Determination of Optimal True Digestible Calcium to True Digestible Phosphorus Ratio in Growing Pigs

by

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ABSTRACT

THE DETERMINATION OF OPTIMAL TRUE DIGESTIBLE CALCIUM TO TRUE DIGESTIBLE PHOSPHORUS RATIO IN GROWING PIGS

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Three studies were conducted. In the first study, 12 Yorkshire barrows (initial BW of 23.2 ± 2.0 kg) were allotted to 2 dietary treatments with 6 replications per treatment using a completely randomized design to determine apparent Ca and P digestibility (AD), true digestibility (TD) and endogenous output (EO) in a corn/SBM-based diet. All pigs were placed in individual feeder pens that allowed for easy collection of individual feces. The AD of Ca and P were 28.4% and 23.9%, respectively. Their TD of 42% and 40% for Ca and P respectively were significantly ($P < 0.05$) higher than the corresponding AD. EO was 0.8g for Ca and 1.3g for P per kg of dry matter intake (DMI).

In the second study, the optimal dietary ratio of true digestible Ca and P was determined in terms of its effect on growing pig performance, excretion of Ca and P in feces and urine in a corn/SBM-based diet using a randomized complete block design. Thirty six growing barrows (initial BW: 24.2 ± 1.9 kg) were allotted to 6 dietary treatments with 6 replications per block. Six corn/SBM-based diets with very similar nutrient contents were formulated but differed in their dietary ratio of Ca to P. The balances of Ca and P and their true digestibility/retention were calculated for each diet. Animal performance and true retention of both Ca and P was optimal ($P < 0.05$) with diet 2 with a true digestible Ca to P ratio of 0.82: 1 compared to other experimental diets.

The third study was conducted with a similar protocol to that used in experiment 1 involving 12 barrows (initial BW: 23.9 ± 1.1 kg) to determine Mg, Cu, Fe, Mn, Se and Zn TD values for the growing pig. Se and Zn AD of 73.9% and 9.5% significantly (*P < 0.05*) underestimated their TD of 82.1% and 15%, respectively. Se and Zn EO were 0.00004mg and 0.01 mg/kg of DMI, respectively. The TD and EO for Mg, Cu, Fe and Mn could not be estimated because of their negative AD.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
AD	Apparent digestibility
ADG	Average daily gain
ADFI	Average daily feed intake
ADV	Apparent digestible value
AFD	Apparent fecal digestibility
ANOVA	Analysis of variance
BW	Body weight
Ca	Calcium
Cd	Cadmium
CP	Crude protein
Cu	Copper
d	Day
DE	Digestible energy
DM	Dry matter
DMI	Dry matter intake
EFL	Endogenous fecal loss
Fe	Iron
FE	Feed efficiency
g	Gram
h	Hour
Hg	Mercury

H_2S	Hydrogen sulphide
Kg	Kilogram
L	Litre
Mg	Magnesium
Mn	Manganese
N	Nitrogen
Na	Sodium
NDF	Neutral detergent fibre
P	Phosphorus
P	Probability
PTH	Parathyroid hormone
S	Sulphur
TFD	True fecal digestibility
TD	True digestibility
SBM	Soybean meal
Se	Selenium
SEM	Standard error of mean
TDV	True digestible value
Zn	Zinc

CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

1.1 Overview of the Current Swine Industry and Its Effects on the Environment

The population of the United States of America and Canada like other parts of the world is growing rapidly, partly through their active immigration policies and programs; this stresses the need for increased food production, such as animal protein in the region to cope with the population increment. These developments also stress the need to increase food production without necessarily increasing the cost of production, such as that of feed components, including calcium (Ca) and phosphorus (P), so as to enable animal products to be within the reach of consumers and simultaneously encourage production and supply to meet demand and support effective marketing.

However, more importantly, it is required that these production activities should be eco-friendly in order to continually sustain optimal productivity and profitability without environmental degradation and deterioration. Therefore, at present the livestock industry, particularly the swine industry faces a great challenge of increasing production and expanding production frontiers by developing solutions to the problems currently facing the industry. One such problem is the determination of optimal true digestible Ca to true digestible P ratio in the diets of growing and finishing pigs to help in addressing the current problem of environmental pollution due to P emanating from intensive swine production. To this end, the NRC (1998) data based on total Ca to available P ratio for pigs are still in use. However, with current modern genetic strains of pigs in addition to improved management industry practices, the NRC (1998) data may be obsolete for supporting optimal pig performances and therefore explain in part the high levels of P excretion in the pig manure that is currently highly undesirable. Thus, in an attempt to meeting the requirements of Ca and P for growing and finishing pigs, these nutrients in most cases are either included in excess or below minimum requirements for pigs of these physiological states. Under these conditions, as previously stated, animal performance is compromised, in addition to more Ca and P being present in animal manure leading to environmental pollution, such as eutrophication (Mallin, 2000). These trends can potentially aggravate the cost of production as a result of the search for optimal Ca and P inclusion levels in swine diets because of their essential physiological functions, such as promoting bone health and supporting optimal growth. Additionally, it has currently resulted in both provincial and federal authorities of most nations, including those of North America, enacting more stringent regulations in the management of farm nutrients to protect the environment in a bid to prevent environmental degradation and pollution which threaten sustainable productivity. From the above discussion, it is clear that the present problem is primarily nutrition related. Accordingly, proper nutritional management remains the core to addressing the problem. Therefore, there is a need to nutritionally strategize for environmental protection in conformation with government regulations.

1.2. The Use of Corn and Soybean Meal in Swine Nutrition

Since the major aim of the swine producer is to make profit without compromising standards of production while meeting government regulations on the environment, there is an urgent need more than ever to strategize for reduction in feed cost emanating from Ca and P ratio levels in swine diets while simultaneously reducing the amount of Ca and P, particularly P in swine manure. Traditionally, the use of corn and soybean meal (SBM) has become very popular in the feeding and nutrition of growing and finishing pigs. Compared with barley and wheat, corn fibre levels are the lowest (NRC, 1998) and are a more efficient source of energy than barley (NRC, 1998). Furthermore, corn is a high yielding cereal grain and results in more pounds of pork being produced per acre than barley. Corn's Ca and P contents are lower compared with barley and wheat (NRC, 1998). SBM with its high P contents complements corn in swine diets, thereby making corn-soybean meal diets excellent for swine. Soybean meal like corn is also low in fibre and therefore is a good source of energy and crude protein (NRC, 1998) for pigs. It is also a rich source of critical or limiting amino acids, such as lysine, threonine and tryptophan that are lacking in corn. Furthermore, the low Ca and P contents in corn are again complemented by the high contents of Ca and P in SBM.

For the fact that both corn and SBM are palatable with very similar high apparent ileal dry matter (DM) digestibilities of 89 versus 90% for corn and SBM, respectively (NRC, 1998), it follows that with better nutrient management strategies, pigs would significantly reduce their dependence on the energy originating from their hindgut by microbial activity, as less nutrients, including fibre would be entering the hindgut (Smith et al. 1999). This source of energy is not as efficiently assimilated as that originating from glucose metabolism in their small intestine. Furthermore, the use of corn-SBM in swine diets can also significantly reduce the odour for neighbours in pig producing areas and therefore serve as one of the main nutritional approaches of managing the odour problems in pig production. Since the problem of pungent odour emanating from pig feces is principally associated with the fermentation of undigested feed materials in the large intestine of the pig, the feeding of corn/SBM diets to pigs has very great potential in reducing odour emissions since much of the feed residues used by microbes in the hindgut would not be available for such fermentative processes. In this way, the use of corn/SBM diets offers another additional advantage in odour management emanating from swine production.

Irrespective of the excellent complementary effect and advantage existing in the use of a corn and soybean meal combination for swine, up to this time swine nutritionists have not established a universally acceptable optimal true digestible Ca to true digestible P ratio that best support animal performances with minimal nutrient losses, such as Ca and P in the pig manure to the environment. At present, nutrient losses emanating from pig production into the environment is a huge concern to all stakeholders, including the nutritionists, producers, feed formulators and the environmentalists, especially when one considers environmental pollution due to excess P in the soil caused by the application of pig manure as a fertilizer. There should be a balance between animal production activities and environmental safety in nutrient management strategies in line with government regulations for sustainable agricultural productivity and profitability. However, the magnitude of this environmental concern will continue to increase with grave consequences (Mallin, 2000), if strategies to curb the challenges in line with government policies are not developed. This observation comes to the fore from the fact that animal production activities are still on the increase (Tamminga, 2003), especially in North America, due to intensive animal production activities in the region.

1.3. Developing a Sustainable and Profitable Eco-Friendly Swine Production System

Nutritional strategies are the fundamental keys to developing sustainable and profitable swine production systems to conform with the current required production standards, particularly as it concerns environmental protection. Therefore, as previously stated, if optimal ratios of true digestible Ca to true digestible P are not used in diet formulations animal performances would be negatively impacted and environmental pollution emanating from Ca and P would increase via their excessive excretion into the environment as a result of their poor retention and utilization by the animal (Reinhart and Mahan, 1986). This would also increase the cost of production with attendant losses of revenue to swine producers. These scenarios present a serious threat to the swine industry, and animal industry generally and therefore require studies geared towards developing nutritional strategies and information to aid in the better management of true digestible Ca to true digestible P ratio in swine nutrition. The success of developing such strategies certainly would better support the health of the animal and subsequently enhance animal performances through improved nutrient retention in addition to reducing foul odour generation and environmental pollution originating from animal production. This therefore would also culminate in a significant boost to the swine industry, especially now that intensive swine production has become a huge source of revenue in many regions, including North America.

Based on these developments, there is a strong need to develop nutritional strategies to effectively manage Ca to P ratio in nutrition for swine. Many nutritional strategies have been examined but none have been found to be very effective in proffering the required solution. A recent nutritional manipulation approach has been shown to be promising in dealing with the situation, such as the unpublished data of Fan (2007). This work is at a preliminary stage and therefore needs to be evaluated further to draw conclusions in the adoption of a strategy for the effective management of Ca and P in swine nutrition. Therefore, in this study, the preliminary data of Fan (2007) will be evaluated in terms of insights it provides into the efficacy of Ca and P utilization, as well as its effects in the utilization of other essential nutrients by the growing pig.

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

During the last few years, swine production has increased tremendously globally. For instance, according to Manitoba Agriculture, Food and Rural Initiatives about 2 million hogs were produced in Manitoba alone in 1999 and by the end of 2005 the production level was more than doubled with about 9 million hogs produced per year. Similarly, according to Hog Statistics, Canada, the total number of hogs on farms in Ontario as of July, 2005 was about 4 million. Furthermore, pork consumption has been estimated to account for over 40% of the annual total meat consumption in the United States (Pond and Lei, 2001). This trend clearly shows that the swine industry currently represents a large source of revenue and therefore has become very lucrative as it has also been shown that in the United States, pork production accounts for over $66 billion in annual economic activity (Hollis and Curtis, 2001). This present trend has therefore resulted in increasing interest in the search for strategies to maximize production of quality pork and its related products at lower cost to producers, especially in an eco-friendly manner in order for the environment to be able to continuously sustain productivity. In fact, nutrition is a very important component of pig production as it represents about 65 – 70% of the total cost of production (Prairie Swine Centre Inc. 2000). Traditionally, cereal grains, particularly corn and SBM are used as sources of energy and other nutrients, such as protein, Ca and P in the rations of pigs. As part of the means of reducing the cost of production and increasing the revenue of the hog farmer, corn and SBM have also traditionally been widely used in swine rations due to their palatability and high digestibility coefficient characteristics (NRC, 1998).

However, these nutritional benefits to the hog producer with respect to the use of corn and SBM in the diet are currently being threatened as a result of environmental pollution emanating from intensive swine production (Mallin, 2000). The intensification of the swine industry has come with negative effects on the environment. Firstly, some researchers have shown that intensive swine production is correlated with some human health problems within the vicinity of production facilities. Health concerns include accelerated decline in pulmonary function and an increased incidence of bronchitis, sinusitis, inflamed nasal mucosa, throat irritation and headaches (Schenker et al. 1998; Donham, 2000). These health problems are further believed to be associated with change from positive mood to negative mood culminating in "stress". More importantly, intensification of swine production has also been strongly shown to be responsible for environmental pollution due to high level of P excreted in the pig manure (Mallin, 2000). Due to these negative effects, it is apparent that in the near future sustainable swine production and the society would experience more constraints reflecting concerns with animal well-being and health, quality of animal product and production system, utilization of nutrients and the fate of the environment or ecosystem.

Meanwhile, swine production activities are still on the increase due to rising demand from consumers for animal protein (Tamminga, 2003) implying that more damage may be done to the environment from the swine industry. Interestingly, it is known that the negative environmental impacts from intensive swine production is as a result of or is influenced by the amount of undigested or non-utilized dietary nutrients and endogenous products generated by the hindgut of the animal that is eventually released into the environment via pigs' manure and urine. To this end, although nutrient losses in the pig manure are inevitable (Tamminga, 2003), nutritional strategies still remain the fundamental key in managing nutrient losses in swine nutrition. For any nutritional strategy, such as feed formulation to be effective in minimizing environmental pollution, it should be targeted at reducing the causative agents, such as the amount of P excreted into the environment via the pig manure and urine. This therefore necessitates that both economic and environmental implications be explored for the development of nutritional strategies in consonance with government regulation for reduction of nutrient in the pig manure in swine nutrition in order to protect the environment from the negative effects of intensive swine production. It is also required that animal production activities should be eco-friendly, especially with neighbours living in the vicinity of production facilities and in the society generally in order to continually sustain and support optimal animal productivity and profitability without environmental menace, particularly now that there is an increasing interface between animal agriculture and the society in many regions. This is important as even more of such interactions are projected to be on the increase due to the intensification and industrialization of the swine industry. All the above raised concepts will be covered in detail in this review.

2.2. Factors Affecting the Feeding Value of Corn and SBM in Swine Feeding and the Nutritional Challenge

As previously discussed, corn and SBM are traditional feed ingredients commonly used in livestock feeds in North America, including Ontario, Canada. They are well recognized as good sources of digestible energy (DE) and also provide other nutrients, including crude protein (CP), Ca and P complementarily in corn-SBM-based diets (**Table 2.2**).

Table 2.2. DE, Ca, P, NDF and ADF contents of barley, corn, SBM and wheat

(data on as-fed basis)

Item	Barley	Corn	SBM	Wheat
DE (Kcal/kg)	3,050; 3,100	3,525; 3,550	3,685; 3,675	3,365; 3,250
CP (%)	11.3; 10.6	8.3; 8.5	43.8; 44	13.5; 12.9
Ca (%)	0.06; 0.07	0.03; 0.02	0.34; 0.29	0.05; 0.10
P (%)	0.36; 0.35	0.28; 0.25	0.69; 0.61	0.36; 0.32
NDF (%)	18.0; 17.8	9.6; 12.0	8.9; 9.3	13.5; 10.8
ADF (%)	6.2; 7.1	2.8; 3.4	5.4; 6.6	4.0; 3.5

References: NRC (1998); Patience et al. (1995)

As shown in **Table 2.2**, corn and SBM have higher DE values compared with barley and wheat but conversely, barley and wheat have higher fibre contents compared to corn and SMB. These factors therefore can partly explain why growing-finishing pigs fed barley rations show inferior performance in contrast to those fed corn. Crude fibre has long been known to be a limiting factor affecting pig performance due to the fact that a high level of this component does not allow pigs to consume enough net energy to enable them to gain at a maximum and efficient rate. Fibre acts as a diluent of available nutrients and may also contain factors which can physically or chemically inhibit nutrient digestion, absorption and utilization. These observations were supported by the results of a study in chickens (Rotter et al. 1989) where digestibility data indicated that the observed effect on growth and feed conversion were primarily caused by lowering the digestibility of energy-supplying nutrient of the diet, a reduction that is greater with fibre from barley hulls than from cellulose. Furthermore, it was suggested that a more pronounced performance depression with fibre could result from a physical interference with the activity of digestive enzymes due to the form of the fibre or from direct chemical inhibition of enzyme action (Low, 1985). Additionally, it has also been identified that grains contain some soluble fibre which are associated with poor utilization of nutrients. This is due to the fact that their inherent indigestibility is aggravated by their solubility which causes increased digesta viscosity in the digestive tract thereby interfering with the availability of energy and nutrients by limiting the rate and amount of both digestion and nutrient absorption. Specifically, the viscous condition in the lumen of the digestive tract disrupts the interaction of endogenous enzymes with substrate resulting in the limitation of nutrient digestion uptake and retention. This condition further enhances endogenous nutrient losses that contribute significantly to high nutrient concentrations in the pig manure (NRC, 1998).

Although nutrient losses to the environment are inevitable in swine production (Tamminga, 2003) the factor that is currently nutritionally more challenging to nutritionists and feed formulators is the establishment of an optimal Ca to P ratio or range over which the ratio may vary without harmful effects on pig performance; while concomitantly reducing P excretion from the animal. It is imperative to state that an inappropriate ratio of Ca to P leads to poor animal performance due to imbalance in Ca and P metabolism that consequently culminates in poor P retention leading to the majority of dietary P being excreted in the pig manure. Part of the reason responsible for this observation is the fact that most P of plant origin is in the form of phytate-P. To aid in matching digestible dietary P in swine diets, optimal digestible P from plant sources such as corn and SBM in swine diets needs to be determined. It has been demonstrated by different independent studies that the apparent digestibility of P in both corn and SBM grossly underestimate true digestible P from these feed ingredients (Ajakaiye et al. 2003; Shen et al. 2003). To this end, a novel method for determining true nutrient digestibility associated with a diet has been established. This is known as the substitution method (Fang et al. 2007) as depicted in figure 2.2.1 with P as the mineral whose true digestibility (D_T) is to be determined.

2.2.1 Determination of True Digestible Ca and P in Diets by the Substitution Method

Phosphorus is both a physiologically and economically important nutrient for swine (NRC, 1998). It is also a major pollutant of the environment emanating from intensive swine production (Mallin, 2000). One of the major reasons to explain this scenario is the fact that most plant P used in swine diets is in the phytate form. Pigs are known to lack the phytase enzyme that hydrolyses phytate to release the phytate-associated P for use by the pig. Therefore, excess P is always included in the pig diet to avoid its deficiency, leading to high levels of P excretion in the pig manure. This is currently perceived as a major negative impact of intensive swine production on the environment since it has been shown to be responsible for environmental degradation and deterioration (Mallin, 2000). The determination of an optimal true digestible Ca to true digestible P ratio for pigs fed corn-SBM-based diets would go a long way in addressing some of the problems associated with P management in swine nutrition.

It has been established from previous studies that there are no differences between the distal ileal and the fecal P digestibility in pigs (Fan et al. 2001; Ajakaiye et al. 2003). Furthermore, it has also been recommended that true rather than apparent fecal P digestibility should be measured in feed ingredients and diets for pigs (Fang et al. 2007). This is based on the premise that true P digestibility will better guide the feed formulator to matching P requirements of pigs using low-P levels in the diet, since apparent P digestibility underestimates true P digestibility (Ajakaiye et al. 2003). True mineral digestibility by the principles of the difference method is based on the premise that true mineral digestibility does not change as well as the inevitable endogenous loss of the mineral and its intake increases. However, as the mineral intake increases the apparent digestibility also increases with increment of the undigested mineral in the total fecal loss.

Therefore, the measurement and quantification of the endogenous P secretions, recycling and losses are very important components in deriving true P digestibility associated with feed ingredients or diets (Fan et al. 2008). Normally, endogenous P loss is the next major route of P inefficiency after the indigestible fecal P (Fan et al. 2008). Additionally, when pigs are fed within the requirement range of the animal, urinary P excretion is usually within 2% of the total manure P loss (Pettey et al. 2006) and thus considered as the third route of P loss from the animal.

Figure 2.2.1. ***Modus operandi*** **of the substitution method**

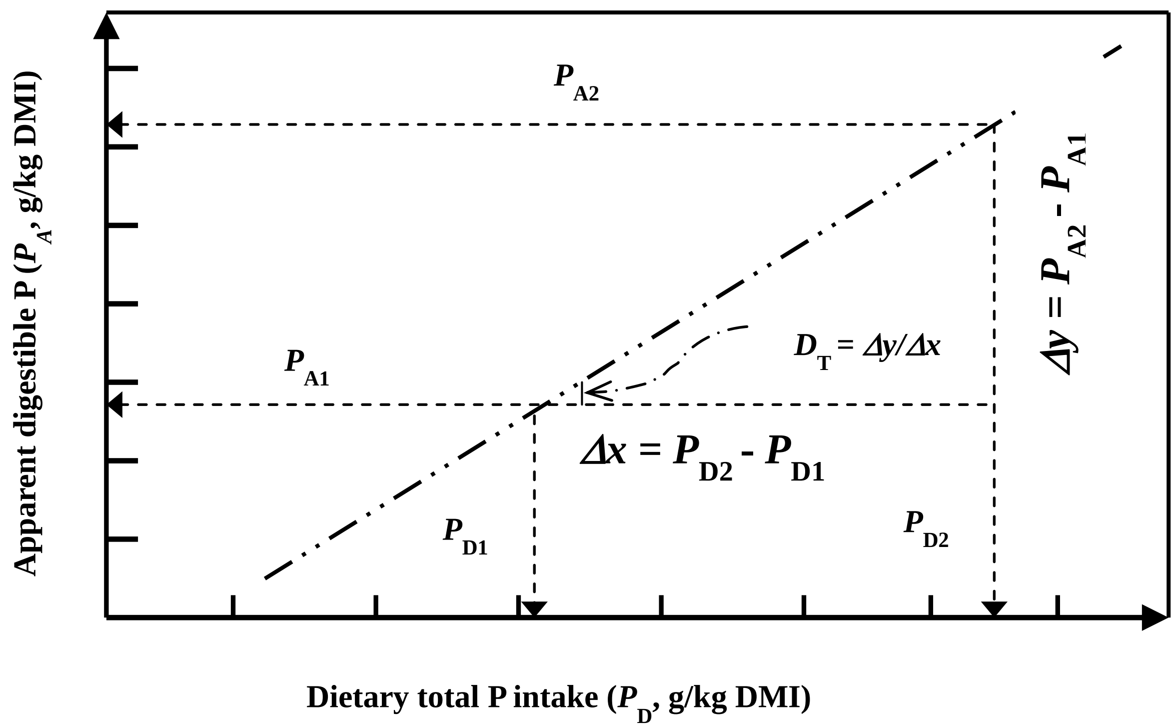


Illustration of the substitution method for measuring true P digestibility (D_T) and endogenous P output associated with a test feed ingredient. Further details are provided in the methodology sections of studies.

