manure high moisture treatments had approximately the same concentration of TIN as the Control high moisture treatments. The low moisture treatment in both the Phytase and No-phytase manure treatments resulted in much higher TIN recovery than the Control treatments. Regardless of soil and moisture level, the Phytase diet had consistently lower levels of TIN remaining at the end of the experiment compared to the No-phytase diet.

Table 6.6. *Analysis of variance for manure, soil type and moisture effects and their interactions expressed as P values on cumulative GHG emissions and their CO<sub>2</sub> equivalent, and extractable P and total inorganic N (TIN)*

Treatment Factors	CO <sub>2</sub> -C	N <sub>2</sub> O-N	CH <sub>4</sub> -C	CO <sub>2</sub> eq.	Olsen P	TIN	eDOC
	----- P > F -----						
Manure	<0.0001	<0.0001	0.5112	<0.0001	<0.0001	<0.0001	0.0510
Soil	<0.0001	0.0010	<0.0001	<0.0001	0.0021	<0.0001	0.0002
Moisture	<0.0001	<0.0001	0.5353	<0.0001	0.0006	<0.0001	0.3922
Manure*Soil	0.2756	<0.0001	0.0300	0.2692	0.0003	0.0118	0.5700
Manure*Moisture	0.0354	0.2783	0.0918	0.0332	0.3217	<0.0001	0.9067
Soil*Moisture	0.0003	0.0232	0.0918	0.0004	0.2514	<0.0001	0.1139
Manure*Soil*Moisture	0.570	0.901	0.243	0.964	0.893	0.487	0.908

ns: not significant

The eDOC contents of the soils were initially 12.7 and 29.7 mg/l for SL-1 and SL-2 soils, respectively. There was no significant effect of manure treatment on the content of eDOC among the two soils types as there was no any type of interaction

effects (Table 6.6). However, after manure treatment, SL-1 soil eDOC increased by 7.00 mg/l and SL-2 increased by 1.66 mg/kg (Table 6.8).

## 6.5 DISCUSSION

Our results contribute to an understanding that addition of phytase to diets to be a means of lowering the amount of P supplemented in swine diets. Our study goes further in showing the impact of diet on GHG from manure added to soil.

The effect of phytase supplementation on the performance of pigs is very well established as various studies have reported an improvement in performance of animals where diets were supplemented with microbial phytase (e.g., Shelton *et al.* 2005, Veum & Ellersieck 2008). In situations where phytase supplementation does not improve performance of starter pigs, lack of gut maturity and insufficient retention time have been suggested as some of the factors associated with such an observation (Jendza *et al.* 2006).

Table 6.7. Comparison of manure and soil interaction effects on  $N_2O$ ,  $CH_4$  and total  $CO_2$  equivalent ( $CO_2$  eq.) emissions, extractable P and  $TIN^1$  recovery

Manure	Soil	$CO_2$ -C	$CO_2$ -C	$N_2O$ -N	$N_2O$ -N	$CH_4$ -C	$CO_2$ eq.	$CO_2$ eq.	Olsen P*	$TIN^*$
		mg/kg/d	ln $mg^2$	$\mu g/kg/d$	ln $mg^2$	$\mu g/kg/d$	mg/kg/d	ln $g^2$	---- mg/kg ----	
Control	SL-1	38.4	7.08	9.60	-1.20	2.80	145	1.50	2.66	-38.1
	SL-2	24.3	6.62	0.67	-3.90	-2.20	89.2	1.02	0.13	-49.5
Average		31.4	6.85	5.14	-2.55	0.30	117	1.26	1.40	-43.8
No-Phytase	SL-1	53.8	7.42	34.7	0.07	2.1	213	1.89	0.50	2.37
	SL-2	38.0	7.07	38.4	0.18	-0.42	157	1.58	9.10	-35.4
Average		45.9	7.25	36.6	0.13	0.84	185	1.74	4.80	-16.5
Phytase	SL-1	62.2	7.56	23.0	-0.34	1.70	239	2.00	6.46	-27.9
	SL-2	39.2	7.1	42.5	0.28	0.50	164	1.63	10.6	-45.3
Average		50.7	7.33	32.8	-0.03	1.1	153	1.81	8.53	-36.6
S.E.D. (D.F. = 48)			0.039		0.189	0.028		0.035	1.011	4.379

<sup>1</sup>TIN = total inorganic nitrogen; <sup>2</sup>Natural logarithm scale reported on a 31 days basis; S.E.D. = standard error of difference reported based on log transformed values; D.F. = degrees of freedom;

\* Values are reported after correcting for initial Olse-P and TIN contents of treatment soils.