Adopted from: Fang *et al.* 2007; Fan *et al.* 2008

According to the data reported in the literature, it is thought that about 22 to 25% of P intake is retained by pigs, especially for corn and SBM, respectively (Shen et al. 2002; Ajakaiye et al. 2003). This results in the majority of the P intake being excreted via feces and urine in corn-SBM diets. Due to the accumulation of P in soil from pig manure, recent researchers have focused on strategies to improve P utilization without compromising the pig's health and productivity with the sole goal of reducing the amount of P excreted in the manure. However, it is a known fact that P together with Ca play essential physiological roles in the pig. For example, they are involved in the development and maintenance of the skeletal system, and in fact about 75% of the body P pool is located in the skeleton (Poulsen, 2000). Furthermore, another structural function of P is that it is an important element of the phospholipids found in cell membranes. In addition, as a constituent of adenosine triphosphate (ATP) and other phosphorylated intermediates, phosphorus also takes part in energy metabolism (NRC, 1998). Therefore, with all these well-documented important physiological roles of P, there are no doubt that its deficiency would lead to impaired metabolism and growth and abnormal bone mineralization. Phosphorus is also known to be an important economic factor in swine production (NRC, 1998), typically being the third most expensive nutrient in the diet after energy and protein. Nevertheless, it is still a common practice to include a plentiful supply of P in swine diets, mainly to ensure that P deficiency does not occur. Since the exact P requirement is not well established in swine nutrition, this common insurance policy approach to dietary P inclusion levels for swine is currently widely used in swine diet formulations, results in excessive P excretion in the manure. Therefore, it is necessary to re-define P requirements in terms of digestible P instead of total P in the bid to insure the so-called safety margins. Accordingly, the determination and use of digestible P in swine diets will enable pig farmers and feed manufacturers to formulate diets that contain the optimal amount of digestible P required by pigs for maintaining normal P physiological roles and therefore reduce the P levels in the manure.

It has been demonstrated that there is no difference between the use of regression analysis technique and the substitution method in the determination of true P digestibility and fecal endogenous P losses associated with feed ingredients for growing pigs (Fang et al. 2007). There are considerable data showing that true P digestibility in SBM is underestimated by about 25% for both post-weaned and growing-finishing pigs (Fan et al. 2001; Ajakaiye et al. 2003). Similarly, in corn true P digestibility is underestimated by 30-38% (Shen et al. 2002; 2003). With these data, there is paucity of information in relation to the true digestible Ca to P ratio, especially for corn and SBM which are common feed ingredients for swine. There is a need to evaluate in detail the preliminary findings of Fan (2007) for the growing pig fed corn-SBM diet before it can be recommended for adoption for managing Ca and P effectively in a more eco-friendly and economical fashion for corn-SBM diet for the post-weaned pig. This first involves determining the optimal digestible values of Ca and P (%).

2.3. Effects of P, Ca and Mineral Content Levels of Diets on P and Ca Digestibility, Diet Quality and Nutrient Utilization in Swine Nutrition

Although minerals are essential nutrients for pigs, they do not need to be in excess of animal requirements as the surplus may result in undesirable accumulation of pollutants in the soil and eutrophication of groundwater and freshwater. This is also related to the impact of dietary mineral content on the bioavailability of other minerals (Hu et al. 2010; Miura et al. 1999). Although excess minerals do not exhibit an anti-nutritional effect *per se*, they nevertheless are considered undesirable as they can potentially cause the dilution of nutrients in the diet (a negative effect that mimics the negative impact of fibre in the diet) as well as diarrhoea. For example, this assertion comes from the fact that among feed effects energy concentration of diets is one of the major nutritional factors causing variation in feed intake (Smith et al. 1999). Consequently, it has been reported that the regulation of feed intake depends on the energy densities of diets. Therefore, at low energy densities, energy intake is reduced and subsequently growth performances of the animals are similarly reduced (Smith et al. 1999). This observation has further been demonstrated in studies with grower-finisher pigs (Brady et al. 2002). In that study the lowering of the total Ca to total P ratio from 1.85: 1 to 1.15: 1 significantly increased the digestible energy content of the diet which also accounted for the superior performance of the pigs on the reduced levels of Ca to P in their diets compared with animals presented with diets of high levels of Ca to P. Another factor that can potentially affect the bioavailability of specific minerals is the interrelation existing between the different minerals within the diet. This implies that the concentrations of the different minerals in the diet should also be within their true digestible levels, since they could be nutritionally antagonistic to each other when supply is below or above the animal requirements. For example both magnesium and P are antagonistic to Ca in swine feeding (Peo, 1991; Jongbloed et al. 1999). Another important angle to this is that due to hormonal regulation, the rate of absorption depends on the level of P supply in relation to the requirement. Similarly, in a study (Atkinson et al. 1993) involving the use of piglets to investigate the effect of a combination of Ca and P on zinc, copper and iron absorption across the intestine, the piglets were fed a complete liquid diet supplemented with Ca and P, as well as one of the following: zinc alone; zinc plus copper; or a combination of zinc, copper and iron. After a 5-day adaptation period the piglets were orally and intravenously dosed with a radio- active isotope mix containing zinc, manganese, iron, selenium and Ca. Isotope levels in the body were measured after 15-day duration during which time fecal excretion of any unabsorbed isotope was monitored. Dietary supplementation with Ca, P and zinc reduced isotopic zinc uptake compared to the control pigs. Increased intake of a combination of Ca, P, zinc and copper tended to reduce iron absorption as well. Unfortunately, due to the study design it was not possible to determine whether only one or a combination of these elements were associated with these alterations in zinc and iron absorption. Nevertheless, the findings of this study demonstrate that the level of the presence of certain minerals in the diet can impact upon the absorption of other minerals. However, at this stage, some of the exact mechanisms modulating these interactions are not clearly understood. But this result is a clear pointer to some of the important implications of some useful interactions among mineral in swine feeding that must be taken into consideration in swine diet formulations along with feed ingredients used.

Ingredients are selected based on their availability, composition and cost (NRC, 1998). Nevertheless, the primary concept behind ingredient selection is that nutrients such as P fed in excess of animal requirements is excreted, indicating that reduced overfeeding when employed can serve as a powerful nutritional manipulation strategy to reduce the P content of the pig manure. Accordingly, in practical swine feeding minerals should not be added haphazardly according to the old adage that “if little is good, then more would be better” as this is not true in mineral metabolism in swine nutrition. Therefore, animal diets should not contain plenty or excess Ca and P. Furthermore, all minerals have a toxic level. For example if Ca is added in excess of animal needs in the diet, it is capable of impairing it’s absorption and utilization in addition to interfering with the absorption of other minerals, such as P, Mg, Cu, Fe and zinc (Peo, 1991; Atkinson et al. 1993). This is of particular importance as Ca competes for active sites of phytase; an exogenous enzyme that aids phytate-P degradation and the release of other phytate trapped minerals (Montminy et al. 2007). This is further explained by the fact that an increase in gastric pH decreases P hydrolysis, especially when diets contain microbial phytase. Similarly, an excess of Ca has been shown to negatively affect P solubility as soon as dietary Ca to P ratio exceeds 2.1 (Montminy et al. 2007). Again, decreased transit time in the stomach has also been shown to linearly decrease P hydrolysis (Montminy et al. 2007). These mechanisms involved in the metabolism of P therefore may also account for excessive P excretion in the pig manure which is one of the major concerns in areas where intensive swine production activities exist. Based on these accounts, to be able to successfully manipulate dietary Ca and P in the diets of pigs to overcome the problem associated with excess P in the pig manure, there is a strong need to define an optimal true digestible Ca to P ratio or range since Ca and P metabolism are interrelated (NRC, 1998). When clearly defined and used in diet formulations for pig, it could serve as a powerful tool to manage P efficiently and thus significantly reduce environmental pollution due to P from swine production.

As a result of the current situation presented above, increased swine and crop production have led to the net export of nutrients from major crop-producing areas to areas with high concentration of animal agriculture, such as swine production. Swine utilize P inefficiently, resulting in the high levels of P in the pig manure, implying that the majority of P brought on to the farm in feed also stays on the farm, rather than being retained by exporting it into meat and milk. Eventually, pig manure is typically applied to the land supplying crops with nutrients for maintenance and growth purposes. However, the high level of P in such manures has resulted in an imbalance between nitrogen (N) and P relative to crop needs. This has also resulted in the over-application of P and therefore led to accumulation of P in soils. In the past, it was primarily thought that P contamination of surface water was related to erosion. Now it is known that the continuous application of P in excess of crop requirements causes the soil to become saturated thereby giving room for run-off of P to occur independently without erosion (Daniel et al. 1992). Since projections support increased swine production (Tamminga, 2003), practices that reduce P losses from farms without compromising animal performance and profitability deserved to be developed and implemented so food supply will not be threatened. These involve the development of improved strategies in understanding the kinetics of P digestion and metabolism in swine nutrition. This would consequently improve the efficiency of P utilization, thereby aid in reducing excess P excretion in the pig manure. It would thus also aid in minimizing the imbalance between N and P in the pig manure. Overall, one of such areas that deserve attention is striking a balance between the levels of Ca to P in the diet via a suitable Ca to P ratio range in the ration that does not interfere with effective metabolism of P as well as other dietary minerals.

2.3.1. Current Status and Environmental Concerns

As previously highlighted the excessive excretion of P in pig manure from intensive swine production activities has become a major environmental concern as it has been shown that it is a major cause of environmental deterioration and degradation (Mallin, 2000). It is known that crops generally require much less P compared with nitrogen. The applications of pig manure to crop fields invariably lead to P accumulation in surface soil; and eventually results in run-off and/or leaching of P into surface water resources causing eutrophication of the surface water system with huge negative impacts on the environment (Mallin, 2000). For sustainable swine agriculture, there is a need for the swine industry to strategize for effective and better management strategies for reducing both total and soluble P levels in pig manure in order to curtail its negative impacts on the environment.

The determination and use of optimal digestible low-Ca and low-P levels in pig diets have the potential of significantly reducing the amount of P in the pig manure. Traditionally, in swine nutrition, calcium phosphate and limestone are normally used to meeting the requirements of these macro-minerals. However, the preliminary data of Fan (2007) indicated that the total amount of calcium phosphate and limestone required for supplementation to meet the requirements for Ca and P is about 5 kg for marketing each pig weighing about 110 kg. Further expansion of the estimation data of Fan (2007) also indicated that an effective strategy in the use of low-Ca and low-P ratio in the formulation of weanling pig diets has the potential of reducing the use of these mineral salts by about 40% based on their current dietary supplementation levels. Consequently, the reduction in the use of these salts by about 40% represents about $4.5 million saving on feeding cost per year, for Ontario pork production alone. This is further explained by the fact that P is the most expensive mineral added to swine diets. Therefore, the potential exists for significant reduction in cost of production to the swine industry by improving the efficiency of P utilization in pigs, especially now that the industry has become one of the major sources of revenue for many economies, globally. This signals a strong scientific challenge to swine nutritionists and feed formulators.

2.4 Current Recommended Ca to P Ratio in Swine Diets

According to NRC (1998) data compiled based on studies conducted many years ago (which might not be adequate for modern strains of pig) suggest total Ca to available P ratio to be from 2:1 to 3:1 and for total Ca to total P ratio, it is recommended to be between 1:1 to 1.25:1 in grain-SBM diets. Peo (1991) indicated that adequate Ca and P nutrition for all classes of pigs is dependent mainly on three major factors: an adequate supply of each element in an available form in the diet; a suitable ratio of available Ca and P in the diet; and the presence of adequate vitamin D. NRC (1998) also reported that narrower calcium-to-phosphorus ratios whether total or available P, probably results in more efficient utilization of P. It means that a narrower Ca to P ratio would not result in excess inclusion of P in the diet at the expense of animal performance. Again, it would not lead to releasing excessive P in the pig manure. Other studies have also shown that the feeding of large amounts of Ca and P to growing pigs does not necessarily improve structural soundness (Eeckhout et al. 1995) nor has it been shown to be necessary for good health or longevity (Kornegay et al. 1984). The initial data of Fan (2007) also agrees with the data of Peo (1991) and that of NRC (1998) that a narrower ratio of available Ca to available P better support animal performance resulting in minimizing P excretion in the pig manure. These may be related to the internal Ca and P homeostasis mechanisms by tight hormonal regulations of parathyroid hormone, calcitonin and vitamin D (Crenshaw, 2001).

It is possible that P excretion can be minimized through better feeding regimes (Poulsen, 2000). Accordingly, one of the major goals of the swine industry in recent times has been the search for a suitable strategy of improving P utilization and consequently reducing its release in the manure to alleviate its negative impacts on the environment. Furthermore, for the strategy to be efficient and suitable for use, it should also be inexpensive and cost-effective to avoid increase in cost of production. It is known that there are two main causative factors influencing the inefficient P utilization and excessive P excretion in pigs, the first being phytate P. The second factor is over supplementation of inorganic phosphate salts and limestone in swine diets as a result of paucity of information on P and Ca requirements based on an optimal true digestible Ca to true digestible P ratio for pigs. This scenario is further compounded as the pig requirement for minerals like other nutrients is also highly dependent on their physiological status, such as growth rate. About 50% of dietary P is in the 'phytate form' and thus cannot be digested by pigs except by the provision of the 'phytase enzyme' since pigs naturally lack this enzyme especially young and growing pig. As a result of this, the different strategies that have been developed to curb P problem include dietary supplementation of microbial phytase (Simons et al. 1990), development of novel phytase pigs (Golovan et al. 2001), and the introduction of new crop cultivars that produce grains with relatively low phytate P content (Spencer et al. 2000). To date, these strategies are only capable of marginally reducing P excretion.

Until a dependable, reliable, cost-effective and commercially applicable strategy is developed, the animal industry, particularly the swine industry, will continue to experience the problems associated with P utilization in relation to excretion. This is where defining P requirements as digestible P instead of total P may enable diet formulation closer to actual physiological P pig requirements. This strategy certainly would come with two major benefits. Phosphorus excretion in the manure will be reduced and secondly, the cost of excess dietary P will be eliminated thereby increasing net income of pork producers because P is the third most expensive nutrient after energy and protein (NRC, 1998). Accordingly, this is where optimal true digestible Ca to true digestible P ratio in swine diets may prove effective as it shows some promise of being effective in minimizing P excretion and also increasing net income for producers (Fan, 2007). An inaccurate Ca to P ratio can also result in bone problems particularly in pigs fed microbial phytase (Veum et al. 2002; Emiola et al. 2009) and the phytase pig (Brady et al. 2002). Inaccurate Ca to P ratio also reduces both Ca and P retention with the resultant effect of increasing their excretion (NRC, 1998). Accurate determination of bioavailability of P in feed ingredients and the formulation of swine diets based on bioavailable P supply are very important in ensuring efficient P utilization (Jongbloed et al. 1991).

Traditionally, P availability in feed ingredients is usually measured using the apparent digestibility studies and the slope-ratio assay (Jongbloed et al. 1991). Relating to the results data of Fan *et al.* (2001) the nutritive value of P in feed ingredients for pigs were considerably underestimated using the traditional method that is widely used in European countries and the United States of America. Accordingly, using the data of that study in conjunction with that of Ajakaiye *et al.* (2003) true P digestibility in SBM was determined to be 50% which is much higher than the average value of 25% reported in the literature. Similarly, true P digestibility in corn was measured to be 57% which is also considerably higher than the average value of 22% reported for corn in the literature using the traditional method (Shen et al. 2002; 2003). Again, Ca digestibility in SBM was determined to be 67 and 97% in post-weaned and growing-finishing pigs, respectively (Fan et al. 2000; Ajakaiye et al. 2001).

Based on the findings of these studies, it is apparent that excessive amounts of supplemental Ca and P salts are used in pig diets partially due to underestimation of the nutritive values of P in feed ingredients, such as corn and SBM. This has resulted in high level fecal P as well as a large proportion of P in the readily mobile and water-soluble form (Fan et al. 2001) leading to environmental pollution with huge negative consequences (Mallin, 2000). Information on the optimal dietary true digestible Ca to true digestible P ratio which could be more eco-friendly and economical in intensive swine production is therefore required; since the current status in use is based on dietary

Ca to P ratio values for pigs at different phases of production being determined on the basis of total Ca to total P or apparent digestible P supply (NRC, 1998). Such information when obtained would guide the use of optimal dietary true digestible Ca to true digestible P ratio in the formulation of low-P diet for minimizing fecal P excretion. Additionally, adequate true digestible Ca to true digestible P ratio would better support efficient Ca and P deposition thereby ensuring good bone health and also enhance animal performance. Therefore, an effective nutritional strategy remains a potential strategy in managing P in swine nutrition to reduce the negative effects of P on environmental pollution.

2.5 Major Strategies for Managing P in Swine Manure

From the situations presented earlier, it is clear that environmental pollution due to swine production is highly undesirable (Mallin, 2000). Although nutrient release into the environment is inevitable (Tamminga, 2003) the potential exists for significant curtailment in the degree of the environmental pollution caused by P released via manure and urine into the environment. As a result of this, one of the major goals of the agricultural industry in recent times has been the search for a suitable means of reducing P level in the pig manure to alleviate the negative impacts it has on the ecosystem.

In order for any strategy to combat P pollution to be viable and effective for adoption for use by the swine industry it needs to be efficient in reducing P and other mineral, such as Ca, levels in pig manure as their metabolism are interwoven (NRC, 1998). Furthermore, the strategy needs to be simple and inexpensive and at the same time be ideally suitable for use in existing technology in the environment so as not to unnecessarily increase the cost of production. Additionally, the strategy should not significantly alter or affect the normal process of nutrient digestibility so as not to compromise animal health and performance. Until a cost-effective applicable strategy of reducing P in the pig manure in order to minimize its negative effect on the environment is available the food and agricultural industries will continue to experience the problems associated with P pollution. This poses a serious challenge and threat to the swine industry and swine nutritionists. To this end, as briefly mentioned above different strategies have been tested and some of them appear promising. These strategies have been categorized into the use of microbial phytase, use of low-phytate crop cultivars, commercialization of phytase enviropig^{TM}, and improving diets based on the use of optimal true digestible Ca to optimal true digestible P ratio.

2.5.1 Use of Microbial Phytase to Release Phytate P

Simple-stomached or non-ruminant animals such as the pig and poultry lack the phytase enzyme that degrades phytate-P. Most of the plant sources of P in swine diets are phytate-associated P which is known to be poorly digested by pigs. This scenario has been identified as one of the major reasons responsible for the problems of P pollution in regions experiencing intensive livestock production. The technique of adding exogenous phytase to swine diets to aid the hydrolysis of phytate-P by rendering it available to the pigs has been employed as a means of reducing P levels in the pig manure (Simons et al. 1990). In that study it was concluded that addition of microbial phytase to diets for growing pigs increased the apparent absorbability of P by 24% resulting in the reduction of P amount in the pig feces by 35% and also improved growth and feed efficiency. However, in the same study, the authors acknowledged that when the same microbial phytase was added to low-P diets for broilers the efficacy of P availability was higher resulting in a significant reduction level of P in the poultry droppings. They stated that when the availability of P improved the availability of Ca also increased. This is an indication that the metabolism of these minerals may be dependent on each other. Therefore, from the results of that study it could further be extrapolated that the use of exogenous phytase in animal diets could be further enhanced in the presence of low-P and low-Ca diets, since Ca and P availability to the animal are positively correlated (Simons et al. 1990). Interestingly, the coefficients of P and Ca availabilities were higher when the ratio of Ca to P was narrower in the low-P diets in the same study. Recently the efficacy of this technology has also been shown to improve P digestibility and utilization resulting in the reduction of P level in the pig manure and growth performance in growing pigs (Emiola et al. 2009). However, other workers have also reported no improvement in Ca digestibility as a result of phytase supplementation (Sands et al. 2001). This apparent inconsistency involving the use of this strategy to improve Ca retention may not be unrelated to the imbalance of Ca to P ratio in these studies.

Although the strategy involving the use of phytase to improve P digestibility and performance appear promising, they do suffer from some drawbacks. For example, the activity of microbial phytase showed pH optima at pH 5.5 and 2.5 (Simons et al. 1990). The jejunum which represents almost 90% of the total length of the small intestine has a mean pH of 5.5 to 6.9 while the ileum has a mean pH of 7.0 to 7.4 (Knowlton et al. 2004). The pH values in these different important absorptive segments of the gastro-intestinal tract certainly have a large impact on the efficacy of the exogenous enzyme use technology. Furthermore, the high temperature involved in feed pelleting could destroy phytase and therefore reduce its activity. Although some feed ingredients may contain native phytase activity; steam-pelleting usually used commercially to manufacture pig feeds results in significant losses of this intrinsic phytase activity. The remaining residual phytase activity often may not be considered in diet formulation when feeds are pelleted. These factors are sources of concern in the use of this strategy in effectively managing P in practical swine feeding. It has been suggested that liquid phytase should be sprayed onto the pellets after cooling (BASF, 1993), which again also increases processing costs. Additionally, the phytate molecule has a high chelating potential to form a wide variety of insoluble salts with di- and trivalent cations at neutral pH. A mole of phytic acid is capable on average of binding 3-6 moles of Ca to form insoluble phytate at the pH of the small intestine (Knowlton et al. 2004). The formation of insoluble phytate makes both Ca and P unavailable to the animal. Other trace minerals like Zn, Cu, Mn, Fe and Mg can also be complexed; however Zn, Cu, Fe and Mg have been shown to have the strongest binding affinity (Lei and Porres, 2003). This phenomenon potentially renders these minerals unavailable for intestinal absorption. Other studies have also shown that Zn is the mineral most influenced by phytate (Pallauf and Rimbach, 1995). To further compromise the use of the phytase strategy is the fact that the optimal digestible ratio of Ca to P in diets with added exogenous phytase is also lacking which increases the chelating potential of phytic acid with concomitant reduced digestibility of dietary minerals (Lei and Porres, 2003). For instance, it is the insoluble Ca-Zn-phytate complexes formed in the intestine that eventually impair Ca and Zn absorption. Insoluble Ca-Zn-phytate complex formations from diets containing phytate depend not only on the concentration of phytate in the diet but also that of Ca. Thus in the past this relationship is usually expressed as a critical molar ratio of phytate/zinc and values exceeding 15 are usually considered to indicate a risk factor of reduced Zn absorption (Lo et al. 1981). It is now known that the dietary Ca concentration is a critical factor leading to the derivation of a composite ratio involving Ca, Zn and phytate (Chesters, 1997). Using the data from at least 40 studies that investigated the effects of dietary Ca, Zn and phytate on growth it has been reported that a better predictor of the dietary effects of Ca, Zn and phytate could be expressed as the ratio of this relationship: (Calcium) x (phytate)/Zinc; where the constituents are expressed as moles per kilogram diet. Ratios in excess of 3.5 are associated with significant reductions in growth rate (Chesters, 1997). Therefore information on true digestible Ca to P ratio is required to enable the use of this technology to enhance P, Ca and other mineral digestibility and absorbability in the pig. When such data are available nutritionists will be able to exploit the full benefits associated with this technology.

Here, it is also worth mentioning that the dietary level of P is an important factor that influences the response to phytase (Rodehutscord et al. 1999), implying further that the effectiveness of phytase in enhancing P digestibility and reducing manure P excretion is dependent on the concentration of P in the diet. In that study, pigs fed a diet with digestible P above requirements and supplemented with phytase responded with increased fecal P and consequently, P digestibility was reduced suggesting that dietary P levels beyond animal requirements limits the animal response and therefore reduces the efficacy of phytase inclusion in the diet. Therefore, extremely high or low levels of dietary P should be avoided in order to fully benefit from the use of phytase in optimizing the P digestibility response by the pig. One major effective strategy that can be very useful in exploiting the advantages that can be accrued through the use of phytase in dealing with P digestibility to reduce fecal P is to define the optimal digestible Ca to P ratio. High levels of Ca or wide Ca to P ratios will reduce the effectiveness of the pig response to phytase. It has recently been demonstrated in both broilers and turkeys that Ca retention as well as P retention linearly increased as the amount of supplemental phytase increased and conversely decreased as the Ca: P ratios became wider (Kornegay, 2001). This has also been observed in swine (Liu et al. 2000). To this extent, the overall effectiveness of this strategy to release P and other nutrients, coupled with the need to reduce P excretion and other elements with high potential for translating into disposal costs in its use in managing P in swine diets is limited.

2.5.2. The Use of Low-Phytate Crop Cultivars

Concerns about how to combat P pollution in the environment have led to strategizing for solutions to this problem. Accordingly, another biological approach that has been adopted for increasing P digestibility to minimize P excretion in the feces is the use of feed ingredients with low phytate P contents. This has led to the research and development of low-phytate crop varieties, such as genetically modified low-phytate corn (Spencer et al. 2000). In that study, the authors concluded that low-phytate corn contains at least five times as much available P as normal corn. The use of low-phytate corn greatly reduced the amount of P excreted by the pig and increased the N: P ratio in the manure.

The findings of Spencer *et al.* (2000) were also substantiated by the studies of Veum *et al.* (2002), who showed that the use of low-phytic acid barley improves Ca and P utilization as well as growth performance in growing pigs. With respect to the importance of Ca to P ratio in that study, no information on the optimal digestible level of Ca and P was provided in clear terms to further guide the process of adopting the use of the technology in dealing with P metabolism in swine production. Although the technology appears promising in addressing P pollution problems by the livestock industry, information regarding the optimal ratio of Ca to P inclusion levels in the diets of low-phytate crop cultivars is still lacking; even though improvement in Ca digestibility was reported. Furthermore, the processes involving the development of modified crop cultivars are time-consuming as well as being expensive. Again for this strategy to succeed, agronomic issues including reduced crop yields need to be overcome. However, the effectiveness and successful adoption of this strategy may be dependent on the ratio of Ca: P in diets where these modified crop cultivars are used as feed ingredients.

2.5.3. Commercialization of the Phytase Enviropig^{TM}

The current challenge for improving agriculture and increasing food production without polluting the ecosystem has also led to the development of the phytase transgenic pig (Golovan et al. 2001). The success recorded here was geared towards using the transgenic pig to produce and release the digestive enzyme phytase endogenously to improve P digestibility and thereby provide a relief from the current dependence on the inorganic sources of P in meeting animal requirements for P. Golovan et al. (2001) also reported that the developed transgenic pigs produced salivary phytase that has enabled the pig to require almost no inorganic phosphate supplementation for normal growth and in fact excrete up to 75% less fecal P than non-transgenic pigs without any negative implication for pig health and performance. As laudable as this breakthrough sounds, these workers echoed that the transgenic phytase pig activity decrease with the age of the pig as a result of the diminishing activity of the promoter gene as also observed in transgenic mice. Additionally, they noted that the fecal P observed in their studies could have been derived from endogenous sources that probably escaped digestion and absorption. This could as well be explained in part by lack of information on the optimal Ca to P ratio in the diet of such animals which of course is important and therefore required to better guide the use of the strategy in dealing with P management in swine nutrition.

The second major drawback that may be associated with the use of these new transgenic pigs would be the efficacy of the indogenous phytase activity in the small intestine. This assertion is derived from the fact that the activity of the natural pig phytase like its exogenous counterpart is favoured by an acidic medium or environment, such as in the stomach. Therefore, since the enzyme is only highly active in low-pH it follows that beyond the stomach and possibly the upper part of the duodenum, the enzyme activity will be significantly reduced. This may explain in part the loss of endogenous P found in the manure of the transgenic pigs in the study by Golovan et al.; 2001 and thus stresses the need to define the optimal digestible ratio of Ca to P in the diets of such animals. In order to overcome the weak points in the successful adoption of this novel technology to address environmental pollution due to P from swine production, the optimal ratio of Ca to P needs to be clearly defined. Like the former strategy of the use of low-phytate crop cultivars, this technology is also time-consuming and expensive in addition to cost of skilled personnel required for such studies.

2.5.4. Improving Diets based on the Use of Optimal True Digestible Ca to P Ratio

From the discussion above, it is very clear that none of the strategies considered can stand independently on their own merit in solving the problem of pollution on the environment resulting from P levels in animal feces. In this way, the option of combining the benefits of these different strategies together appear very plausible in using nutritional principles to address the problem, since the core of the problem itself is nutrition centred. Combined supplementation with phytase and improved diets based on the use of optimal true digestible Ca to P ratio may work synergistically together to improve the digestibilities of P, Ca, other macro- and micro-minerals and in fact other nutrients, such as N and energy. Nutrition still remains the key factor to reducing nutrient environmental load from pig production. For instance according to current practices at the industry level, nutrition strategy via diet formulation is already being used to reduce nitrogen pollution and other environmental loads from swine production facilities by meeting the animal minimal crude protein intake or requirements by fortifying diets with adequate amino acids required by the animals depending on their physiological status. This nutritional manipulation has resulted in presenting, to the hindgut, lower amounts of feed products or indigestible feed materials such as N and precursors of odor producing compounds that are eventually released in the pig slurry (Dourmad and Jondreville, 2007; Aarnink and Verstegen, 2007). Indicators from the literature also point to the fact that a similar nutritional strategy via diet formulation can be used to control or reduce P pollution of the environment. Accordingly, from available literature data, the use of low-Ca to low-P diet contents have been shown to have a very high potential for improving Ca and P solubility in the stomach thereby enabling their effective digestion and absorption in the small intestine, especially when phytase is involved (Kornegay, 2001; Brady et al. 2002). It has also been reported that by combining the currently available diet modification strategies, in conjunction with other available technologies, such as the development and commercialization of the enviropig™ and the use of exogenous phytase, it should be possible to decrease the total P concentration in manure by 40% for poultry, 50% for swine and 30% for dairy cattle (Council for Agricultural Science and Technology, 2002). One option to address excess manure P level has been to manipulate animal diets to decrease P concentrations fed to the animal and consequently decrease P excretion in the pig manure without compromising animal health and growth performance. The nutritional logic of reduced P diets is based on the premise that if the concentration of P in the manure is decreased then dietary P is close to matching animal P requirement resulting in nutrient manure loads more closely matching crop P use, thereby culminating in removing P surpluses from the soil. Though the use and implementation of this strategy (reduced P diets) may vary from region to region, may be more effective than the use of a single strategy as earlier stated in managing P control in the manure, as it has been shown to be a very effective method in reducing P in manure while simultaneously enhancing animal performance and feed efficiency in post-weaned pigs (Fan, 2007). The green light indicated in the findings of that study relating to the use of the strategy in curbing P pollution from the swine industry needs to be explored further in managing Ca and P in swine nutrition. Such data when available would effectively serve as good working tools in addressing P nutrition in the livestock industry, including the poultry industry, thereby aiding in changing the negative effect of swine production on the environment to a more environmentally-friendly gesture as prediction indices indicate that swine production is still on the increase and the trend is expected to continue in the future (Tamminga, 2003).

It is also very apparent that this strategy of improving diet would not only be limited to managing P and Ca in swine nutrition but also some other macro-minerals, such as Mg and other trace minerals, such as Cu, Fe, Mn, Se and Zn as the levels of Ca and P in a diet has been shown to also impact these other minerals (Miura et al. 1999; Snedeker et al 1982). These relationships or interactions existing between minerals when well-established will also be useful in effectively managing mineral nutrition in pigs as well as in humans (Wood and Zheng, 1997). At present, due to the fact that calcium is an important mineral in the maintenance of sound bone structure as different independent studies have shown, especially in preventing age-associated bone loss particularly in women; this awareness has resulted in the significant production and sales of calcium supplements as well as the introduction and sales of calcium-fortified foods. However, high intake of Ca and P can potentially impede the absorption of other minerals, such as Mg and other trace minerals as previously mentioned (Brink et al. 1992; Matsuzaki et al. 1997; and Snedeker et al. 1982). For example, high intake of Ca can induce reduction of intestinal Mg absorption which is capable of causing or triggering various diseases as Mg is a vital mineral that plays some important physiological functions in many fundamental biological processes (Rude, 1998). Furthermore, Mg is one of the minerals whose low intake and impaired absorption in mammals may be highly correlated with the pathogenesis of coronary heart disease, hypertension, cancer and in fact kidney calcification (Rude, 1998). It is also known that high intake of Ca in the long-term reduces Mg retention and utilization (Miura et al. 1999). This also implies that taking high Ca during one's life time can in fact antagonize the metabolism of Mg. It has been demonstrated that ingestion of high Ca and P causes a negative effect on Mg metabolism by lowering the solubility of Mg in the intestinal lumen, thereby triggering reduction in Mg absorption (Brink et al. 1992). This is primarily because increasing the amounts of Ca and P in the diet causes the formation of an insoluble Ca-Mg-P complex, thereby significantly reducing Mg solubility and also potentially reducing Ca and P availabilities as well. This again points to the fact that animal, including human diets should be well fortified with optimal levels of Ca and P, since they determine the level of the efficient metabolism of other minerals such as Mg and trace minerals (Peo, 1991). To this extent, defining Ca and P in their optimum digestible ratios in animal diets formulated for different physiological phases should aid in enhanced retention of these minerals by the animals and thus improve the nutritional management of these minerals and reduce their effects on the environment as a result of their improved retentions by the animal. This information may also be useful for humans in the nutrition of Ca, P, Mg and other trace minerals like Fe, Zn, and Cu (Wood and Zheng, 1997; Snedeker et al. 1982); particularly now that there is a growing awareness of the need for high amounts of dietary Ca especially among women to prevent bone loss which has led to significant increase in the sale of Ca supplements and the introduction of various new Ca-fortified foods as earlier stated.

At present, the accumulation of trace minerals, such as Cu, Fe and Zn in soils are potential pollutants as they may impose medium- as well as long-term toxicity risk to plants and soil micro-organisms (Dourmad and Jondreville, 2007). However, the nutritional strategy also has a great potential for the effective management of these minerals in swine nutrition. This would rely on improvements in our current knowledge of the physiology of pigs to enable nutritionists to achieve a better agreement between supply and requirement. Similarly, the situation also requires better understanding of the factors that affect trace mineral availability and a more precise evaluation of the requirements.

To this point therefore, one of the fundamental means of achieving this will be to determine the endogenous fractions of these trace minerals voided in the feces. This undoubtedly will provide further insight and thus more vital nutritional information on the dietary requirements for these trace minerals to better guide feed formulators to match dietary supply with the animal requirements. It can be concluded that improving the efficiency of nutrient utilization by livestock is a very efficient strategy to reduce the excretion of these minerals in the pig slurry. From a whole-farm perspective, this strategy is really a more efficient means to reduce the import of nutrients, including P and trace minerals from outside the farm.

2.6. Calcium to Phosphorus Ratio for Swine

The understanding of nutrient efficiencies may be essential for a significant breakthrough in the effective management of the economics, health problems and environmental pollution by the livestock industry, particularly the swine industry. To this extent, from the economic point of view, dietary Ca supplements in swine diets are achieved through the use of limestone; a well known and relatively cheap ingredient. The consideration of calcium availability is modest (Crenshaw, 2001). Consequently, producers will supplement Ca freely without paying much attention to the efficiency of the use of this mineral. Conversely, the problem with P is that it is expensive and at the same time is of less benefit to the animal if it is deficient or in excess in the diet. Swine producers are prone to improving their cost-benefit ratios by having a better understanding of the kinetics of nutrient efficiencies, especially as it concerns the levels of Ca and P in the diets of their animals.

For example, health problems, such as osteoporosis can be prevented by fortifying the animal diets with adequate levels of Ca in combination with P as well as vitamin D (Peo, 1991; NRC, 1998). When such background information regarding nutrient efficiencies and requirements are lacking or not available, it becomes difficult to prevent such bone diseases leading also to poor animal performances. Here it is important to have nutritional information or knowledge about the combination of nutrients and nutrient availability with respect to diet ingredients in order to formulate diets that match animal requirements depending on their physiological states.

At present there is considerable concern regarding environmental pollution (Mallin, 2000) emanating from excess P in pig feces as previously discussed. The excess P in the pig manure is as a result of dietary P that is not utilized by the animals and consequently, is excreted in the manure and urine leading to the undesirable incidence of P pollution (Mallin, 2000) as also discussed earlier. Hence P pollution can be significantly reduced by also reducing inefficiencies of P retention by optimum diet formulations. This option of improving nutrient efficiencies is continuously being explored and bringing about new antidotes to address the problem of P pollution, such as that of Fan (2007). Minimizing over-formulation is the easiest and cheapest means to reduce P excretion and waste by pigs. This is where the ratio of available Ca to available P in the animal diets becomes important.

The P requirements as provided in the latest edition of NRC (1998) are the best available estimation. It is also vital to note that the accuracy of the requirements is limited by available data. In NRC (1998), there are 151 references regarding P requirements. Out of this figure, only 37 were from the 1990s and more so only 5 of the 37 addressed the P requirements (Knowlton et al. 2004). Of more importance to this observation is the fact that only one of the 37 really addressed the bioavailability of P from feed ingredients (Knowlton et al. 2004). With increased in knowledge, it is now known that bioavailability of P differs among feed ingredients resulting in a better estimate of P requirements on a digestible or available P basis. More importantly, another factor that needs serious attention in formulating precise diets for any of the physiological phases of the animal to meet P requirements for pigs is the availability of Ca. This requires that diets should be formulated based on P-available to Ca-available basis or ratio. In this way, as previously discussed, environmental pollution due to P would be reduced in addition to more economic gains to producers. This is where the improvement of diets based on the use of optimal true digestible Ca to P ratio may prove effective as it shows promise of being very effective in dealing with P management in swine nutrition as previously stated.

2.6.1. Studies on Ca to P Ratio

Feeding is the major cost associated with livestock production, including swine production. As a result of this, one of the major challenges to nutritionists is to develop less wasteful and more cost-effective diets for pigs. Phosphorus has been identified as an economic ingredient in swine diets (NRC, 1998) but it is often included in excess in swine diets leading to significant wastes of the ingredient to the environment resulting in P environmental pollution, especially in areas of intensive swine production. One of the main causes of P waste in swine diets is the paucity of information on the Ca to P ratio in the diets which may lead to P excess inclusion level. This is done to avoid P deficiency due to P importance from the physiological standpoint (Poulsen, 2000). It is well known that the metabolism and utilization of Ca and P are highly correlated and interdependent. Therefore, in order to reduce feeding costs and waste of P emanating from its excess inclusion level in the diet, dietary P should be matched with dietary Ca according to the needs of the animal (Hall et al. 1991; Crenshaw, 2001). This will better support pig performance and as well as sustainability of the ecosystem. For this to be done successfully requires accurate information on the true digestibility of P based on diet type as well as on the limitations of ingredients used to formulate the diet. It also requires formulation of cost-effective diets based on accurate information of Ca to P ratio in the diet also based on a well-defined requirement of the physiological class of the animals in question. This will improve the composition and digestibility of economic ingredients and their nutrients, including the plant sources that contain some P in a phytate form, such as SBM and corn.

To this point, in growing poultry, a total Ca: total P ratio of 1.1: 1 to 1.4: 1 has been recommended. Accordingly, feeding diets with wider ratios between Ca and P has been shown to reduce performance, P utilization and bone mineralization. In expanding this assertion further using the data reports of Qian *et al.* (1996b), it has been demonstrated in turkeys that 8.7, 10.8 and 6.6% reduction was calculated for BW gain, P retention and total ash percentage, respectively, when Ca : total P ratio was increased from 1.1 : 1 to 2.0 : 1. The findings of these results therefore, suggest that wider ratios between Ca and P would be detrimental to animal performance, P utilization and negatively affect the bone health of the animal. This situation may not be limited to poultry species alone as this finding has been shown to be similar in the literature with pig data studies as presented below:

Earlier studies have recommended that the level of Ca should be in a close relationship with the level of P in the diet. An optimal ratio range between total Ca and total P of about 1.2 – 1.4: 1 was recommended (Jongbloed, 1987). However, it is now known that the ratio when expressed as digestible Ca to digestible P is more accurate for meeting animal requirements. For the success of supplying these minerals in the appropriate ratio in terms of digestible Ca to digestible P, it is required that the digestibility of the Ca source, intrinsic phytase activity of the feed, the concentration of digestible P and the type of production pig category be known to better guide the inclusion levels of the minerals on digestible basis.

The data of Hall *et al*. (1991) further corroborated the earlier findings of the studies of Jongbloed (1987) on the required levels of Ca and P in swine diets. In the studies of Hall *et al*. (1991) it was shown that high intake of Ca is detrimental to the health of the animal. The findings of that study are also of particular significance to human health. In human nutrition it is a common practice to consume high levels of Ca supplements usually in an attempt to either prevent osteoporosis in post-menopausal women or for the control of hypertension. In the light of this, the data of these studies (Hall et al. 1991) suggest that high intake of Ca may trigger or increase the risk of prolonged blood clotting time, especially when the intake of vitamin K is low or marginal. Expanding the results of these studies further, demonstrated that pigs that consumed diets deficient in P grew at a significantly slower rate indicating that the diets were less efficient compared with those containing adequate levels of P. These findings also suggest that an adequate level of P in swine diets is important for the animals' optimum performance. Furthermore, increasing the Ca: P ratio from 1: 1 to 2: 1 or 3: 1 significantly depressed both the rate and efficiency of gain and lead to excreting more Ca and P in the manure, perceived as one of the major detrimental environmental effects of intensive swine production. In terms of economic swine production, observations in these studies which involve high ratios of Ca: P result in reduction of revenue for the pig farmer; as the pigs fed the 2: 1 and 3: 1 ratios of Ca: P gained 3 and 7% more slowly and also required 5 and 12% more feed per unit of gain, respectively, than those fed the 1: 1 ratio of Ca: P. The slower growth of pigs fed the high Ca and low P diets in these studies can be attributed in part to the high amounts of dicalcium phosphate and ground limestone in the diets. High levels of Ca in the diets may have dilute the energy content of the diets as suggested by earlier studies (Reinhart and Mahan, 1986) which observed that increasing the Ca: P ratio from 1.3: 1 to 3: 1 resulted in a 16% decrease in daily gain and an 11% higher feed: gain ratio in growing pigs.