Table 6.8. Comparison of soil type and moisture level interaction effects on CO<sub>2</sub>, N<sub>2</sub>O, CO<sub>2</sub> equivalent (CO<sub>2</sub> eq.) emissions and TIN

Soil	Moisture	CO <sub>2</sub> -C	CO <sub>2</sub> -C	N <sub>2</sub> O-N	N <sub>2</sub> O-N	CO <sub>2</sub> eq.	TIN*	eDOC
		mg/kg/d	ln mg <sup>1</sup>	μg/kg/d	ln mg <sup>1</sup>	mg/kg/d	mg/kg	mg/L
SL-1	Low	37.2	7.10	4.20	-2.10	138	9.83	16.1
	High	68.4	7.70	92.4	1.10	294	-52.3	23.2
Average		52.8	7.40	48.3	-0.50	216	-21.2	19.7
S.E.D			0.030		0.185		3.59	
SL-2	Low	21.4	6.50	3.40	-2.30	80.5	6.34	32.4
	High	51.0	7.40	31.2	-0.03	201	-93.2	30.3
Average		36.2	6.95	17.3	-1.17	141	-43.4	31.3
S.E.D.			0.028		0.152		3.58	

(D.F. = 48)<sup>2</sup>

<sup>1</sup>Natural logarithm scale reported on a 31 days basis; <sup>2</sup>S.E.D. = standard error of difference reported based on log transformed values; D.F. = degrees of freedom.

\* Values are reported after correcting for initial TIN content of treatment soils.

Phytase is believed to improve digestibility of amino acids in swine diets by minimizing formation of precipitates of phytic acid and protein in the GI tract, thereby improving the availability and reducing N excretion (Kies *et al.* 2006). There was a 44% reduction in total N in manure collected from growing pigs fed a phytase supplemented diet in the study of Gilley & Eghball (1998). The lower rate of reduction in this study was mainly because the pigs in the experiment were younger than those used by Gilley &

Eghball (1998). Concentrations of VFA tend to be higher in pig slurry and their rapid oxidation is important in explaining CO<sub>2</sub> and CH<sub>4</sub> production (Dendooven *et al.* 1998). However, the relationship between the VFA concentration in the manure treatments and CO<sub>2</sub> and CH<sub>4</sub> emissions showed an inconclusive observation in the current study.

The GHG emission results showed that manure treatment of soils could result in higher emissions of CO<sub>2</sub> during wet season conditions. The effect of the spread of manure, from feeding phytase amended diet, on CO<sub>2</sub> emissions cannot be fully explained at present as effects of phytase on energy components in diets of swine are inconclusive. There appears to be a counterbalance in energy expenditure when phytase is supplemented in the diet with a reduced energy expenditure of the digestive tract and increased metabolic activity in the visceral organs (Kies *et al.* 2005). Therefore, with more elucidations on this effect, CO<sub>2</sub> emissions when manure is spread to soils in Manitoba would need to be assessed for a better mitigation option.

Moisture content of soils greatly influenced emission of N<sub>2</sub>O possibly due to increased denitrification with increasing WFPS. A similar increase in N<sub>2</sub>O emission was shown in the study of Ciarlo *et al.* (2007), where cumulative total N emissions at 0.40 WFPS were significantly lower ( $P = 0.02$ ) than those at 0.80, 1.00 and 1.20 WFPS, with the N<sub>2</sub>O emissions in the 0.80, 1.00 and 1.20 WFPS not being statistically different. The results from this study also agree with other reports where higher emissions of N<sub>2</sub>O was observed in soils treated with animal slurry and a higher soil moisture content, resulting possibly from denitrification with a maximum value being reached at around 0.80 WFPS (Maag & Vinther 1999, Skiba & Ball 2002, Dobbie & Smith 2003).

Table 6.9. Comparison of manure and moisture level interaction effects on CO<sub>2</sub>, CO<sub>2</sub> equivalent (CO<sub>2</sub> eq.) emissions, and changes in Olsen P and TIN<sup>1</sup> after incubation