Following the work of Jongbloed (1987), Ketaren et al. (1993) studied the metabolism of P in pigs based on the available P requirements of grower and grower/finisher pigs used four Ca: available P ratios as: 1.7, 2.1, 2.5 and 2.9. These authors observed a significant linear depression effects with increasing Ca: available P feed ratios on carcass gain and feed conversion ratios. However, the majority of these effects were seen to only occur when the ratio increased from 2.5:1 to 2.9:1. Based on these observations, it was concluded that a Ca: available P ratio of between 1.7 and 2.5:1 seemed suitable for grower and grower/finisher pigs. Furthermore, it was reported that the depression effect with increasing the Ca: available P ratio on growth rate and increased feed conversion ratio highlighted the fact that the grower pig is not tolerant of high Ca to low available P ratios. In that study, it was shown that the highest utilization of available P was found in pigs receiving the lowest dietary P with a narrower Ca: P ratio, where in fact the entire available P intake was retained. This result therefore is an indication that the available P was completely utilized when the pigs were supplied with only a low-P diet and low-Ca concentration diets. In the study, it was also highlighted that at the low-P dietary intake bone development of pigs was impaired; therefore, the available P intake may have been primarily utilized for the growth of soft tissue which might have resulted in bone resorption (Shoback and Sellmeyer, 2010). Conversely, as the available P intake of the pigs increased, bone development improved but available P utilization decreased. The earlier results of Jongbloed (1987) also identified this consequence that the absorption and retention of P decreased with the increase in dietary P level and offered corresponding explanation for this as: firstly, P intake exceeding the P requirement; and secondly, P intake exceeding the absorption capacity of the pig. From here, it can be deduced that P absorption efficiency is reduced with increase in available P intake above P requirement as substantiated in the studies of Ketaren *et al.* (1993) which therefore may explain why excess P level in the pig diet results in high levels of P in the pig manure.

The above conclusions in the two studies referred to in the literature were again corroborated by the studies of Liu *et al.* (1998) using growing-finishing pigs. These researchers investigated the effect of three dietary Ca: total P (tP) ratios, viz: 1.5:1, 1.3: 1, or 1.0: 1 on P utilization in low-P corn-SBM diets supplemented with microbial phytase at 500 phytase units/kg. In that study, the lowering of Ca: tP ratio showed a significant linearly increase in average daily gain (ADG) during the growing phase and overall; gain: feed ratio during the growing phase; and P absorption during the finishing phase. Additionally, the lowering of the Ca to tP ratio also significantly linearly increased body weight (BW) at slaughter; carcass weight; bone breaking strength; and bone ash weight. However, dressing percentage and back fat depth were unchanged. Therefore, these authors concluded that pig performance and P utilization were increased by lowering the Ca: tP ratio from 1.5:1 to 1.0:1 in low-P corn-SBM diets supplemented with microbial phytase. Simultaneously, Ca excreted daily was reduced by 54% by lowering the Ca: tP ratio from 1.5: 1 to 1.0: 1 as a result of decrease in Ca intake and the increase in Ca digestibility and retention. These findings clearly demonstrate that P utilization was increased by lowering the dietary Ca: tP ratio from 1.5: 1 to 1.0: 1 in low P corn-SBM diets fed to growing-finishing pigs based on growth performance, P absorption, bone strength, and bone ash criteria.

Three possible mechanisms were advanced as possible explanations for the above observed results. It has been proposed that a wide range of Ca to P ratios has a detrimental effect on P utilization (NRC, 1998), especially when phytase is involved. The extra Ca is known to form an insoluble phytate-complex thereby making it inaccessible for hydrolysis by phytase. In addition to this, high dietary Ca level increases the pH of the intestinal contents that in turn decreases microbial phytase activity. The excess Ca in the diet could potentially depress phytase activity by competing for the active sites of the phytase enzyme (Qian et al. 1996a). The highlights of some of the findings of Liu *et al.* (1998), therefore are one of the bases on which the high recommended Ca level for grain-SBM diets by National Research Council (NRC) can be challenged. Hence, dietary Ca must be less than NRC (1998) recommended concentrations in low-P corn-SBM diets for pigs, particularly when such diets are supplemented with microbial phytase for growing-finishing pigs as discussed below.

According to the NRC (1998) data, it was recommended that the total Ca to available P ratio should be from 2: 1 to 3: 1. At present, although these ratio ranges are observed to support production, they are not eco-friendly. However, NRC (1998) also recommended that for total Ca to total P ratio, the ratio should be between 1 : 1 to 1.25 : 1 in grain-SBM diets. Additionally, NRC (1998) further reported that a narrower Ca to P ratios, be it total or available P, probably results in the use of P more efficiently by the animal. This assertion was in agreement with the earlier reports of Peo (1991) that first stated that adequate Ca and P in the diet in a suitable available ratio are very fundamental to their efficient uses by the animal with the implication that less P would be found in the pig manure. NRC, (1998) also reported that P absorption is impaired in pigs if the Ca-to-P ratio is too wide. The reasons for these observations in these reports are not farfetched. There is an important relationship between Ca and P nutrition and metabolism. The absorption of both dietary Ca and P is usually influenced by the concentration of dietary P (Summers, 1997). The absorption of P is reduced with increasing levels of dietary Ca. A wide Ca-to-P ratio lowers Ca and P absorption whereas a narrow ratio is more correlated with increase in Ca and P retention (Li et al. 2000). Achieving proper balance between Ca and P will invariably favour reductions in dietary P and P excretion with no detrimental effect on skeletal growth and development as well as bone soundness (Eeckhout et al. 1995).

Again, the findings that the lowering of Ca to P ratio favours improved P utilization were further supported by the studies of Liu *et al.* (2000) involving three levels of Ca: tP ratio treatments as: 1.5: 1; 1.3: 1; and 1.0: 1, respectively. In that study, the lowering of the dietary Ca: tP ratio in the diets containing phytase significantly linearly increased the apparent absorption of P in the small intestine. However, Ca absorption was not affected. Pigs fed the low-P diet with a Ca: tP ratio of 1.0: 1 had an apparent absorption of P or Ca similar to that of pigs fed the control diet that was adequate in Ca and P. In conclusion the lowering of the dietary Ca: tP ratio to 1.0: 1 in a low-P diet containing phytase increased the apparent absorption of P in the small intestine. Furthermore, a significant amount of P was also absorbed in the cecum in that study signifying that the cecum-colon region of the digestive tract may after all play a role in maintaining P and Ca homeostases in pigs, especially under conditions of marginal P and Ca status.

Brady et al. (2002) evaluated the effect of phytase inclusion and Ca/P ratio on the performance and nutrient retention of grower-finisher pigs fed barley/wheat/SBM-based diets found that phytase significantly increased P digestibility and evidently reduced the amount of P excreted when added to the diet in a 1.15 : 1 ratio but had no effect when added to the diet with a 1.85 : 1 ratio; suggesting that imbalance of Ca to P ratio level in the diet is capable of causing interference of phytase activity by rendering its effect completely inefficient. In that study the lowering of Ca/tP ratio increased the DE content of the diet, digestibility of protein, DE, Ca and resulted in a significant improvement in feed conversion ratio. Reducing the dietary Ca/tP ratio from 1.85:1 increased the overall performance of growing-finishing pigs which is also a clear indication that the beneficial effects of microbial phytase supplementation of pig diets are adversely affected by a wide Ca/tP ratio as earlier stated above.

Wang *et al.* (2001) also examined the effect of dietary P levels (0.3%, 0.6% and 0.9%) and the ratio between Ca and P as: 1:1, 1.25:1 and 1.5:1 on the activities of alkaline phosphatase (AKP) and serum Ca and P in miniature pigs (Xiang pig) using the activities of AKP in serum, duodenum and the sixth rib and on serum Ca and P levels as yardsticks. The researchers found that the activity of AKP in the serum and in the sixth rib decreased significantly as dietary P increased from 0.3% to 0.9%, but the activity of duodenal AKP and serum Ca level were not affected by the different levels of dietary P. Similarly, there were no effects of the ratio between Ca and P on AKP activity in serum, duodenum or the sixth rib. However, in that study, it was observed that serum P level increased as dietary P increased and decreased as Ca to P ratio increased. Serum Ca level was highest with the 1.25:1 Ca: P ratio. Therefore, from these results it could be inferred that the ratio of 1.25: 1 was optimum for the pigs.

Again, in another independent study (Hanni et al. 2005) that investigated the effects of increasing Ca-to-P ratio in diets containing phytase on finishing pig growth performance in two different experiments also came to the conclusion that a wide ratio of Ca to P in swine diets suppresses performance resulting in high levels of P that are excreted in the pig manure. In their first experiment involving Ca: P ratios of 1.0:1, 1.25:1, 1.5:, or 2.0: 1 in the diets fed to the pigs from 38.6 to 113 kg body weight (BW) found that increasing Ca: P ratio decreased average daily gain (ADG), average daily feed intake (ADFI) and carcass weight significantly, with the greatest reduction observed when Ca: P ratio increased from 1.5:1 to 2.0: 1. Similarly, in their second experiment in which they also evaluated the effect of diets that contained Ca: P ratios: 0.75:1, 1.0:1, 1.25: 1, 1.5: 1, or

2.0: 1 to determine whether diets were formulated above the pig's requirement found that increasing Ca: P ratio significantly decreased ADG and gain-to-feed ratio (G: F), with the greatest decrease observed as Ca: P also increased from 1.5:1 to 2.0: 1. Based on these results, the researchers concluded that finishing pig diets containing phytase should not have a Ca: P ratio of more than 1.5: 1.

Han and Thacker (2006) examined the effects of Ca and P ratio in high zinc diets on performance and nutrient digestibility in weanling pigs using four corn-SBM-based diets containing 20.4% CP and 0.60 – 0.63% P formulated to contain 0.83%, 0.91%, 1.01% and 1.17% of Ca resulting in 1.31: 1, 1.51: 1, 1.65: 1 and 1.91: 1 ratios of Ca to P, respectively. They observed that although daily gain and feed intake were unaffected by the dietary treatments, feed conversions were significantly affected as dietary Ca concentration increased throughout the duration of the study. In fact, in that study the digestibility of fat and Ca in the 1.17% Ca diet were significantly lower than for the other diets. There was also the tendency for reduced P retention by animals on the 1.17% Ca diet compared with the other three diets with lower Ca concentration levels in the diet. These results suggest that more Ca and P would have been released via manure into the environment by the pig. Furthermore, based on the findings of that study as earlier stated, the feeding of higher levels of Ca decreased the digestibility of crude fat. These effects are potent factors that cause environmental pollution due to increased nutrient loads on the environment. The fact that crude fat digestibility was reduced can potentially reduce the DE of the diet, especially from the dietary fat sources of the diet which is also capable of affecting animal performance. This finding of reduced fat digestibility in the study was however not surprising. One possible mechanism that has been suggested that can explain at least a part of this negative or detrimental effect of high Ca level in the diets used in this study is the fact that a higher Ca level causes Ca to complex with fatty acids in the lumen of the animal to form insoluble metallic soaps, thereby reducing both fat and Ca digestibility as confirmed also by the results of that study (Han and Thacker, 2006). To avoid Ca complexes with fatty acids high or excess Ca in pig diets should also be avoided requiring that the animal diets should only be fortified with or only matched by the dietary requirements of the animal which has been shown to improve nutrient digestibility and utilization.

Improving the efficiency of a nutrient, such as P utilization by pigs is a very sound and efficient means to reduce its excretion in the manure. From the whole-farm perspective it is an effective strategy to reduce the import of nutrients from outside the farm. Fan (2007) investigated the optimal true digestible Ca to true digestible P ratio in post-weaned pigs; and the effect of the determined optimal Ca to P ratio on growth performance, efficiency of Ca and P utilization and Ca and P excretion in the pig manure as well as the effect of altering the determined Ca and P ratio on the above stated parameters. Based on these parameters, Fan (2007) found that the true digestible Ca to true digestible P ratio range was from 0.96 to 1.35: 1 and therefore recommended that this ratio range should be used to fortify the diets of post-weaned pigs during diet formulation to reduce the level of Ca and P excreted in the manure. This ratio range agrees with the earlier reports of Peo (1991) and NRC, (1998) that a narrow Ca to P ratio better supports P retention and animal performance and would therefore minimize P levels in the pig manure.

The summing up of results that evaluated Ca to P ratios in pigs of all physiological phases generally blend with the fact that a wide ratio range between Ca and P in pig diets adversely affect the efficiencies of the utilization of Ca and P as well as other nutrients in the diets, such as protein and DE (Brady et al. 2002). The overall impact being a huge price on the environment. As a first step guide to using nutritional strategy to curb environmental pollution emanating from the swine industry, a suitable range of Ca to P ratio in the diet is very critical and central to the success and implementation of using nutritional manipulation to control or reduce P levels in the pig manure and thereby aiding in reducing environmental pollution due to P. It does not gainsay that low-P content diets based on the use of optimal digestible Ca to true digestible P ratio in weanling, grower and finisher pigs will not compromise animal health and performance but consequently reduce excess P in the diet leading to minimized P availability in the hindgut and ultimately reduce P level excreted in the manure and urine of the pig. When P as well as Ca levels in the diets are low but meet the requirement of the animal lower amounts of inorganic P and limestone would be used in compounding the diet. Economically, this has a very high potential of increasing the net income for producers as previously discussed.

2.7. Conclusions

In light of the above discussions, the determination of an optimal true digestible Ca to P ratio for swine is essential. Such information when obtained will serve as a working guide for improving swine growth performance, bone health and the reduction of Ca, P and other nutrients excreted in swine manure for the swine industry. Therefore, a series of studies will be conducted to examine the true digestible Ca, P, Mg and some trace-minerals for the growing pig in corn-SBM-based diets. Also, the impact on manure Ca and P excretions as well as the effects on animal performance will be studied. Furthermore, it will also inform the inclusion levels of these essential macro-and micro-minerals in meeting the nutritional requirements for the growing pig without over supplementation. In these ways, there would be reduction in the cost of production and more importantly intensive swine production would become more eco-friendly. Therefore, it can be hypothesized that:

1. The determination and use of true digestible Ca to true digestible P ratio values in swine diets would better support animal performance and health.
2. In addition to 1 above, the determination and use of true digestible Ca to true digestible P ratio in swine diets would better support Ca and P retentions and therefore aid in better P management through the reduction of total and soluble P excretions in manure.

3. The determination of true digestible Ca to true digestible P for swine would provide essential data to support reduction of current dietary supplementation levels of mineral salts and therefore reduce cost of production via improvement in the efficiency of P utilization in pigs.

Based on these hypotheses, the objectives of this thesis are:

1. To determine optimal dietary true digestible Ca to true digestible P ratio values for the growing pig.

2. To also determine the optimal digestible level of Mg, Fe, Cu, Mn, Se and Zn in the growing pig fed corn-SBM-based diets based on the principles of the difference method.

3. To examine the effect of changing dietary true Ca to true digestible P ratio on growth performance, efficiency of Ca and P utilization and manure Ca and P excretions in the growing pig.

4. To recommend an optimal dietary true digestible Ca to true digestible P ratio for the growing pig fed corn-SBM-based diet for use by the swine industry to reduce excess P and Ca excretions in the manure as well as improving the metabolism of other nutrients, such as DE, N and trace minerals, thereby aiding in using nutritional management in reducing the nutrient environmental loads emanating from the swine industry.

CHAPTER 3

ESTIMATION OF TRUE CALCIUM AND PHOSPHORUS DIGESTIBILITY AND THEIR ENDOGENOUS OUTPUTS BY THE SUBSTITUTION METHOD IN GROWING PIGS FED CORN/SOYBEAN MEAL (SBM)-BASED DIETS

3.1 Abstract

Twelve Yorkshire barrows with initial and final body weights of 23.2 ± 2.0 and 31.1 ± 1.9 kg respectively were allotted to two dietary treatments with six replications per treatment according to the principles of the difference method to determine: dry matter (DM) digestibility, apparent Ca and P digestibility (AD), true Ca and P digestibility (TD) and their endogenous losses (EL) associated with a corn-SBM-based diet for the growing pig. The two diets were formulated at 100 (diet 1) and 70 % (diet 2), respectively, of Ca and P requirements for growing pigs. Results showed that the DM digestibility of diet 2 (84%) is significantly higher ($P < 0.05$) than that of diet 1 (81%). Ca and P AD are 28.4% and 24%, respectively. However, their TD values are about 42% and 40% which are significantly ($P < 0.05$) higher than their AD values. Their EL are 0.8g/kg DMI and 1.3g/kg DMI for Ca and P, respectively. Results also demonstrated that AD values underestimate TD value of Ca by 33% and that of P by 40%. It was thus concluded that in a corn-SBM-based diet Ca and P TD values should be employed in diet formulation for the growing pig to better manage Ca and P nutrition for the swine industry.

Key Words: Apparent and True Ca and P digestibility, Ca and P Endogenous Losses, Pigs

3.2. Introduction

Corn and soybean meal (SBM) are common feed ingredients for swine. Corn is primarily used as an energy source while SBM contains both energy and protein. These two ingredients also serve as sources of other nutrients, such as phosphorus and calcium for pigs. Phosphorus and calcium act together in the development and maintenance of the skeletal system. One of the major drawbacks in using these sources for supplying P in swine diets is the fact that most of the P is in the phytate-associated form and is not readily available to the animal, since pigs naturally lack the phytase enzyme that hydrolyzes phytate-P. There is however considerable data demonstrating that apparent digestibility of P in corn and SBM by growing pigs grossly underestimates the true P digestibility (Shen et al. 2002; Ajakaiye et al. 2003) because of a large endogenous loss of P in feces. The use of apparent digestibilities may result in excess inclusion of P in swine diets to ensure that P deficiency does not occur, leading to excretion of excess P intake into feces and urine in corn/SBM-based diets. Therefore, the quantification of endogenous fecal loss (EFL) by pigs fed a corn/SBM-based diet would allow animal nutritionists to better match dietary P intakes with animal requirements. A better P balance has the potential of increasing producers' net income (Fan, 2007) considering the fact that P is the third most expensive nutrient in swine diets (NRC, 1998) while also making intensive swine production more eco-friendly.

It has recently been shown that there is no difference between the use of the regression analysis technique and the substitution method in the determination of true P digestibility and fecal endogenous P losses associated with feed ingredients for growing pigs (Fang et al. 2007). The objectives of this study were to determine, by the Substitution Method of Adeola (2001), true Ca and P digestibilities and their EFL associated with a corn/SBM-based diet for growing pigs since they act together in their functions.

3.3. Materials and Methods

3.3.1. Animals, Housing and Experimental Design

Twelve Yorkshire barrows with an average initial BW of 23.2 ± 2.0 (mean ± SD) kg were acquired from Arkell Swine Research Station, University of Guelph. The barrows, on arrival at the Animal Wing of the Department of Animal and Poultry Science, University of Guelph were weighed to obtain initial BW and randomly assigned to pens. They were housed individually in tender-foot^{TM} plastic floor pens with smooth transparent plastic sides in a room that was mechanically ventilated to provide an ambient temperature of 20 - 22^{0}C. Six pigs were randomly assigned to each of two experimental diets and fed at 5% of BW (as-fed basis) twice daily at 0900 h (half of the daily meal) and 1600 h. Animals received their assigned diets for a total of 15 d, consisting of 10 d of adaptation followed by 5 d of fecal collection. They each had unlimited access to water from a low-pressure drinking nipple. Animal pens were cleaned regularly. Average BW at the end of the experiment was 31.1 ± 1.9 (mean ± SD) kg. All procedures used in the management of the animals were reviewed and approved by the University of Guelph Animal Care Committee and pigs were cared for with compliance to the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

3.3.2. Experimental Diets

The basis of the substitution method to estimate true digestibility of a mineral is that the increment in fecal mineral output when mineral intake is increased comes exclusively from the diet, representing truly indigestible mineral, and the endogenous loss remains constant. At intakes below the requirement, the assumption is considered valid (Fan et al., 2001). To estimate the true Ca and P digestibility by the substitution method, two diets were formulated at 100 and 70 %, respectively, of Ca and P requirements for growing pigs NRC, (1998). The low-nutrient diet was formulated by removing corn, SBM, limestone and dicalcium phosphate, and replacing them with low-ash corn starch and cellulose to balance DE and NDF contents (**Table 3.3.2**). Titanium oxide was included at 0.3% of diet DM as an indigestible marker (**Table 3.3.2**).

3.3.3. General conduct of study:

Feed intake was normalized between the high- and low-nutrient diets. Pigs had similar BW and diets were offered at 5% of BW. This resulted in similar intakes for all pigs throughout the study duration. At the beginning of each day of the experimental period, orts from the previous day were collected, dried at 105^{o}C, and weights were recorded. The difference between dry feed delivered and the next day’s orts represents the DM consumed by the animal for the day. On the last day of the study, all animals were weighed. The difference between final and initial BW represents weight gained during the study period. Accordingly, the average daily feed intake (ADFI), average daily gain (ADG), as well as gain to feed ratio for the study period were obtained.

3.3.4. Diet and Fecal Samples Collections and Processing:

Samples of the dietary treatments used in the study were collected immediately after each diet mixing and stored in sealed sample bags at 4^{o}C. They were later dried and ground in a Wiley mill through a 1-mm screen, thoroughly mixed and stored again at 4^{o}C.

From the 11th to the 15th day in the study, fresh representative fecal samples were spot collected from each pen at 2-h intervals. Fecal samples were sealed in sample containers and immediately frozen at -20^{o}C. They were later freeze-dried, ground in a Wiley mill through a 1-mm screen, and stored at 4^{o}C until analysed.

3.3.5. Chemical Analyses

Dry matter (DM) content of samples was determined according to AOAC (2000) by oven-drying at 105^{o}C for 24 h. After drying, samples were removed, cooled in a dessicator and re-weighed. The DM content was determined as final weight/initial weight × 100.

Calcium concentration in diet and fecal samples was analysed by Inductively Coupled Plasma-Optical Emission Spectrometer (Varian Vista Pro 2001, Varian Canada, Mississauga, ON, Canada). Following a wet-ash digestion with hydrochloric and nitric acids, phosphorus concentrations in diet and fecal samples were analysed by a colorimetric assay. Acetone-Acid-Molybdate solution was added to samples to form a phospho-molybdenum complex (Akinmusire and Adeola, 2009). Aliquots of sample solutions so obtained together with similarly prepared standard solutions were used to determine P concentrations. Color intensity proportional to P concentration was determined on a UV/Visible Spectrophotometer (Ultrospec 2000, Biochrom Ltd., Cambridge, UK) at 355 nm. Titanium dioxide concentrations in diet and fecal samples were analysed according to the method of Short *et al.* (1996). Briefly, samples were weighed, ashed and digested in concentrated sulphuric acid. Hydrogen peroxide (30% vol.) was added to develop an orange colour that was read at 410 nm using a UV/Visible Spectrophotometer (Ultrospec 2000, Biochrom Ltd., Cambridge, UK).

3.3.6. Calculations and Statistical Analyses

The apparent fecal DM, Ca and P digestibilities (AFD; %) were calculated using the marker technique according to equation (1):

$$AFD = \frac{N_{Diet}/M_{Diet} - N_{Feces}/M_{Feces}}{N_{Diet}/M_{Diet}} \qquad (1)$$

Where N_{Diet} = the concentration of nutrient in the diet (%), M_{Diet} = the concentration of titanium marker in the diet (% titanium), N_{Feces} = the concentration of nutrient in the feces (%), and M_{Feces} = the concentration of marker in feces. All percentages were on a DM basis.

Based on average AFD of Ca and P, true fecal digestibilities (TFD; %) were estimated as:

$$TFD = \frac{\overline{AFD_{Diet1}} N_{Diet1} - \overline{AFD_{Diet2}} N_{Diet2}}{N_{Diet1} - N_{Diet2}} \qquad (2)$$

where Diet 1 is the high-nutrient diet and Diet 2 is low-nutrient.

Endogenous fecal losses (EFL; g/kg DMI) of Ca and P estimated as:

$$EFL = \left(\overline{TFD_{Diet}} - \overline{AFD_{Diet}}\right) N_{Diet} 10 \quad (3)$$

Data were subjected to ANOVA using PROC GLM of SAS (SAS Inst. Inc., Cary, NC) according to:

$Y_{ij} = \mu + D_i + E_{ij}$:

where Y_{ij} is the observation; μ is the overall mean common to all treatments, D_i is the effect of the i^{th} diet, and E_{ij} is the error term. Furthermore, homogeneity of variances across the two diets was tested for and confirmed (*P* > *0.05*) by Levene's test using SAS (SAS Inst. Inc. Cary, NC). The pig was the experimental unit and an α-level of 0.05 was used for all statistical comparisons to represent significance.

3.4. Results and Discussion

3.4.1 Animal Performance

All animals in the two dietary treatments readily consumed their respective diets and grew throughout the 15-d experimental period. The average daily feed intakes of pigs on the two dietary treatments (**Table 3.4.1)** were similar (*P* > *0.05*). There was a trend for better ADG (*P* = *0.07*) for pigs that consumed the HN diet compared with pigs that consumed the LN diet that resulted in a significantly (P = 0.02) superior gain: feed ratio for pigs on the HN diet compared with pigs on the LN diet.

3.4.2 Apparent DM, Ca and P Digestibility

Apparent digestibility of DM was significantly (P = 0.03) higher in the LN diet than in the HN diet. The higher digestibility may have been due to the presence of cornstarch in the LN diet (**Table 3.3.2**) which contain higher digestible energy that is also 100% digestible (NRC, 1998) compared to corn and SBM with lower digestible energies that are 96% and 92% digestible respectively (NRC, 1998). On the other hand, the apparent P digestibility of the HN diet was significantly higher ($P = 0.007$) than that of the LN diet (24% vs. 13%), respectively. According to the assumption that EFL of P are equal between HN and LN, apparent P digestibilities will be elevated at higher P intakes. Thus, the higher AFD of P on HN supports a key assumption of the substitution method to estimate TFD. Apparent Ca digestibilities for HN and LN diets were 28% and 17%, respectively (**Table 3.4.2**), which showed no significant difference ($P > 0.05$) but a lack of effect could not be declared either ($P < 0.15$).

The apparent digestibility values of P and Ca determined in this study are similar to values found in the literature. For corn, Jongbloed and Henkens (1996) showed P digestibility value of 17%; Widmer et al. (2007) showed 28.6%; Shen et al. (2002) showed -41.4 to 39.1%; Dugelhoef et al. (1994) and Pointillart et al. (1984) showed 18% and 29%, respectively. For SBM, Jongbloed and Henkens (1996) showed apparent P digestibility of 38%; Ajakaiye et al. (2003) showed 3.73 to 48.05%; Jongbloed (1987) showed 24% and Jongbloed et al. (1991) showed 15 to 35%. Also, apparent Ca digestibility has been shown to be within the range demonstrated for P in these feed ingredients. Widmer et al. (2007) showed this value to be 35% in corn. These values therefore would be reflected in the digestibilities of P and Ca in diets involving the additivity of these ingredients.

3.4.3 True Ca and P Digestibilities and Their Endogenous Losses

True Ca and P digestibility estimates were 42% and 40%, respectively while their EFL were 0.8 g/kg DMI for Ca and 1.3 g/kg DMI for P (**Table 3.4.3)**. These values correspond with 5.3 mg/kg BW and 8.3 mg/kg BW per day for Ca and P, respectively. True digestibilities correct apparent digestibilities for those portions of Ca and P that have been absorbed into the pig's body and subsequently excreted back into the gastrointestinal tract. This portion of the total fecal excretion, known as metabolic or endogenous fecal loss, is not a constant proportion of the mineral intake and causes AFD of the mineral to vary according to its intake. True digestibilities, on the other hand, are constant across mineral intake level and have more utility in diet formulation and evaluation. The true digestibilities of Ca and P estimated in this study were 42% and 40%, respectively (Table 3.4.3). The true Ca digestibility value estimated in this study, when compared with other values in the literature, was somewhat lower. Traylor et al. (2001) demonstrated that Ca true digestibility in cornstarch/dehulled SBM-based diets ranged from 61.6 to 69.3%. In the same study, true Ca digestibility in cornstarch/soy protein concentrate-based diets was 61%. These higher values, compared with our estimated value, might be due to the fact that the diets used in the study of Traylor et al. (2001) were mainly semi-purified diets. The value of 40% determined as the true P digestibility in our study agrees closely with the value of 40.9% as the true digestibility value in SBM (Akinmusire and Adeola, 2009) that also used the marker method. Other studies have shown slightly higher values. For example, Fan et al. (2001) demonstrated a value of 48.5 to 50.7% in a cornstarch-SBM based diet; Ajakaiye et al. (2003) showed 51 to 59% in SBM using the regression method and Fang et al. (2007) showed 49.4 and 50.6% in SBM using both the regression and substitution methods and Shen et al. (2002) showed 53.9 to 59.8% in corn using the regression method. Petersen and Stein, (2006) using 5 inorganic P sources: dicalcium phosphate (DCP), monocalcium phosphate (MCP) with 50% purity (MCP50), MCP with 70% purity (MCP70), MCP with 100% purity (MCP100) and monosodium phosphate (MSP), showed high true total tract P digestibility values of 88.41, 89.45, 88.64, 94.93 and 98.2%, respectively. The true digestibility values reported in this study for Ca and P can be used in combination with other TFD estimates for dietary Ca and P supplements to formulate a complete diet for pigs.

Endogenous fecal losses of Ca and P were estimated to be 0.8 g/kg DMI and 1.3 g/kg DMI, respectively, corresponding to about 5.3 mg/kg and 8.3 mg/kg BW/d, respectively. The value for endogenous fecal P loss estimated in the current study is in close agreement with those found in the literature, such as that of Jongbloed and Everts (1992) who estimated 9.0 mg/kg BW/d day for growing pigs and that of Rodehutscord *et al.* (1998) who estimated 5.9 mg/kg BW per day on average for growing pigs weighing between 30 and 70 kg but higher and thus at considerable variance with that of Shen *et al.* (2002) who showed 0.57 to 0.84 g/kg DMI for the growing pig. The difference between our values and those of Shen et al. (2002) might be due to the fact that they fed the animals below requirements and more importantly at variable levels. Therefore the difference may be due to the fact that endogenous loss is not a fixed component of dietary intake (Braithwaite, 1985). There is a paucity of information in the literature concerning estimation of the endogenous fecal Ca loss in the growing pig. Nevertheless, Traylor et al. (2001), who investigated Ca endogenous fecal loss in semi-purified diets in growing pigs, estimated a value of 349 mg/kg of feed. This value is lower than ours, probably due to the fact that they used semi-purified diets in their study.

3.5 Conclusions

The results of this trial demonstrate that true Ca digestibility in corn/SBM-based diet for the growing pig (20 -50 Kg) BW is 42% whereas the true P digestibility in corn-SBM-based diet is 40%, respectively. Endogenous fecal loss of Ca is 0.8 g/DMI and that of P is 1.3 g/DMI respectively. These estimates can be adopted in formulating ideal corn/SBM-based diets for growing pigs to reduce Ca and P excretion into the environment without compromising pig performance and welfare.

Table 3.3.2: Diet formulation for measuring true Ca and P digestibility and the endogenous Ca and P outputs in grower pigs (20 – 50 Kg) by the Substitution (30%) Method

Ingredients (kg)	High-nutrient (HN)	Low-nutrient diet (LN)
Soybean meal	27.07	18.95
Corn	66.00	46.2
Cornstarch	2.80	28.32
Solka-Flock^{TM}(100% cellulose)[1]	0.00	2.98
L-Lys.HCL (79% L-Lys)	0.17	0.119
L-threonine (100%)	0.05	0.035
Animal fat	1.10	1.10
Iodized salt	0.40	0.40
Limestone (38.5% Ca)[2]	0.84	0.59
Dicalcium phosphate (22 % Ca)	0.86	0.60
Vit-Mineral premix[3]	0.50	0.50
Antibiotics mixture	0.00	0.00
Titanium oxide[4]	0.30	0.30
Total (100 Kg)	100.09	100.09
Calculated nutritive values (as-fed basis):		
DE (MJ/Kg)	14.58	14.74
CP (%)	17.33	12.13
Total Ca (%)[5]	0.62	0.43
Total P (%)[5]	0.52	0.36
Total Ca/total P ratio	1.19	1.19
NDF (%)	9.94	9.94
ADF (%)	4.39	3.07
Cellulose+hemicelulose (%)	5.54	6.86

[1]Solka-Flock^{TM}: commercial cellulose fibre product from International Fibre Corporation.

[2]Commercial feed grade limestone; contained 38.5% Ca.

[3]Vit-mineral premix contained per kg pre-mix: vitamin A, 2,000,000IU; vitamin D3, 200,000IU; vitamin E, 8,000IU; vitamin K, 500mg; pantothenic acid, 3,000mg; riboflavin, 1,000mg; folic acid, 400mg; niacin, 5,000mg; thiamine, 300mg; pyridoxine, 300mg; vitamin B12, 5,000mcg; biotin, 40,000mcg; Se, 60mg; choline, 100,000mg; I, 100mg; Cu, 3,000mg; Fe, 20,000mg; Mn, 4,000mg; Zn, 21,000mg.

[4]Titanium oxide used as a digestibility marker. [5]Analyzed values.

Table 3.4.1. Mean ± SE growth responses of pigs fed high- and low-nutrient corn/SBM-based diets (n = 6)

Item	High-Nutrient (HN)	Low-Nutrient (LN)	*P- value*
ADFI (DM basis; kg/d)	1.3 ± 0.02	1.3 ± 0.03	0.22
Initial BW (kg)	22.8 ± 1.6	23.5 ± 2.6	0.56
Final BW (kg)	31.3 ± 1.4	30.7 ± 2.6	0.64
ADG (kg/d)	0.58 ± 0.04	0.48 ± 0.03	0.07
ADG/ADFI	0.54 ± 0.03	0.42 ± 0.04	0.02

Table 3.4.2: Mean ± SE apparent fecal digestibility values of dry matter (DM), calcium (Ca) and phosphorus (P) of pigs fed high- and low-nutrient corn/SBM-based diets (n = 6)

Item	HN	LN	*P-value*
	%		
DM	80.8 ± 0.9	83.6 ± 0.6	0.03
Ca	28.4 ± 4.7	17.2 ± 5.0	0.13
P	23.9 ± 1.9	13.4 ± 2.3	0.007

Table 3.4.3 Mean true fecal digestibility values and the fecal endogenous outputs of calcium (Ca) and phosphorus (P) of pigs fed corn/SBM-based diet

Items	True digestibility (%)	Fecal endogenous loss (g/kg DM intake)
Ca	41.8	0.8
P	39.7	1.3

CHAPTER 4

THE EFFECT OF CALCIUM TO PHOSPHORUS RATIO ON GROWTH PERFORMANCE, Ca AND P UTILIZATION IN GROWING PIGS FED CORN-SBM-BASED DIETS

4.1 Abstract

An experiment was conducted to investigate the hypothesis that true dietary ratio of Ca to P influences pig performance, Ca and P retentions and Ca and P levels excreted through the pig manure and urine into the environment. The experiment was conducted as a randomized complete block design with 36 growing pigs (initial BW: 24.2 ± 1.9 kg) allotted to 6 dietary treatments. All pigs were placed in their individual metabolic crates that allowed for individual pig's fecal and urine sample collections. Six corn/SBM-based diets were similarly formulated to contain all nutrients at the same level except for the dietary ratios of Ca: P as: 0.78: 1, 0.98: 1, 1.19: 1, 1.40: 1, 1.61: 1 and 1.81: 1 for diets 1, 2, 3, 4, 5, and 6, respectively. Animal performance, and Ca and P true retentions were calculated for each diet. Results of the experiment showed that animals on diet 2 had the best ADG and FE ($P < 0.05$). These were further confirmed by the significant true Ca and P retentions that increased linearly from diet 1 to diet 2 and beyond diet 2 decreased linearly ($P <. 0.0001$) for Ca, and linearly ($P = 0.006$) for P, with increasing levels of Ca and P in the manure. The true Ca and P retentions are 50.8% and 50.1%, respectively found with diet 2 with true dietary ratio of 0.82: 1. It was concluded that true Ca: true P range of 0.82 to 1 is optimal for the growing pig.

Key Words: True Ca to P ratio, Performance, Retentions, Environment and Pigs

4.2. Introduction

The Ca to P ratio in the bone is about 2:1. In the past, it was recommended to feed diets or mineral mixes to pigs with a Ca: P similar to that found in bone. Later literature reviews (Kornegay, 1985) on the role of Ca and P in swine nutrition, however, revealed the fallacy of thinking that the ratio of Ca and P in bone is the ratio that should be used in feed formulation. A more appropriate indicator probably is the composition of these minerals in sows' milk, where the Ca: P varies from 0.6 to 1.2:1 (Peo, 1991). When one considers the purpose and importance of sows' milk to the neonates for their rapid development and survival, it is quite reasonable to postulate that its Ca to P ratio is near optimal. Recent findings confirm that dietary Ca concentration affects P digestibility and P digestibility was optimized when the true dietary Ca to P ratio was around or slightly less than 1.1 to 1 (Stein et al., 2011).

The use of high dietary Ca concentration relative to P reduces P digestibility and retention by the animal due to the formation of insoluble Ca-P-complexes (Lei and Porres, 2003; Knowlton et al. 2004; Montminy et al. 2007). To enhance dietary P digestibility, and thereby reduce losses of P to the environment, the optimum dietary Ca to P ratio needs to be determined. To this point, true Ca and P digestibility values compared to their apparent digestibility values are more accurate and thus more reliable regarding their relevance to the growing pigs, since they correct for endogenous losses. Therefore the determination and use of the true digestibilities for Ca and P would enable the feed formulator to formulate diets that have a dietary Ca to P ratio very close to animal requirement. The objectives of this study were to determine a practical, optimal dietary ratio of truly digestible Ca and P in a corn/SBM-based diet for growing pigs, and to determine the effect of truly digestible Ca and P on growing pig performance and excretion of Ca and P in feces and urine.

4.3. Materials and Methods

4.3.1. Animals and Management

Thirty six Yorkshire barrows of 24.2 ± 1.9 kg body weight were acquired from Arkell Swine Research Station, University of Guelph. All pigs were individually weighed and randomly assigned to their metabolic crates (0.8 x 2.0 m) housed in the experimental room that is environmentally and mechanically ventilated to provide an ambient temperature of 21 ± 2^0C. The pigs had free access to water from automatic water nipples. During the experimental period, the pigs were fed at 5% of their individual BW according to the method of Akinmusire and Adeola (2009). One-half of measured feed was fed twice daily at 0900 and 1600 h, respectively. The experimental period consisted of 15 d, with 10 d of adaptation to diets and 5 d of feces and urine sample collection. The day prior to the collection period the crates were thoroughly cleaned to ensure spot sample collections of fresh fecal material that were not contaminated and also to reduce urine contamination with fecal materials. All experimental procedures were reviewed and approved by the University of Guelph Animal Care Committee and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

4.3.2. Experimental diets

Six corn/SBM-based diets were formulated to meet or exceed the NRC (1998) recommended levels of nutrient requirements for 20 to 50 kg body weights pigs. They were formulated to be isocaloric, isonitrogenous, isofibrous and differing in Ca content and Ca: P ratio. Ca content was manipulated by gradient increases in the level of limestone, at the expense of corn starch, the energy of which was replaced by animal fat (**Table 4.3.2 a**). The analysed values for P were similar to calculated values while analysed values for Ca were somewhat lower than their calculated values (**Table 4.3.2 b)**. Titanium oxide was added at 0.3% of diet DM as an indigestible marker (**Table 4.3.2 a**).

4.3.3. Experimental Design

The experiment was designed and carried out as a randomized complete block design (RCBD) with 6 pigs per block and a total of 6 blocks. The diets were each mixed as a single batch for the entire trial. Pigs were housed individually in tender-foot^{TM} plastic floor pens and fed at 5% of BW (as-fed basis) in two equal meals per day. Experimental periods were 15 d in length with feces and urine collection during the last 5 d. On the last day of each period, body weights were recorded.

Fecal samples were immediately frozen at -20^{0}C, later freeze-dried and finally ground in a Wiley mill through a 1-mm screen and stored at 4^{0}C until analysis. Urine samples were collected via funnel-shaped metal trays secured to the base of the metabolic crate and covered with a netted screen mesh to prevent urine contamination with fecal materials. The containers contained 6N hydrochloric (HCL) acid solution made with milli-Q water (Millipore Corporation). The total volume of urine collected each day/pig/treatment/block was recorded and a sub-sample of 10% for each pig was stored at -23^0C.

4.3.5. Chemical Analyses

Dry matter content for each diet and fecal samples was analysed according to AOAC (2000). Ca and P concentrations in diets, feces and urine were analysed by Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES)], Laboratory Services Department, University of Guelph. Samples were microwave digested (MARSxpress, CEM Corporation, Matthews, NC, USA) with nitric acid. The microwave digested sample was brought to volume with Nanopure water. The clear supernatant was measured without further processing by ICP-OES (Varian Vista Pro, Varian Canada, Mississauga, ON, Canada) according to AOAC (1996).

4.3.8. Calculations and Statistical Analyses

Apparent nutrient digestibility (AFD) was calculated as:

$$AFD = \frac{N_{Diet}/M_{Diet} - N_{Feces}/M_{Feces}}{N_{Diet}/M_{Diet}}$$

where N_{Diet} = the concentration of nutrient in the diet (%), M_{Diet} = the concentration of titanium marker in the diet (% titanium), N_{Feces} = the concentration of nutrient in the feces (%), and M_{Feces} = the concentration of marker in feces.

Based on endogenous fecal losses (EFL) of Ca and P of 0.8 and 1.3 g/kg DMI, respectively true fecal digestibilities (TFD) of dietary Ca and P were computed using equation 2 as:

$$TFD = AFD + EFL/(N_{Diet}\ 10)$$

Apparent and true nutrient retention were calculated by subtracting urinary loss from the apparent and truly digested Ca and P intakes, respectively.

The experimental data were analyzed as a randomized complete block design. Data were subjected to analysis of variance (ANOVA) using mixed model procedure of SAS. Pigs served as the experimental unit for all the growth parameters assessed and nutrient digestibility responses. Orthogonal linear and quadratic contrasts were used to separate means and significance level was set at $P \leq 0.05$. Homogeneity of variances for the performance endpoints assessed in this study was also tested for and confirmed ($P > 0.05$) by the Levene's test using SAS (SAS Inst Inc. Cary, NC).

4.4. Results and Discussion

4.4.1 Animal Performance

All animals in each dietary treatment group consumed their rations normally and thus grew throughout the experimental period. The ADFI, ADG and FE determined in this study are shown in **Table 4.4.1**. Throughout the duration of the study period, changes in the dietary Ca to P ratio showed no difference in the ADFI between treatments groups. However, ADG declined in a linear fashion (P = 0.02). Feed efficiency, expressed as ADG/ADFI decreased quadratically (P = 0.02) across treatments with pigs on diet 2 exhibiting the highest feed efficiency. The FE for diet 2 was 60.3% while those of diets 5 and 6 were 50.4% and 50.6%, respectively. The dietary ratio of Ca to P at which animal performance was optimised for all parameters measured in this study was with diet 2 with dietary ratio of Ca to P as 0.82 to 1. This range is in close agreement with other studies in the literature. Hall et al. (1991) demonstrated that growth performance and bone strength were best when dietary Ca to P was 1:1 and were reduced significantly when the ratio increased to 2:1 or 3:1. Brady et al. (2002) also showed optimised animal performance when the dietary ratio of Ca to P was 1.15:1.