Manure	Moisture	CO <sub>2</sub> -C	CO <sub>2</sub> -C	CO <sub>2</sub> eq.	CO <sub>2</sub> eq.	Δ Olsen P*	Δ TIN*
		mg/kg/d	ln mg <sup>2</sup>	mg/kg/d	ln mg <sup>2</sup>	----- mg/kg -----	
Control	Low	21.9	6.50	80.4	0.91	2.95	-24.7
	High	42.5	7.20	156	1.60	-0.17	-63.0
Average		32.2	6.85	118	1.26	1.39	-43.9
No-Phytase	Low	32.0	6.90	117	1.30	5.96	33.0
	High	63.9	7.60	234	2.00	3.63	-66.0
Average		48.0	7.25	176	1.65	4.80	-16.5
Phytase	Low	32.1	6.90	118	1.30	11.5	15.9
	High	75.9	7.80	278	2.20	5.51	-89.1
Average		54.0	7.35	198	1.75	8.51	-36.6
S.E.D. <sup>3</sup>			0.039		0.039	0.240	4.38
(D.F. = 48) <sup>2</sup>							

<sup>1</sup>TIN = total inorganic nitrogen; <sup>2</sup>Natural logarithm scale reported on a 31 days basis; <sup>3</sup>S.E.D. = standard error of difference reported based on log transformed values; D.F. = degrees of freedom.

\* Values are reported for changes (Δ) of Olsen-P and TIN after correcting for initial contents in soils.

Total eDOC was associated with N<sub>2</sub>O emissions in the soils, with higher emissions in SL-2 soil than SL-1 as eDOC concentration increases. As eDOC of soils increases with the application of manure (Wood *et al.* 1996), it acts as an electron donor for denitrifiers and the capacity of soils to hold water increases which can have an effect on the emission of N<sub>2</sub>O (Hudson 1994). The SL-1 soil showed higher emissions of all the gasses compared to the SL-2 possibly due to the higher clay content in the former than the latter. Velthof *et al.* (2005) hypothesized that addition of degradable C with application of manures strongly affected the potential denitrification in clay soils than sandy soils. In the present study, higher correlation between emissions of N<sub>2</sub>O and CO<sub>2</sub> was observed in SL-2 than SL-1. As denitrification is a major N<sub>2</sub>O production process, this relationship between the two gases might be explained by the parallel dynamics of N<sub>2</sub>O and CO<sub>2</sub> concentrations in soil atmospheres where concentration of both gases increase with depletion of oxygen and the carbon substrate encourages denitrification, resulting in higher N<sub>2</sub>O emissions with higher organic carbon (Swerts *et al.* 1996, Rochette *et al.* 2000, Franzluebbers 2005). Furthermore, the acidic condition of SL-1 might have resulted in increased emission of N<sub>2</sub>O in that soil. When denitrification is the main process, N<sub>2</sub>O emissions tend to decrease with increasing pH (Yamulki *et al.* 1997). Weier & Gillam (1986) reported that acidification of soils may severely inhibit N<sub>2</sub>O reductase which immediately results in higher N<sub>2</sub>O than N<sub>2</sub> yields from denitrification. Effects of phytase supplementation on emissions of N<sub>2</sub>O were not clearly observed possibly due to different factors in the soil that influence the dynamics and conversion of manure N<sub>2</sub> into N<sub>2</sub>O suggesting a soil specific management strategy with a good understanding of all interactions in the soils.

Although the difference in emission of  $\text{N}_2\text{O}$  between the two manure types was not significant, the reduced  $\text{NO}_3^-$  in phytase manure treated soil and the numerically lower emissions of  $\text{N}_2\text{O}$  in the same treatment show a potential for  $\text{N}_2\text{O}$  emission mitigation by using phytase amended diets with reduced inorganic P content in the diet. Compared to  $\text{CO}_2$  and  $\text{N}_2\text{O}$ ,  $\text{CH}_4$  emissions from soils amended with hog manure seem to play a minor role in atmospheric loading of GHG, hence is not to be considered important in the mineral soils used here and collected from fields cultivated to annual crops. Other studies have also shown that emissions of  $\text{CH}_4$  from manure treated soils were minor compared to other agricultural sources/sinks (Rochette & Côté 2000).

Dietary modification with phytase and phytase manure amendments can prolong the capacity of soils for receiving manure applications and reduced runoff P. However, reports on the effect of phytase on runoff P after manure application to soils have been mixed. Smith et al. (2004) indicated that manure treatment from nursery swine fed a phytase diet might release up to 25% more P than manure from animals fed a normal diet, however, the difference was not statistically significant. This observation is in agreement with the current study where approximately 40% more Olsen P was recovered in the Phytase treatment which may result in exceeding the threshold of soils for the loss of P due to erosion or leaching. Other studies showed no effect on runoff P in soils amended with manure from phytase supplemented diets even at a higher value of 300,000 FTU/kg of diet (Gilley & Eghball 1998). Furthermore, Gilley (2001) observed that the Phytase diet led to consistently lower concentrations of dissolved runoff P initially than high available phosphorus (HAP) corn or traditional corn diets, even though not always significantly lower.