4.4.2 Apparent DM Digestibility

Increases in dietary Ca to P ratio were associated with a linear ($P < .0001$) decrease in fecal DM digestibility (**Table 4.4.2)**. At present the biological mechanisms modulating the effect of dietary Ca to P ratio on DM digestibility are not clearly understood. However, Qian *et al.* (1996a) demonstrated that as apparent digestibilities of Ca and P decreased due to wider ratios of Ca to total P in the diets of growing pigs, DM digestibility linearly increased.

4.4.3 Efficiency of Digestive and Post-Absorption of Dietary Ca and P

The results of the efficiency of digestive and post-absorptive utilization of dietary Ca are shown in Table 4.4.3 (a). Increases in the dietary Ca to P ratio showed linear ($P < .001$) and quadratic ($P = 0.009$) responses. True Ca digestibility increased to a maximum at a 0.82: 1 ratio of Ca: P, beyond which a further increment in the ratio resulted in a reduction of efficiency of Ca assimilation by the animal, leading to linear ($P <. 0001$) increases in the concentration of Ca in the pig manure. True Ca retention was also maximized at 0.82: 1 Ca: P.

The results of the efficiency of digestive and post-absorptive utilization of dietary P are shown in Table 4.4.3 (b). Increases in the dietary ratio of Ca to P showed linear responses. Increases in the dietary ratio of Ca to P linearly ($P = 0.0003$) increased true P digestibility and also linearly (P = 0.006) increased true P retention. Interestingly, the maximum true P digestibility and retention were also observed at the same ratio of 0.82 to 1 of Ca to P where maximum true Ca digestibility and retention were demonstrated.

The use of truly digestible P and Ca in swine diet formulation aids in meeting dietary P and Ca requirements for growing pigs. For corn/SBM-based diets, the true Ca to P ratio was optimal at 0.82 to 1 as evidenced by superior growth performance of pigs on diet 2 with concomitant true Ca digestibility and retention of 51 and 50.8%, respectively. This was similarly mimicked by true P digestibility and retention of 51.3 and 51.1%, respectively. The optimal ratio of Ca: P determined in this study are in agreement with other literature data, such as those of Liu et al. (2000) who demonstrated that lowering Ca: P ratio from 1.5: 1 to 1: 1 significantly improved Ca and P absorption. Stein et al. (2011) found that P was best digested when the dietary Ca to P ratio was around or slightly less than 1.1: 1.

Also, with diet 2 the true digestibilities of Ca and P were optimised and demonstrated close similarities 51 and 51.3% leading to reduced Ca and P levels in the excreted manure (Tables 4.4.3 a and b). This further confirms the fact that at optimal ratio of Ca to P, the two minerals are similarly metabolised (Kaune, 1996). Furthermore, these values of true digestibilities are in agreement with values also found with other studies in the literature. For example Fan et al. (2001) showed true P digestibility value of 48.5 to 50.7% in a cornstarch/SBM-based diet for the growing pig; Fang et al. (2007) demonstrated values of 49.4 to 50.6% and Ajaikaye et al. (2003) showed 51 to 59%, respectively.

Optimal true digestible ratio of Ca to P is very critical in the ability of the pig to efficiently utilize Ca and P. Overall, the higher digestibilities of Ca and P in this study were mainly observed with diet 2 where the optimal true digestible Ca to P ratio occurred. Ca and P digestibilities outside the optimal ratio were similar to Ca and P digestibilities estimated in our first study. Therefore, the higher Ca and P digestibility in this study, especially with diet 2, may have been a result of the agreement physiologically between Ca and P (Stein et al. 2011; Peo, 1991 and Kaune, 1996). When the true Ca digestibility value determined in this study was compared to literature value, our value of 51% was somewhat lower than values of Traylor et al. (2001) who demonstrated a range from 61 to 69.3%. The difference between our value and those of Traylor et al. (2001) could have been due to their semi-purified diets compared with our study that used a typical corn/SBM-based diet. Their dietary sources of Ca and P were inorganic dicalcium phosphate and ground limestone, whose Ca and P contents are 95 to 100% digestible (NRC, 1998). Additionally their diets were cellulose-free. Cellulose is known to significantly impede nutrients' digestibility, particularly minerals' digestibility via adsorption and transit time (NRC, 1998; Calvert, 1991). Stein et al. (2011) reported Ca retentions from 46.1% to 62% and P retentions from 43.5% to 57.7%, with the best retentions being at a ratio of dietary Ca to P at or slightly less than 1.1 to 1. This finding is also in close agreement with our study findings with the best Ca and P retentions obtained when the true digestible ratio of Ca to P was 0.82 to 1.

Improvement of diets based on optimal truly digestible Ca to P ratio presents economic opportunities to the swine industry. Preliminary studies (Fan, 2007) showed that the total amount of calcium phosphate and limestone supplementation needed for meeting NRC (1998) Ca and P requirements is about 5 kg for the production and rearing of a pig to a market weight of 110 kg. According to Fan (2007), the dietary inclusion level of these minerals could be reduced by about 40% in comparison with the NRC (1998) recommended levels. This reduction level, considering that P is the most expensive mineral added to swine diets, translates into about $4.5 million saving on feed cost per year for Ontario swine producers. Extrapolation and further expansion of these data indices indicate a significant shift from high cost of production to savings that can be further transformed to industry expansion resulting in more economic gains for the industry.

4.4.4 Volume of Urine Excretion

Changes in the true digestible Ca to true digestible P had no effects ($P > 0.05$) on total volume of urine excreted. Nevertheless, pigs on diets 1, 3, 4, 5 and 6 had numerically higher volume of urine excretion compared with pigs on diet 2, respectively (**Table 4.4.4**). For Ca and P, the indigestible fecal loss, the endogenous fecal loss and the post-absorptive urinary loss are the major, the intermediate and minor routes of excretion, respectively. Accordingly therefore, urinary loss is really a minor route for the excretion of these two macro-minerals and is often used in determining net retention of the minerals. Although the collection of urine during absorption studies enables net retention to be derived and therefore becomes important in interpreting results, net retention *per se* appears to have little value in determining bioavailability of a mineral, including Ca and P (Ammerman, 1995). It has been reported in various studies that mineral element excreted in the urine represents a portion that is potentially nutritionally effective and that has been involved in, or was available for use in metabolism (Ammerman, 1995). This was also observed in this current study. As shown in **Table 4.4.3 b**, it was in diet 1 and at the determined true dietary digestible Ca to the true dietary digestible P ratio level (diet 2) that P urine loss peaked with linear ($P <. 0001$) and quadratic ($P = 0.0005$) decreases as the ratio of Ca to P increased in the diets. Therefore, it would be an error in this case to include the P urinary fraction as part of the unavailable portion of total P dietary intake (Ammerman, 1995).

It is important to note that under normal conditions, the water content of the body is maintained within relatively narrow limits as variation in the rate of water consumption is matched by appropriate changes in the output of urine. According to Mroz *et al.* (1995) there are three primary factors influencing spontaneous drinking of water and its equilibrium in the body, namely: metabolic regulation of the effective circulating volume of fluid in the vascular system; changes in plasma osmolality; and changes in acid-base equilibrium within and between particular compartments. However, at present information on the underlying physiological mechanisms regulating water consumption is limited coupled with the difficulties in establishing the impact of some extrinsic factors, such as ambient temperature, humidity, diet quality and quantity, frequency of water provision, maintenance conditions or stress. In this current study however, pigs on diet two had the least total volume of urinary excretion. A high concentration of water in the manure usually increases costs of storage and disposal (Mroz et al. 1995). Water in manure originates mainly from excreted urine that is known to be closely related to water intake (Mroz et al. 1995). Therefore, it can be concluded that the inappropriate true dietary digestible Ca to true dietary digestible P ratio in diet 1 coupled with higher concentrations of Ca in diets 3, 4, 5 and 6 might in part have been responsible for higher amounts of water intake by those pigs based on the numerically higher volumes of urine excreted by the pigs on those diets compared with pigs on diet 2, respectively. The higher water intake by the pigs observed in this study might be a physiological response or adaptation to consume adequate water to detoxify them in order to be able to tolerate inappropriate electrolyte balance (Mroz et al. 1995). This may be an additional economic benefit in using true digestible Ca to true digestible P ratio in the formulation of diets for pigs to reduce the level of urine in the pig manure leading to reduced costs of manure storage and disposal.

In summary, one of the major challenges currently militating against mineral nutrition in swine production systems is the ratio of Ca to P in their diets, involving the interactions with other minerals. A proper Ca to P ratio therefore is one of the major problems related to dietary adequacy of Ca and P for pigs. As previously stated, P is an expensive mineral element to add to swine diets whereas Ca is inexpensive. The economics of these two important minerals therefore has resulted in swine diets often approaching or exceeding the upper limits for Ca but barely meeting the minimum requirements for P. This has also resulted in feeding excess Ca with a minimum level of P leading to a wide ratio between Ca and P. This trend leads to poor weight gain, poor feed conversion and potentially structural unsoundness (Peo, 1991, Qian et al. 1996b). However, it may be possible for pigs to perform reasonably well on high levels of dietary Ca only if a desirable ratio exists between Ca and P (Peo, 1991), nevertheless with huge negative effect on the environment as a result of high concentrations of P in the pig manure (Mallin, 2000) as also observed in this study with diets 1 and diets 3 to 6 compared with diet 2, respectively.

4.5 Conclusions

The optimal truly digestible Ca to P ratio in our study was 0.82:1. At this ratio animal performance, true Ca and P digestibilities and retentions were maximized. Furthermore, it is at this ratio that the Ca and P levels released in the pig manure were lowest. This ratio is also within the range of Ca to P ratios in sow's milk. This data agreement makes our result very dependable and therefore, it is recommended for adoption for use by the swine industry to better manage the nutrition of Ca to P ratio for the growing pig. Additionally, it should be used to further refine Ca and P dietary requirements for other physiological classes of swine for value capture as well as sustainability of the environment in compliance with the government regulations on our ecosystem.

Table 4.3.2 (a): Diet formulation for Optimal Ca/P Ratio in Growing Pigs (20 – 50 kg body weight)

Ingredients (kg)	**Diet 1**	**Diet2**	**Diet 3**	**Diet 4**	**Diet 5**	**Diet 6**
Soybean meal	27.07	27.07	27.07	27.07	27.07	27.07
Corn	66.00	66.00	66.00	66.00	66.00	66.00
Cornstarch	3.87	3.29	2.71	2.13	1.55	0.97
L-Lys.HCL (79% L-Lys)	0.17	0.17	0.17	0.17	0.17	0.17
L-threonine (100%)	0.05	0.05	0.05	0.05	0.05	0.05
Animal fat	0.50	0.80	1.10	1.40	1.70	2.00
Iodized salt	0.40	0.40	0.40	0.40	0.40	0.40
Limestone (38.5% Ca)[1]	0.28	0.56	0.84	1.12	1.40	1.68
Dicalcium phosphate (22% Ca)	0.86	0.86	0.86	0.86	0.86	0.86
Vit-Mineral premix[2]	0.50	0.50	0.50	0.50	0.50	0.50
Antibiotics mixture[3]	0.00	0.00	0.00	0.00	0.00	0.00
Titanium oxide	0.30	0.30	0.30	0.30	0.30	0.30
Total (100 Kg)	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.0**
Calculated nutritive values (DM basis):						
DE (MJ/Kg)[4]	16.52	16.54	16.55	16.56	16.57	16.60
CP (%)[4]	19.69	19.69	19.69	19.69	19.69	19.69
Total Ca (%)[5]	0.45	0.57	0.70	0.83	0.94	1.07
Total P (%)[5]	0.59	0.59	0.59	0.59	0.59	0.59
Total Ca/total P ratio	0.78	0.98	1.19	1.40	1.61	1.81
NDF (%)[6]	11.29	11.29	11.29	11.29	11.29	11.29
ADF (%)[6]	4.98	4.98	4.98	4.98	4.98	4.98
Cellulose+hemicellulose (%)[6]	6.30	6.30	6.30	6.30	6.30	6.30

[1]Commercial feed grade limestone; contained 38.5% Ca.

[2]Vit-mineral premix contained per kg of pre-mix: vitamin A, 2,000,000IU; vitamin D3, 200,000IU; vitamin E, 8,000IU; vitamin K, 500mg; pantothenic acid, 3,000mg; riboflavin, 1,000mg; folic acid, 400mg; niacin, 5,000mg; thiamine, 300mg; pyridoxine, 300mg; vitamin B12, 5,000mcg; biotin, 40,000mcg; Se, 60mg; choline, 100,000mg; I, 100mg; Cu, 3,000mg; Fe, 20,000mg; Mn, 4,000mg; Zn, 21,000mg.

[4,5,6]Calculated values. [3]Not added to diets.

Table 4.3.2 (b). Calculated and analysed concentrations of Ca and P in experimental diets (DM basis)

Item	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Ca concentration, %						
Calculated value	0.45	0.57	0.70	0.83	0.94	1.07
Analysed value	0.35	0.49	0.57	0.67	0.60	0.61
P concentration, %						
Calculated value	0.59	0.59	0.59	0.59	0.59	0.59
Analysed value	0.59	0.60	0.63	0.63	0.61	0.59
Calculated Ca: P	0.78: 1	0.98: 1	1.19: 1	1.40: 1	1.61: 1	1.81: 1
Analysed Ca: P	0.59: 1	0.82: 1	0.90: 1	1.06: 1	0.98: 1	1.03: 1

Table 4.4.1. Mean ± SE growth responses of growing pigs fed corn/SBM-based diets but with varied Ca to P ratios

Item	0.35 n = 6	0.49 n = 6	0.57 n = 6	0.67 n = 6	0.60 n = 6	0.61 n = 6	SEM	*P*-value	
								linear	quadratic
ADFI (kg/d)	1.24	1.18	1.14	1.22	1.18	1.18	0.03	0.40	0.33
ADG (kg/d)	0.64	0.71	0.67	0.66	0.59	0.60	0.03	0.02	0.18
ADG/ADFI	0.52	0.60	0.58	0.54	0.50	0.51	0.02	0.03	0.02

Table 4.4.2: Effects of six gradient levels of dietary calcium (Ca) to phosphorus (P) ratios on apparent fecal dry matter (DM) digestibility in growing pigs fed corn/SBM-based diets (n = 6)

Item	0.35	0.49	0.57	0.67	0.60	0.61	SEM	*P-value*	
								linear	quadratic
DM digestibility	79.3	72.8	77.6	76.0	74.0	73.4	0.65	<.0001	0.65

Table 4.4.3 (a): Effects of six gradient levels of dietary calcium to phosphorus ratios on efficiency (% of total dietary intake) of digestive and post-absorptive utilization of dietary Ca in growing pig fed corn/SBM-based diets (n = 6)

Item	0.35	0.49	0.57	0.67	0.60	0.61	SEM	*P-value*	
								Linear	Quadratic
Apparent Ca digestibility	22.34	34.47	35.46	32.02	17.02	13.08	3.86	0.004	0.0004
True Ca digestibility	45.29	51.04	49.75	44.04	30.46	26.27	4.07	<.0001	0.009
Urinary Ca loss	0.37	0.25	1.14	1.74	5.84	5.56	0.63	<.0001	0.05
Dietary Ca fecal loss	54.34	48.71	49.11	54.22	63.70	68.17	1.32	<.0001	0.72
Apparent Ca retention	21.97	34.22	34.32	30.28	11.18	7.52	1.95	<.0001	0.62
True Ca retention	44.92	50.79	48.61	42.30	24.62	20.72	3.97	<.0001	0.003

Table 4.4.3 (b): Effects of six gradient levels of dietary calcium to phosphorus ratios on efficiency (% of total dietary intake) of digestive and post-absorptive utilization of dietary P in growing pig fed corn/SBM-based diets (n = 6)

								P-value	
Item	0.35	0.49	0.57	0.67	0.60	0.61	SEM	linear	Quadratic
Apparent P digestibility	18.95	30.42	22.87	14.28	8.9	13.65	2.92	0.0004	0.37
True P digestibility	40.24	51.34	42.73	34.34	29.42	34.97	3.26	0.0003	0.64
Urinary P loss	3.93	1.26	0.66	0.09	0.00	0.06	0.44	<.0001	0.0005
Dietary fecal P loss	55.83	47.40	56.61	65.57	70.58	64.97	1.41	0.05	0.66
Apparent P retention	15.02	29.16	22.21	14.19	8.9	13.59	1.21	<.0001	0.56
True P retention	36.32	50.07	42.07	34.25	29.42	34.91	3.28	0.006	0.32

Table 4.4.4: Effect of six gradient levels of dietary calcium (Ca) to phosphorus (P) ratios on urinary excretions (litre) in growing pig (20 – 50 kg) fed corn/SBM-based diets (n = 6)

								P-value	
Item	0.35	0.49	0.57	0.67	0.60	0.61	SEM	Linear	Quadratic
Volume of urinary excretion	15.37	10.44	14.37	11.43	13.77	13.87	0.22	0.89	0.24

CHAPTER 5

ESTIMATION OF TRUE Mg, TRACE MINERALS (Cu, Fe, Mn, Se AND Zn) DIGESTIBILITY AND THEIR ENDOGENOUS OUTPUTS IN GROWING PIGS FED CORN-SBM-BASED DIETS BY THE SUBSTITUTION METHOD

5.1 Abstract

Twelve Yorkshire barrows with known initial BW of 23.9 ± 1.1 (mean ± SD), were assigned to two dietary treatments with six replications per treatment. The two diets were formulated in accordance with the principles of the substitution method. Diets' DM digestibility, animals' performance, the minerals' apparent and true digestibility (AD and TD) values as well as their endogenous fecal losses (EFL) were investigated. The pigs were randomly allotted to their individual feeder pens which enabled individual pig fresh fecal sample collections. The experiment was designed and carried out as a completely randomized design. Results demonstrated that the DM digestibility of diet 1 (79%) is significantly lower ($P < 0.05$) than that of diet 2 (86%). However, the animals on diet 1 had superior (P < *0.05*) ADG and FE compared with animals on diet 2; despite the fact that both groups of animals had similar ($P > 0.05$) ADFI. The AD values of Se (73.9%) and Zn (9.5%) were significantly lower ($P < 0.05$) than their TD values of 82.1% and 15%, respectively. Se and Zn EFL were 0.00004 mg and 0.01 mg/kg of DMI, respectively. The TD values and their EL for Mg, Cu, Fe and Mn were not estimated because of their negative AD values. It was concluded that the TD values of minerals be employed in diet formulation for the growing pig to avoid their mutual antagonism during metabolism.

Key Words: Apparent and true minerals digestibility, Endogenous losses, Pigs

5.2. Introduction

Although trace mineral losses from animals into the environment are inevitable (Tamminga, 2003), there is a need to determine means of supplying these minerals based on the true animal requirements. Nitrogen and P pollution is well documented. Similarly, the accumulation of some trace minerals in the soil, particularly Cu and Zn, can cause medium to long term toxicity effects on plants and soil micro-organisms (Dourmad and Jondreville, 2007). To this end, it has been demonstrated that more than 90% of ingested Cu and Zn by pigs is excreted in manure (Aarnink and Verstegen, 2007). These indices are pointers to the fact that in the near future legislation in trace mineral contents in swine diets may be enacted to mitigate their effects on environmental pollution. The quantification of true mineral digestibilities and endogenous losses will help meet the dietary requirements for these trace yet very important minerals without excessive loss into the environment. The usefulness of this finding may not be limited to swine diets alone but also in human nutrition, particularly for those whose diets are composed mainly of plant-based ingredients, as the pig model has been demonstrated to be a useful model to elucidate mechanisms governing dietary influences on mineral metabolism and absorption in humans (Patterson et al. 2008). One of the main causes of trace mineral losses from the digestive tract has been high dietary levels of Ca and P in the diets (Snedeker et al. 1982; Wood and Zheng, 1997). This implies that with a properly defined digestible Ca to P ratio, the associated insoluble-complex formation with minerals and chelating effects of phytate that aggravate endogenous losses of trace minerals can be effectively controlled and thus aid in reducing nutrient environmental loads from swine production. It has been shown that high levels of Ca and P in animal and human diets decrease Mg, Cu, Fe, Mn and Zn absorption (Snedeker et al. 1982; Wood and Zheng, 1997; Miura et al. 1999; and Han and Thacker, 2006).

To our knowledge there is no information to date on the true digestibility of Mg, Cu, Fe, Mn, Se and Zn in a corn-SBM-based diet for growing pigs. The objectives of this study were to determine the true digestibility and the endogenous outputs of Mg, Cu, Fe, Mn, Se and Zn associated with a corn/SBM-based diet for growing pigs, using the substitution method outlined by Fang *et al.* (2007).

5.3. Materials and Methods

5.3.1. Animals, Housing and Experimental Design

Twelve Yorkshire growing barrows with an average initial BW of 23.9 ± 1.1 (mean ± SD) kg were acquired from Arkell Swine Research Station, University of Guelph and used in this current study. The animals were housed in tender-foot™ plastic floor pens (1.5 x 2.1 m) with smooth transparent plastic sides in a room that was mechanically ventilated to provide an ambient temperature of 20-22^{0}C. All procedures used in the management of the pigs were reviewed and approved by the University of Guelph Animal Care Committee. Animals were cared for with strict compliance to the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

5.3.2. Experimental diet

Two dietary treatments were formulated at 100% (HN diet) and 60% (LN diet), respectively, of the NRC (1998) requirements for Mg, Cu, Fe, Mn, Se and Zn for the growing pig. The LN diet was formulated by partially replacing corn, SBM, limestone, dicalcium phosphate and vitamin-mineral premix with increased cornstarch and Solka-Flock^{TM} to balance for DE and NDF contents (**Table 5.3.2**). Titanium oxide was also added at 0.3% of diet DM as an indigestible marker (**Table 5.3.2**)

5.3.3. General conduct of study for computing performance parameters

Feed intake was normalized for both HN- and LN-diets offered at 5% of BW. At the beginning of each day during the experiment, orts from the previous day were collected, dried at 105^{o}C, and weights were recorded. The difference between dry feed delivered and the next day's orts represented DM consumed by the animal for the day. On the last day of the study, all animals were weighed. Average daily feed intake (ADFI), average daily gain (ADG), as well as gain to feed ratio for the study period were obtained.

5.3.4. Diet and Fecal Samples Collections and Processing

Feed samples were collected immediately after mixing each diet and stored in sealed sample bags at 4^{o}C. They were later ground in a Wiley mill through a 1-mm screen and stored at 4^{o}C until analyzed.

From the 11th to the 15th day in the study, fecal samples were collected from all the animals on the two dietary experimental treatments. The fecal samples were spot collected from each pen during this period at 2-h intervals. Fecal samples collected were sealed in the fecal sample containers and were immediately frozen at -20^{o}C. They were later freeze-dried and ground in a Wiley mill through a 1 mm screen and stored in the cool room (4^{0}C) until required for analyses.

5.3.5. Chemical Analyses

Dry matter of samples was determined according to the method of AOAC (2000). Diet and fecal sub-samples were oven-dried at 105^{0}C for 24 h. After drying, samples were removed from the oven, cooled using a dessicator and re-weighed. The DM content was determined as final weight/initial weight x 100.

Titanium dioxide concentrations in diet and fecal samples were analysed according to the method of Short *et al.* (1996). Briefly, samples were weighed, ashed and digested in concentrated sulphuric acid. Hydrogen peroxide (30% vol.) was added to develop an orange colour that was read at 410 nm using a UV/Visible Spectrophotometer (Ultrospec 2000, Biochrom Ltd., Cambridge, UK).

Dietary mineral contents of the two dietary treatments and fecal sub-samples (Mg, Cu, Fe, Mn, and Zn) were determined according to AOAC (1996). The samples were first microwave digested (MARSxpress 2009, CEM Corporation, Matthews, NC, USA) with nitric acid. The microwave digested sample was brought to volume with Nanopure water. The clear extract supernatant was measured by Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (Varian Vista Pro (radial torch) 2001, Varian Canada, Mississauga, ON, Canada) without further processing. Mineral contents of pig drinking water was also analysed according to AOAC (1996) using ICP-OES.

Dietary selenium (Se) contents of the two dietary treatments and fecal sub-samples were also determined according to AOAC (1996) by spectrofluorimetric analysis. Samples were digested in perchloric/nitric acid and converted to Se^{4+} by reduction with hydrochloric acid. Samples were then chelated using ammonia/EDTA solution using a hot block. They were then reacted with 2,3 diaminonaphthalene and extracted in cyclohexane. The cyclohexane extract was analysed spectrofluorometrically (Spectrofluorometer: Varian Cary Eclipse, 2001, Varian Canada, Mississauga, ON, Canada).

5.3.6. Calculations and Statistical Analyses:

The apparent fecal DM, Mg, Cu, Fe, Mn, Se and Zn digestibilities (AFD; %) were first calculated using the marker technique according to:

$$AFD = \frac{N_{Diet}/M_{Diet} - N_{Feces}/M_{Feces}}{N_{Diet}/M_{Diet}} \qquad (1)$$

where N_{Diet} = the concentration of nutrient in the diet (%), M_{Diet} = the concentration of titanium marker in the diet (% titanium), N_{Feces} = the concentration of nutrient in the feces (%), and M_{Feces} = the concentration of marker in feces. All percentages were on a DM basis.

Based on average AFD of Mg, Cu, Fe, Mn, Se and Zn, true fecal digestibilities (TFD; %) were estimated as:

$$TFD = \frac{\overline{AFD_{Diet1}} N_{Diet1} - \overline{AFD_{Diet2}} N_{Diet2}}{N_{Diet1} - N_{Diet2}} \qquad (2)$$

where Diet 1 is the high-nutrient diet and Diet 2 is low-nutrient.

Endogenous fecal losses (EFL; g/kg DMI) of Mg, Cu, Fe, Mn, Se and Zn were estimated as:

$$EFL = \left(\overline{TFD_{Diet}} - \overline{AFD_{Diet}}\right) N_{Diet} 10 \qquad (3)$$

Data were subjected to ANOVA using PROC GLM of SAS (SAS Inst. Inc., Cary, NC) according to:

$Y_{ij} = \mu + D_i + E_{ij}$:

where Y_{ij} is the observation; μ is the overall mean common to all treatments, D_i is the effect of the i^{th} diet, and E_{ij} is the error term. Furthermore, homogeneity of variances across the two diets was tested for and confirmed ($P > 0.05$) by Levene's test using SAS (SAS Inst. Inc. Cary, NC). The pig was the experimental unit and an α-level of 0.05 was used for all statistical comparisons to represent significance.

5.4. Results and Discussion

5.4.1 Animal Performance

All animals consumed their diets normally throughout the 15-d experimental duration and also grew during the period. The performance of the animals on the two dietary treatments is shown in **Table 5.4.1**. The ADFI of animals on the two dietary treatments were very similar ($P = 0.93$). The ADG of pigs on the HN diet was superior ($P = 0.0003$) compared with pigs that consumed the LN diet. This resulted in a better feed efficiency ($P < .0001$) for the HN diet compared with the LN diet, respectively (**Table 5.4.1**).

5.4.2 Apparent DM and Mineral Digestibility

The final actual analysed dietary contents of Mg, Cu, Fe, Mn, Se and Zn for the HN and LN diets are shown in **Table 5.4.2 a**. The results of the apparent fecal digestibility values of dry matter (DM), Mg, Cu, Fe, Mn, Se and Zn in the experimental diets are shown in **Table 5.4.2b**. The apparent DM digestibility for the HN diet was 79% while that of the LN diet was 86% which is significantly higher ($P < .0001$) than that of the HN diet. This may have been due to the higher cornstarch content of the LN diet compared with the HN diet because the energy of cornstarch is approximately 100% digestible compared to that of corn and SBM that are 96 and 92% digestible, respectively (NRC, 1998).

The apparent digestibility values of most of the minerals studied were negative except for Se and Zn (Table 5.4.2 b). The negative apparent mineral digestibilities observed have also been shown with studies in the literature (Woyengo et al. 2009 and Jongbloed et al. 2004). The apparent digestibility of Zn (9.5%) is in agreement with NRC (1998) values. NRC (1998) reported apparent digestibilities of Mg, Cu, Fe and Zn from 5 to 30%. Also, the apparent Se digestibility value of 73.9% estimated in this study is in agreement with literature data, such as those of Wright and Bell, (1966) who found apparent digestibility of Se of 73.5%, on average.

5.4.3 True Mineral Digestibility and Their Endogenous Losses

True fecal digestibility values and fecal endogenous outputs of Se and Zn are shown in **Table 5.4.3**. The true fecal digestibility of Se was 82.1% and that of Zn was 15%. Furthermore, Se fecal endogenous loss was 0.00004 mg/kg DMI and that of Zn was 0.01 mg/kg DMI. The animals thus demonstrated low endogenous losses of these minerals. This may be related to animal physiological adaptation in the metabolism of these minerals probably in response to their micro-dietary concentrations compared to Ca and P that are usually provided at macro-dietary levels for the animals (NRC, 1998). However, the true digestibility values for Mg, Cu, Fe and Mn and their endogenous losses could not be estimated in this study because of their negative apparent digestibility values. The true digestibility and the endogenous losses of Se and Zn could not be compared with any literature data as this is the first study, to our knowledge, to investigate true Se and Zn digestibility and their endogenous losses in growing pigs.

It has been shown that more than 90% of the ingested trace-minerals, particularly Cu and Zn, are excreted (Aarnink and Verstegen, 2007) in pig feces. The percentage absorptions of ingested trace minerals are influenced by their dietary concentrations and the presence of inhibiting or promoting dietary components (Peo, 1991; Brink et al., 1992; Liu et al., 2000). For instance, phytate is an effective chelator of positively charged molecules and thus has a high potential to form stable insoluble complexes with minerals, especially with divalent ions (O'Dell, 1997; NRC, 1998), including proteins (Maga, 1982). The insoluble complexes confer on phytate anti-nutritional properties for pigs as the animals lack the hydrolytic enzyme phytase in their digestive tract. The negative digestibilities of Mg, Cu, Fe and Mn observed in this current study could have been due to mutual antagonisms that affected their availability for digestion or formation of insoluble complexes with phytate. Woyengo et al. (2009) showed that increasing dietary phytate level significantly reduced Mg digestibility from 29% to a negative value by increasing endogenous losses.

The negative apparent digestibility values estimated for most of the minerals studied were somewhat surprising initially, since their dietary contents were within requirements and the calculated Ca: P was 1.19:1 (**Table 5.3.2**). The ratio of dietary Ca: P has been shown to be very important in Ca, P, and Mg digestibility (Brink et al., 1992; Liu et al., 2000) as well as for trace-mineral digestibility (Hu et al., 2010). In fact, in the study of Hu et al. (2010), it was shown that as the ratio of dietary Ca: P gets wider, Cu, Fe, Mn and Zn showed negative apparent digestibility values, to an even greater extent than observed in the study reported here.

Traditionally, mineral contents of the animal drinking water are not considered in diet formulation and mineral digestibility studies. One of the interesting findings in our current study strongly disagrees with this traditional nutrition practice. To this point, the mineral contents of our pigs' drinking water, especially for Ca, Mg and P were very revealing as shown in **Table 5.4.4.**

According to NRC, (1998) growing pigs will consume 3.7 kg of water per kg of feed on an as-fed basis. A recent study (Li et al., 2005) estimated average water intake of 4 to 5.38 kg water/kg DMI. Additionally, growing pigs normally have a tendency to drink more water, especially in the afternoon, when feed is restricted as in this study, due to a desire for abdominal fill (NRC, 1998). Thus, although the exact volume of water our pigs consumed was not measured, there is no doubt that the quantity consumed by our experimental pigs for the entire experimental duration would have been high enough to provide excess Ca (7.3g; 4.5g) and Mg (2.4g; 1.6g)/kg DMI for the HN and LN diets, respectively and, to a lesser extent, Zn, Cu, Mn and Fe for the animals. To this point there was a need to investigate the effect of minerals from the pig drinking water on the mineral digestibility. When total estimated mineral intakes from both ingested water and diet sources was considered in the estimation of mineral digestibilities, there was little effect compared to digestibilities when water source minerals were ignored (Table 5.4.5). In this study, P content of drinking water did not appear to be a problem since it was less than 0.1 ppm. However, the amount of Ca consumed via water significantly altered the dietary ratio of Ca: P from 1.19:1 to approximately 1.26: 1 and 1.25: 1 for the HN and LN diets, respectively, and possibly causing more Ca to precipitate and instigate a series of antagonistic interactions, especially in the presence of P and Mg, with the dietary minerals, rendering them unavailable for absorption (Brink et al. 1992; Liu et al. 2000; Hu et al. 2010). Solubility of these minerals is required for digestion. Their insolubility therefore further decreases the animal's ability to digest the minerals (Knowlton et al. 2004) coupled with a possible negative hormonal feedback mechanism because of their effects on increasing the pH of the stomach (O'Dell, 1997; Montminy et al., 2007; Shoback and Sellmeyer, 2010). Our suggestion of a negative effect of drinking water minerals on mineral digestibility has been substantiated by previous researchers (Flipot and Ouellet, 1988; Castillo et al., 2007). Therefore, estimates from our current study alert us to the importance of mineral content of drinking water to be known and included accordingly in estimating mineral supplies to the animal. Overall, water source mineral should always be considered where appropriate in feed formulation to better guide the feed formulator in meeting animal mineral requirements as to avoid excess minerals intake to enable the reduction of minerals export from the pig barn into the environment.

5.5 Conclusions

The results of this study show that true Se digestibility in corn/SBM-based diet for the growing pig (20 -50 Kg) BW is 82% whereas the true Zn digestibility in corn-SBM-based diet is 15%, respectively. Endogenous fecal loss of Se is 0.00004 mg and that of Zn is 0.01 mg/DMI respectively. It is also further concluded that the determination of true digestibility of trace minerals and their endogenous losses require measurement of mineral intakes via the animal drinking water.

Table 5.3.2: Diet formulation for measuring true Mg, Cu, Fe, Mn, Se and Zn digestibility and their endogenous outputs in grower pigs (20 - 50 Kg) by the Substitution (40%) Method

Ingredients (kg)	**HN (Diet 1)**	**LN (Diet 2)**
Soybean meal	27.07	16.24
Corn	66.00	39.6
Cornstarch	2.71	36.93
Solka-Flock^{TM} (100% cellulose)[1]	0.00	3.98
L-Lys.HCL (79% L-Lys)	0.17	0.102
L-threonine (100%)	0.05	0.03
Animal fat	1.10	1.10
Iodized salt	0.40	0.40
Limestone (38.5% Ca)[2]	0.84	0.50
Dicalcium phosphate (22% Ca)	0.86	0.52
Vit-Mineral premix[3]	0.50	0.30
Antibiotics mixture	0.00	0.00
Titanium oxide[4]	0.30	0.30
Total (100 Kg)	100.00	100.00
Calculated nutritive values (as-fed basis):		
DE (MJ/Kg)	14.57	14.81
CP (%)	17.33	10.40
Total Ca (%)	0.62	0.37
Total P (%)	0.52	0.31
Total Ca/total P ratio	1.19	1.19
NDF (%)	9.94	9.94
ADF (%)	4.39	2.64
Cellulose+hemicellulose (%)	5.54	7.31

[1]Solka-Flock^{TM}: commercial cellulose fibre product from International Fibre Corporation. [2]Commercial feed grade limestone; contained 38.5% Ca.[3]Vit-mineral premix contained per kg pre-mix: vitamin A, 2,000,000IU; vitamin D3, 200,000IU; vitamin E, 8,000IU; vitamin K, 500mg; pantothenic acid, 3,000mg; riboflavin, 1,000mg; folic acid, 400mg; niacin, 5,000mg; thiamine, 300mg; pyridoxine, 300mg; vitamin B12, 5,000mcg; biotin, 40,000mcg; Se, 60mg; choline, 100,000mg; I, 100mg; Cu, 3,000mg; Fe, 20,000mg; Mn, 4,000mg; Zn, 21,000mg.

[4]Titanium oxide used as a digestibility marker.

Table 5.4.1: Mean ± SE growth responses of pigs fed high- and low-nutrient corn/SBM-based diets (n = 6)

Item	High-Nutrient (Diet 1)	Low-Nutrient (Diet 2)	*P-value*
ADFI (DM basis; kg/d)	1.3 ± 0.03	1.3 ± 0.03	0.93
Initial BW (kg)	23.7 ± 1.2	24.2 ± 1.1	0.48
Final BW (kg)	32.3 ± 1.3	30.2 ± 1.6	0.03
ADG (kg/d)	0.58 ± 0.01	0.40 ± 0.03	<0.001
ADG/ADFI	0.51 ± 0.01	0.35 ± 0.02	<0.001

Table 5.4.2 a. Analysed dietary mineral contents of diets

Item	HN diet (amount/kg diet)	LN diet (amount/kg diet)
Mg	1.6 g	0.88 g
Cu	30 mg	6.6 mg
Fe	260 mg	130 mg
Mn	51 mg	36 mg
Se	0.47 mg	0.19 mg
Zn	150 mg	68 mg

In the vitamin-mineral premix Cu was provided by copper sulphate (25%), Fe was provided by ferrous sulphate (30%), Mn was provided by manganese sulphate (31.5%), Se was provided by sodium selenite (4.5%) and Zn was provided by zinc sulphate (35.5%)

Table 5.4.2 b. Apparent fecal digestibility values of DM, Mg, Cu, Fe, Mn, Se and Zn in the experimental diets measured for the growing pig fed corn/SBM-based diets by the Substitution Method

Items	Diet 1 (n = 6)	Diet 2 (n = 6)	*P-value*
	%		
DM	78.74 ± 0.66	85.63 ± 0.42	*P < .0001*
Mg	-29.70 ± 3.68	-10.17 ± 3.1	*0.0023*
Cu	-10.68 ± 1.85	-139.2 ± 4.42	*P < .0001*
Fe	-20.14 ± 9.15	-21.17 ± 2.52	*0.9164*
Mn	-22.29 ± 3.39	18.70 ± 2.08	*P < .0001*
Se	73.86 ± 1.0	61.59 ± 1.81	*0 .0001*
Zn	9.45 ± 3.31	2.66 ± 1.25	*0 .0839*

Table 5.4.3: The true fecal digestibility values and the fecal endogenous outputs of Se and Zn associated with corn/SBM-based diet for the growing pig measured

by the Substitution Method (n = 12)

Items	True digestibility %	Fecal endogenous loss (g/kg DM intake)
Se	82.08	0.00004 mg
Zn	15.00	0.01 mg

Table 5.4.4. Ca, Mg, P, Fe, Cu, Mn and Zn content of pig drinking water.

Mineral	Water content (ppm)
Ca	51
Mg	15
P	< 0.10
Fe	< 0.01
Cu	0.08
Mn	0.01
Zn	0.25

Table 5.4.5. Effect of water mineral intake on apparent mineral digestibility

Mineral	Diet 1 (n = 6)	Diet 2 (n = 6)
	%	
Mg	-25.35	-3.63
Cu	-9.60	-128.91
Fe	-20.13	-21.13
Mn	-22.20	18.78
Zn	10.00	3.97

CHAPTER 6

GENERAL DISCUSSION

Corn and SBM are usually used as energy, protein and mineral sources in swine rations. There are nutritional, economical and environmental benefits in the use of corn and SBM in swine nutrition. These benefits include: low fibre contents in corn compared with barley and wheat to provide more available energy to pigs (NRC, 1998). In terms of economic advantages, corn has a very high yielding value within a short growing period which is an indicator that it can quickly be translated into more pounds of pork per acre than barley. Nevertheless, it has low Ca and P contents compared to barley and wheat (NRC, 1998).

However, SBM plays a complementary role in corn/SBM-based diets for swine. This is because SBM is also low in fibre and serves as a very good source of energy, protein and some essential amino acids that are limiting in corn (NRC, 1998). More importantly, the low contents of Ca and P in corn are compensated for by the high contents of these minerals in SBM. Due to the low fibre contents in corn and SBM, corn/SBM-based diets enable nutrients to be more digestible, primarily as a result of reduced effect of fibre during fibre-nutrient interactions which are known to encapsulate nutrients and thus significantly reduce substrate-enzyme interactions (Low, 1985). Therefore, in a corn/SBM-based diet, the negative effects of fibre-nutrient interaction on nutrient digestion and absorption are significantly reduced in the pig, particularly in weanling and growing pigs (Low, 1985). This results in better nutrient availability to the animals, including minerals (Calvert, 1991). This is also expected to reduce nutrient concentrations, especially those of minerals, in the manure that is eventually excreted into the environment in swine-producing areas. To date, the reduction of mineral levels in the pig manure has not been fully achieved and currently it has become a serious source of concern to all stakeholders. Here, the major concern relates mostly to the high levels of P in the manure resulting in environmental pollution (Mallin, 2000). Additionally, the high level of trace-minerals in the pig manure is also gradually becoming another source of concern to the swine industry (Dourmad and Jondreville, 2007). This is also thought to be related to high dietary mineral contents antagonizing absorption, and the presence of phytate as an effective chelator of minerals, particularly P and cations, such as Ca, Mg, Cu, Fe, Mn, and Zn which renders the minerals unavailable to the animals.

Thus, irrespective of the complementary roles existing between corn and SBM in a corn/SBM-based diet for pigs, the presence of dietary fibre and phytate help to explain the high mineral concentration levels in pig manure (Lei and Porres, 2003). The overall resultant effects of these further aggravate the antagonisms among dietary minerals as they induce nutritional distortions leading to physiological mineral imbalances during the process of mineral flux from their dietary source to the animal (Peo, 1991; Petersen, 2010). This observation becomes worse when dietary minerals are included in the diet based on their apparent digestibility values (ADV). To this point, based on mineral wheel study interactions/interrelations it has been reported that when dietary minerals are provided based on the animal true dietary requirements they would show mineral-ion synergy relationship during their normal course of metabolism thereby physiologically leading to the majority of the minerals being made bio-available to the animal for assimilation; and inversely demonstrate mineral-ion antagonisms when dietary mineral supplies are not balanced based on the animal true dietary needs (Peo, 1991; NRC, 1998; Knowlton *et al.* 2004). Animal production and particularly that of swine production has been projected to be on the increase (Tamminga, 2003). Furthermore, although nutrient release via animal manure to the environment is inevitable, the possibility exists from nutritional standpoint to significantly reduce nutrient manure levels into the environment. This is possible by improving swine diets according to their phases in production by fortifying their diets with true dietary nutrient requirements, including minerals. This has the effect of favouring improved mineral metabolism by triggering mineral synergies in the course of their normal metabolisms and thus significantly reduces the formations of insoluble-phytate-mineral-complexes (NRC, 1998; Lei and Porres, 2003) that impede mineral solubility for absorption. Minerals' antagonisms and especially in the presence of phytate consequently inducing the formation of insoluble-phytate-mineral-complexes has been identified as one of the major factors responsible for the high levels of mineral levels in the pig manure causing environmental pollution (Mallin, 2000; Lei and Porres, 2003) as previously mentioned. As a result of these observations, the overall primary objective of mineral intake therefore should be based on maintaining proper mineral balance to avoid the fore stated problems associated with mineral nutrition in the pig.