## 6.6 CONCLUSION

Phytase supplementation slightly increased GHG emissions in manured soils compared to soils amended with manure from diets without phytase. This was attributed to greater CO<sub>2</sub> liberation. Spreading manure originating from pigs fed phytase amended diet did not result in a significant increase in emission of N<sub>2</sub>O compared to preading manure from pigs fed standard diet. Olsen P concentrations were lower with phytase supplemented manure application to SL-1 soil than SL-2.

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## 7.0 GENERAL DISCUSSION

Environmental concerns arising from expansion of swine operations has put pressure on the industry to adopt alternative feeding strategies to minimize nutrient outputs, and legislation has been put in place to limit and account for nutrient outputs to the environment. The main nutrient of interest, P, affects waterways and water bodies where eutrophication is observed with increased input of P to those waters. The main reason behind the threat of excessive P output from intensive swine operations is because monogastric animal diets are mainly comprised of seeds (cereal grains) or their products (oil seed meal and grain by products) in which about two-thirds of the P is present in the form of phytate, and has a low bioavailability due to lack of the enzyme phytase in the gastro-intestinal tract of these animals (Simons *et al.* 1990; Kies *et al.* 2005). Moreover, in order to ameliorate this limitation in swine diets, inorganic P is supplemented at a higher safety margins that has been increasing in its use for the last five decades (Barnett 1994). Most of the intensively managed swine operations in Manitoba produce excessive P to the environment because transporting excess P to areas of low P load is economically unsustainable. The threat of animal manure to surface waters is also further exacerbated because of the need for adequate water supplies that often results in swine operations being established near easily accessible streams and sources of water (Pip 2005). For swine producers to understand and manage nutrient outputs, simple and user friendly manure nutrient determination tools are required for successful nutrient management planning (NMP). Manitoba conservation adopted a model developed for Quebec to predict P and N output that was based on mass balance approaches where nutrients in manure are determined by the difference between nutrient intake and retention. Such

models are important tools because they can help to explain the variability of farms due to climate, storage and handling practices which can affect nutrient output determinations. However, with adoption of models from another region comes the requirement to evaluate these models using data from the region of interest, and if necessary, modify to take into account the unique characteristics of a particular region. The Manitoba Conservation-adopted model was therefore evaluated using various statistical tools that included regression analysis, partitioning of mean square prediction error (MSPE) and concordance correlation coefficient (CCC). Experiments were conducted to obtain data from starter to finisher pigs and sows, and model predictions compared to observed values under Manitoba conditions. With the extensive use of phytase for the mitigation of P output from swine operations, the model was also evaluated in conditions where phytase was supplemented to diets of all categories of pigs and P level reduced in the diets. Finally, the effect of phytase supplementation to swine diets on net GHG emissions due to application of manure to soil was determined.

In Chapter 3, nutrient flow in swine fed typical and phytase supplemented diets was assessed. The results showed that across the nursery to finisher phases, there was no significant difference in performance parameters measured with regard to average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR), in both treatment diets. These observations were in agreement with the studies of Harper *et al.* (1997), Braña *et al.* (2006) and Beaulieu *et al.* (2007) where supplementation of phytase was observed to have a similar effect on ADFI, ADG and FCR. Variable average g P/kg body weight was observed with a range of 4.21 g P/kg body weight (grower phase) to a 7.49 g P/kg body weight (finisher phase). The average retention of P was 5.77 g/kg for a

pig from starter to the finisher production, which was not different from the model assumption of 5.30 g/kg. Variable observation on the g/kg retention of P have also been reported in other studies; Han *et al.* (1997) reported a 7.02 g P/kg for finisher pigs; O'Quinn *et al.* (1997) reported a 4.16 g P/kg for starter pigs and Harper *et al.* (1997) reported a 4.25 g P/kg for grower pigs. The variability observed mainly due to the physiological status of the pigs at different phases warrants a phase specific nutrient, P, management strategy to alleviate the pressure on water bodies in Manitoba.

Total P excretion was significantly reduced with the addition of phytase to the diet in all phases. This observation is in agreement with other studies (Cromwell *et al.* 1993 and O'Quinn *et al.* 1997). Phytase supplementation did not affect the N excretion in all categories of pigs. Ketaren *et al.* (1993), Kies *et al.* (2005) and Liao *et al.* (2005) also showed that supplementing phytase to swine diet has no effect on N excretion. The observations were satisfactory for use in evaluating the model adopted by Manitoba Conservation.

Model evaluation for the excretion of P and N from starter to finisher pigs was performed in Chapter 4. In this study, observed values from Chapter 3 were compared to model predicted values using various evaluation techniques that included regression analysis, partitioning of MSPE and CCC. The model was found to be satisfactory for the prediction of P excretion from starter to finisher pigs when the standard diet, but not the phytase-supplemented diet, was used. This was based on model evaluation results which showed the majority of the error in MSPE analysis coming from random sources for pigs fed the unsupplemented diet. In the phytase-supplemented diet, a systematic deviation of model predictions from observed values were observed. The failure of the model to

predict the excretion of P in the Phytase diet could possibly be explained by the inability of the model to take into account the improvement of bioavailability, hence retention, of P in the body of animals. The model was not satisfactory for the prediction of N excretion in both the No-phytase and Phytase diets in all categories of pigs. Observations of model predictions were also found to be in agreement with other studies (Bridges *et al.* 1995, Carter *et al.* 2003 and Dourmad *et al.* 2003).

In Chapter 5, model evaluation for the excretion of P and N from sows was assessed. In this chapter, as with Chapter 4, observed values from the sow study were compared to model predicted values using various evaluation techniques that included regression analysis, partitioning of MSPE and CCC. The model was found to be unsatisfactory for the prediction of both P and N in both treatment diets, and was recommended not to be used until further assessment is performed to confirm or reject the current observations. The land base requirement for the optimal spread of manure was predicted satisfactorily by the model in the starter to finisher pigs, but not in sows.

Finally, in Chapter 6, the effect of phytase supplementation on GHG emissions from swine manure application to sandy loam soils with high and low moisture levels in the soils was assessed where manure originated from pigs fed standard and phytase-supplemented diets. The results showed that high moisture content of soils produced more GHG than low moisture content with CO<sub>2</sub> contributing the most towards the total emission. Manure addition generally increased N<sub>2</sub>O emissions. Even though not significantly different, phytase supplementation showed numerical reduction in emission of N<sub>2</sub>O. Plant available-P concentrations were lower with Phytase manure possibly due to lower total P added with the manure.

It can, therefore, be concluded that the Manitoba conservation adopted model can be used satisfactorily in starter to finisher operations under Manitoba conditions; while care needs to be taken in the use of this model in sow operations until further assessment on the performance of the model can be carried out to support or refute observations made in the current study. Furthermore, with a wide use of phytase in any operation, this model is not sufficient as a tool in NMP for the prediction of P outputs in all swine operations in Manitoba.

## 8.0 CONCLUSIONS

Based on the results observed in this thesis, it can be concluded that:

1. A variable retention of P, 4.21 to 7.49 g/kg weight gain, was observed across the starter to finisher phases. The average retention of P was 5.77 g/kg for a pig from starter to the finisher production, which was not different from the model assumption of 5.3 g/kg.
2. The Manitoba Conservation-adopted model can be used to predict the excretion of P from starter to finisher pigs under Manitoba conditions.
3. The model cannot predict the excretion of P from sows satisfactorily under Manitoba conditions based on the current experimental observations. Further assessment and experiment with regard to the behaviour of the model in sow operations is required.
4. Model predictions were not satisfactory for the prediction of N excretion from all categories of pig (starter to finisher and sows).
5. The land base requirement can be predicted fairly well by the Manitoba Conservation-adopted model in the starter to finisher pigs.
6. When swine operations use phytase, the model was found to be poor in the predictions of P and N in all the categories of pigs.
7. Phytase supplementation to swine diet resulted in a slight increase in GHG emissions attributed mainly to CO<sub>2</sub> liberation but not N<sub>2</sub>O.

### Future Research:

1. The variability in retention of P for each weight gain across the phases of production warrants for phase specific assumptions of P retention per weight gain when developing or utilizing the model for nutrient management strategies in Manitoba.
2. More information about the effect and availability of endogenous phytase in cereal grains and supplementation of exogenous phytase must also be included for accurate estimation of P output.
3. This study evaluated the model under controlled experimental conditions. Evaluation in commercial conditions is further warranted due to the variation in the feed wastes and other sources of nutrient outputs that can affect the concentration of P and N in slurry before land application. This would allow for a rapid on farm determination of total P and N concentrations in swine slurry.
4. It is recommended that the model be tested under conditions of reduced N intake by lowering the dietary CP and supplementing with AA.
5. A comprehensive nutrient management plan in which mathematical models take into account the changes in nutritional programs to match improving genetic potential, and feeding strategies designed to reduce nutrient excretion is recommended.
6. Development of a mechanistic model of nutrient flow in swine will improve predictions of N and P output and manure composition that would in turn improve predictions of land base requirement for spreading manure.

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