The main strategy in dealing with the problem of mineral nutrition therefore is the fortification of swine diets based on the true digestibility values (TDV) of individual minerals in the diets. Traditionally, swine diets are formulated based on the animal mineral apparent digestible value (ADV). The ADV of the minerals from a nutritional point of view underestimates the true digestibility values of the dietary minerals were confirmed in our studies 1 and 3. Nutrients supplies beyond requirements ultimately give rise to the excretion of the excess in the pig manure (NRC, 1998). This is why the use of TDV is to the rescue as it is always higher compared with the ADV. Additionally, it is more accurate and therefore more reliable in meeting the animal true requirements (Ammerman, 1995). This is true because according to Ammerman (1995) ADV is defined as the difference between the total intake of a mineral or element and the total fecal excretion of the element. The value obtained by this difference is usually expressed as a percentage of the intake. On the other hand, TDV corrects for the portion of the mineral which has been absorbed into the animal's body but subsequently excreted back (inevitable loss) into the gastrointestinal tract as a component of the physiological consequence involving the metabolism of the mineral. Different researchers referred to this portion of the mineral as the total endogenous fecal excretion (Ammerman, 1995; Rodehutscord *et al.* 1998). Therefore, TDV is derived by the difference between the mineral total intake minus the total fecal and total endogenous excretions combined of total intake multiplied by 100 and thus TDV is always expressed in percentages just like the ADV counterparts, resulting in a higher TDV compared with ADV.

Thus, the TDV is more dependable estimate of the mineral presented to the body tissues for metabolic purposes. Therefore, it has been recommended that for the effective utilization and thus better management of ingested dietary mineral for non-ruminants such as the pig, the TDV of the mineral should be determined and employed in diet formulations to better support improved mineral metabolism (Ammerman, 1995). This is also supported by the studies of Rodehutscord et al. (1998) as well as our findings that TDV is a more accurate estimate of the dietary mineral available for the pig.

In these regards, experiment 1 was conducted to determine the TDV for Ca and P. These two macro-minerals, particularly P have recently been the driving minerals in the studies of mineral metabolisms in swine nutrition as they are the most included in swine diets (NRC, 1998). More importantly, P is both an essential and economic nutrient for swine yet it is also the major environmental pollutant in intensive swine production regions, globally. Furthermore, it is the third most expensive nutrient after energy and protein in swine diets (NRC, 1998). Although Ca and P are the most abundant minerals in the body of the pig compared to other minerals, they are mutually antagonistic to each other (Peo, 1991; O'Dell, 1997; NRC, 1998). Therefore, their antagonisms would no doubt negatively affect the metabolisms of other macro-and micro-minerals as a result of the interplay between Ca and P involving more complex cascades' of mechanisms, such as the ones involving metal-ion interactions and hormones as they relate to the entire dietary minerals' metabolism by the animal (Peo, 1991; O'Dell, 1997; Shoback and Sellmeyer, 2010). These scenarios are even worse with the minerals whose dietary concentrations are marginal or at borderline, such as those of Mg and Zn whose deficiencies may thus be induced. This is because both Ca and P are also mutually antagonistic to Mg and Zn (Peo, 1991; O'Dell, 1997; Rude, 1998). Overall, in such conditions dietary requirement for Zn for instance is often three to four fold higher in growing pigs fed conventional diet, such as that of corn-SBM-based diet compared to those fed phytate-free diets (NRC, 1998).

Again, to this point therefore experiment 1 was conducted using a completely randomized design and involved two corn-SBM-based diets hinged on the principles of the difference method to estimate true Ca and true P digestibility values, including their endogenous outputs associated with corn-SBM-based diets for the growing pig. The findings from this study showed that the apparent digestibility values for Ca and P in a corn-SBM-based diet are 28.4% and 24%, respectively. Furthermore, their true digestibility values are 42% and 40%, respectively. Additionally, their daily fecal endogenous losses are 0.8 g/kg DMI and 1.3 g/kg DMI for Ca and P, respectively. These results demonstrate that the true digestible values for Ca and P are actually significantly ($P < 0.05$) higher than their apparent digestibility values. These findings are supported by other independent workers (Ammerman, 1995; Rodehutscord et al. 1998) who also found that apparent digestibility value of an element significantly underestimates the true digestibility value of the element. As previously discussed therefore, the true digestibility value of a mineral, including Ca and P is a more reliable value in meeting the animal true requirement for these minerals and as such are better indicators in the formulation of swine diets that meet the animal true requirements for the mineral with resultant effect of minimizing mineral excretion in the pig manure. This is true because the excess of the mineral normally included in the diets when formulation is based on apparent digestibility values are voided or entirely eliminated and thus leads to practical digestible low-P and low-Ca diets developed to solely meet the nutritional needs of Ca and P of the animals without compromising their performance, health and well-being. This also comes with additional economic and environmental benefits. Economically, when the true digestibility values of Ca and P as found in this study are adopted for growing pigs it would result in a significant reduction of the amounts of both Ca and phosphate salts, particularly that of P used in formulating rations for growing pigs culminating in the increase of profit margins for the hog producer as P has been shown to be the third most expensive nutrient for pigs after energy and protein (NRC, 1998). Environmentally, it would also minimize the amounts of Ca and P in the pig manure that are released into the environment and thus significantly reduce the negative impacts of swine production on the environment (NRC, 1998; Mallin, 2000).

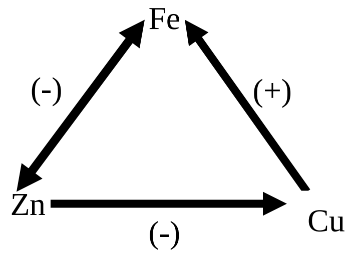
Ca and P are major macro-minerals in swine rations as their dietary ratios in the rations are very critical to their enhanced metabolism that is also known to affect both the metabolisms of other essential dietary minerals and nutrients (NRC, 1998; Liu et al. 2000). For the effective utilization of Ca and P true digestibility values determined in the first study the ratio in which they should be optimally provided in swine rations need to be established for the grower pig.

Therefore, for the better use and adoption of the findings of the first study, experiment two was designed with the following objectives:

to determine an optimal true digestible Ca to true digestible P ratio in grower (20 – 30 kg) pigs fed corn-SBM-based diets; and to also investigate the effect of altering true digestible Ca to true digestible P ratio on growth performance, efficiency of Ca and P retentions as well as to measure Ca and P excretions in grower pigs fed corn-SBM-based diets. The study was conducted as a randomized complete block design with different dietary Ca to P ratios of the six dietary treatments as the only source of variation since all the pigs used in the study were of similar age, sex and breed and thus assumed to be homogenous and also similar to those used in studies 1 and 3. The six Ca to P dietary ratios of the diets were: 0.78: 1, 0.98: 1, 1.19: 1, 1.40: 1, 1.61: 1, and 1.81: 1, for diets 1, 2, 3, 4, 5 and 6, respectively. However, the 'actual' analysed ratios were lower than the calculated ratios, particularly with diets 5 and 6 (Table 4.3.2b). This might not be unconnected with mixing and sampling errors with diets 5 and 6 in comparison with the other diets. Accordingly, the six diets were similarly formulated to meet the NRC, (1998) recommended levels of nutrient requirements for 20 to 50 kg BW pigs except for the ratios of Ca to P in the diets as previously mentioned. Titanium oxide (0.30%) was added to all dietary treatments as the indigestibility marker to measure Ca and P digestibility values.

The results of this second study demonstrated that diet 2 with a true digestible Ca to P ratio of 0.82: 1 best supports animal performance as measured by the significant superior ($P < 0.05$) ADG and FE of the animals that consumed diet 2 compared with the other five dietary treatments and similarly concomitantly resulted in minimized Ca and P excretions in the pig manure as a result of better Ca and P retentions by the animals on diet 2. Furthermore, it resulted in the lowest volume of total urinary excretions by animals on diet 2 which is also an additional advantage in terms of costs associated with manure storage and disposal (Mroz et al. 1995).

In the light of these findings in experiments 1 and 2, experiment 3 was conducted to determine the true digestible values of Mg, Cu, Fe, Mn, Se and Zn also in a corn-SBM-based diet for the growing pig, since Ca and P are known to be mutually antagonistic to these minerals during their metabolism when dietary supplies are based on their apparent digestibility values (Peo, 1991; Ammerman, 1995). As was in experiment 1, this third experiment was designed and carried out as a completely randomized design using the principles of the difference method (Ammerman, 1995; Rodehutscord et al. 1998; Fang et al. 2007). The complex interactions that also occur among trace-minerals further support the determination and use of the true digestible values of the minerals as briefly depicted in figure 6.1. Figure 6.1 shows in brief three trace-mineral wheel diagrams and the nature of the extremely large and complex relationships of mineral-ion interactions could be with diverse effects on their metabolism with huge negative effects on animal performance/welfare and the environment. It further suggests the extent of the complexity when all dietary minerals are involved. It thus further emphasizes that dietary mineral intake should nutritionally be based on maintaining proper mineral balance strictly based on the true digestible values (Knowlton et al. 2004).

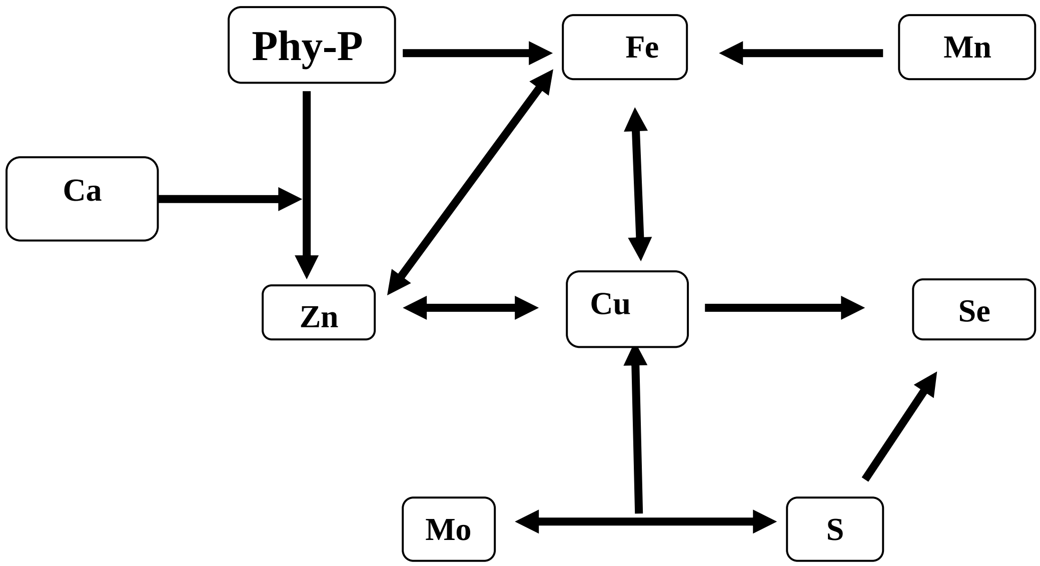


Typical types of ion interactions (positive, negative, reciprocal and multiple) that affect bioavailability

Figure 6.1 (O'Dell, 1997)

Figure 6.1 also demonstrates that there are two major types of interactions; positive and negative. The former is usually synergistic while the latter is commonly antagonistic. Additionally, within these two types there may be multiple or one-on-one interactions. Here, the latter may be one-way or even reciprocal. Accordingly, in figure 6.1, Zn interacts negatively with both Cu and Fe whereas Cu interacts positively with Fe indicating that the relationship when Cu and Fe dietary concentrations are in their true animal requirements' states Cu shows a positive (synergistic) effect on the metabolism of Fe (Peo, 1991; O'Dell, 1997). Furthermore, there is a reciprocal interaction between Zn and Fe. The multiple nature of interactions existing among dietary minerals is further illustrated from figure 6.1 by the fact that if Zn dietary digestibility is antagonised as demonstrated in this example, zinc will in turn antagonistically affect the digestibility and thus the assimilation of Cu which in fact is essential for effective utilization of Fe. This may also explain in part the negative AD values of most metal-ions in our third study. Therefore, all physiologic responses in ion interactions are significantly concentration dependent. Hence from a nutrition viewpoint, when dietary minerals are considered on the bases of TDV the antagonistic effects are overcome resulting in improved mineral retention leading to their minimal excretions in the pig manure (Peo, 1991; Ammerman, 1995). This was why the major objective of experiment 3 was to determine the TDV values of the essential minerals investigated in that study. As demonstrated by the findings of that study Se and Zn ADV are significantly lower ($P < 0.05$) compared with their TDV. These are indications that the excess inclusion levels of these minerals would be avoided with the use of their TDV and subsequently eliminate the inhibition of other ions metabolism when ADV are used in diet formulations (O'Dell, 1997; Rudehutscord et al., 1998).

Overall, since dietary mineral nutrients cannot be considered separately and because of the known interacting forces existing among them in addition to their additive effects there is a need to ensure that the dietary supply pattern does not inhibit their solubility and subsequently impede their absorption and utilization. To this end, the findings of these three studies would aid in better understanding of other essential minerals whose true digestibility values were determined in our studies to further reduce the negative effects of other dietary components known in affecting mineral bioavailability to the animals, such as fibre and phytate. Here, the latter as previously stated more readily comes to mind as it is a strong chelator being a polyanion and therefore capable of forming phytate-mineral-complexes with dietary minerals (NRC, 1998; Lei and Porres, 2003) as also shown in figure 6.2. In this way, it significantly impedes mineral solubility thereby rendering the minerals unavailable to the animal. Figure 6.2 also sheds further insights on the commonly recognized in-vivo interactions involving the essential trace-minerals, especially in the presence of phytate and dietary P and Ca. The absorption and assimilation of Fe are affected negatively by the use of ADV of Zn, Mn and Cu. Phosphate in both its inorganic and organic (phytate) forms interacts with other minerals as well, such as Fe,



Implication of phytate-P on essential micro-minerals interactions. Phytate-P designates both phytate and inorganic phosphate. The effect of phytate on Zn is accentuated by Ca.

Figure 6.2 (O'Dell, 1997)

with huge implications on mineral absorption and utilization. Cu bioavailability is affected negatively by Zn, and Fe as well as the Cu-Mo-S complex. The use of ADV of Cu or Zn can also induce Se deficiency. Therefore, it is not a gainsaying that the determination and use of the TDV of these minerals in diet formulation for swine, as demonstrated in these three studies, are to contribute to the better management of mineral nutrition for the swine industry.

According to NRC (2005) projections, ten minerals (micro- and macro-minerals) that would be of main concern on the environment and subsequently have significant effects on crop production and environmental pollution have been identified. These minerals include: Cd, Cu, Hg, Fe, Se, Zn, Na, K, P and S. Among these minerals P and the trace-minerals (Cu, Fe, Se and Zn) are already identified as currently causing environmental pollution. This is further compounded by phytate associated P from grains and legumes which presently form the bulk of swine diets. This situation has resulted in federal, state and counties in most countries promulgating environmental regulations that swine producers should conform to regarding the concentrations of nutrients in the pig manure in order to protect the environment from pollution and for the sustainability of the ecosystem (Petersen, 2010). These policies when properly implemented no doubt would aid optimize mineral digestion and retention by pigs and thus better enhance animal health and therefore better support animal performance. Furthermore, it would increase hog farmers profit margin due to reduction in cost of production as excess use of minerals particularly that of P are avoided while also minimizing P and trace-minerals excretions in the pig manure leading to better mineral management on the farm.

Additionally, the use of the TDV of these minerals determined in these studies, including the optimal true digestible ratio of Ca to P in swine diets for growing pigs, would also help to reduce the odor menace associated with pig production. This is because the use of the findings of these studies would lead in the improvement of dietary nutrients' digestibility resulting in less dietary nutrient components entering the hindgut of the pig. Since pungent odors emanating from pig feces are principally associated with the fermentation of undigested feed materials in the large intestine, the use of our result data in the feeding of pigs have great potential to reduce odor emissions since less feed residues used by the micro flora in the large intestine would be available for such fermentative processes.

REFERENCES

Aarnink, A. J. A., and M. W. A. Verstegen, 2007. Nutrition, key factor to reduce environmental load from pig production. Livest. Sci. 109: 194-203.

Adeola, A. 2001. Digestion and Balance Techniques in Pigs. In: Swine Nutrition (Lewis, A. J. and Southern, L. L. 2nd Ed.) pp. 903 – 916. CRC Press, Boca Raton

Ajakaiye, A., M. Z. Fan, T. Archbold, R. R. Hacker, C. W. Forsberg, and J. P. Phillips. 2003. Determination of true phosphorus digestibility and the endogenous loss associated with soybean meal for growing-finishing pigs. J. Anim. Sci. 81: 2766-2775.

Ajakaiye, A., M. Z. Fan, T. Archbold, R. R. Hacker, C. W. Forsberg, and J. P. Philips. 2001. Determination of true calcium digestibility and the endogenous calcium loss associated with soybean meal for growing-finishing pigs. Can. J. Anim. Sci. 81: 612 (Abstract).

Akinmusire, A. S., and O. Adeola. 2009. True digestibility of phosphorus in canola and soybean meals for growing pigs: Influence of microbial phytase. J. Anim. Sci. 87: 977-983.

Akinson, S. A., J. K. Shah, C. E. Webber, I. L Gibson, and R. S Gibson. 1993. A multi-element isotopic tracer assessment of true fractional absorption of minerals from formula with additives of calcium, phosphorus, zinc, copper and iron in young piglets. J. Nutr. 123: 1586-1593.

Ammerman, C. B. 1995. Methods for estimation of mineral bioavailability. In: Bioavailability of Nutrients for Animals (Ammerman, C. B., Baker, D. H. and Lewis, A. J. Ed.) p. 83. Academic Press. San Diego.

AOAC. 2000. Official Methods of Analysis. 17th ed. Assoc. Off. Anal. Chem., Arlington, VA.

BASF. 1993. Provisional technical leaflet-Natuphos@5000 phytase. BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany.

Brady, S. M., J. J. Callan, D. Cowan, M. McGrane, and J. V. O'Doherty. 2002. Effect of phytase inclusion and calcium/phosphorus ratio on the performance and nutrient retention of grower-finisher pigs fed barley/wheat/soybean meal-based diets. J. Sci. Food. Agric. 82: 1780-1790.

Braithwaite, G. D. 1985. Endogenous faecal loss of phosphorus in growing lambs and the calculation of phosphorus requirements. J. Agric. Sci. 105: 67-72.

Brink. E. J., A. C. Beynen, P. R. Dekker, E. C. H. van Beresteijn, and R. van der Meer. 1992. Interaction of calcium and phosphate decreases ileal magnesium solubility and apparent magnesium absorption in rats. J. Nutr. 122: 580-586.

CCAC. 1993. Canadian Council on Animal Care. Guide to the care and use of experimental animals. Vol. 1, 2nd Ed. CCAC Ottawa, ON.

Calvert, C. C. 1991. Fiber utilization by swine. In: E. R. Miller, D. W. Ulrey, and A. J. Lewis (Ed.) Swine Nutrition. Pp285-296. Butterworth-Heinemann, Stoneham, MA.

Castillo, A. R., J. E. P. Santos, and T. J. Tabone. 2007. Estimation of mineral balances in dairy herds including minerals in the drinking water. Calif. Agric. **61(2):** 90-95.

Chesters, J. K. 1997. Zinc. Pp. 185 230. In: Handbook of Nutritional Essential Mineral Elements (O'Dell, B. L. and Sunde, R. A. Ed.) Marcel Dekker, Inc. New York.

Council for Agricultural Science and Technology. 2002. Animal diet modification to decrease the potential for nitrogen and phosphorus pollution. Issue Paper no. 21. CAST, Ames, IA.

Crenshaw, T. D. 2001. Swine Nutrition: 2nd edition; "Calcium, Phosphorus, Vitamin D, and Vitamin K in Swine Nutrition." CRC Press, New York, pp. 187-212.

Daniel, T. C., A. N. Sharpley, and T. J. Logan. 1992. Effect of soil test phosphorus on the quality of the runoff water: Research needs. Pages 155-160 in National Livestock, Poultry and Aquaculture Waste Management. Proc. Natl. Workshop. Kansas City, Mo. ASAE, St. Joseph, MI.

Donham, K. J. 2000. The concentration of swine production. Effects on swine health, productivity, human health and the environment. Veterinary Clinician of North America. Food Animal Practice, 16: 559-597.

Dourmad, J. Y., and C. Jondreville. 2007. Impact of nutrition on nitrogen, phosphorus, Cu and Zn in pig manure, and on emissions of ammonia and odours. Livest. Sci. 112: 192-198.

Dugelhoef, M., M. Rodehutscord, H. Spidkers, and E. Pfeffer. 1994. Effects of supplemental microbial phytase on availability of phosphorus contained in maize, wheat and triticale to pigs. Anim. Feed Sci. Technol. 47: 19-29.

Eeckhout, W., M. de Paepe, N. Warnants, and H. Bekaert. 1995. An estimation of the minimal P requirements for growing-finishing pigs as influenced by the Ca level of the diet. Anim. Feed Sci. Tech. 52: 29-40.

Emiola, A., O. Akinremi, B. Slominski, and C. M. Nyachoti. 2009. Nutrient utilization and manure P excretion in growing pigs fed corn-barley-soybean based diets supplemented with microbial phytase. Anim. Sci. J. 80: 19-26.

Fan, M. Z., T. Archbold, and R. R. Hacker. 2000. Determination of true calcium digestibility in feedstuffs for pigs by regressional analysis technique. Can. J. Anim. Sci. 80: 780 (Abstract).

Fan, M. Z., T. Archbold, W. C. Sauer, D. Lackeyram, T. Rideout, Y. Gao, C. F. M. de Lange, and R. R. Hacker. 2001. Novel methodology allows simultaneous measurement of true phosphorus digestibility and the gastrointestinal endogenous phosphorus outputs in studies with pigs. J. Nutr. 131: 2388-2396.

Fan, M. Z. 2007. Final Project Report to “Ontario Pork Producers’ Marketing Board – Ontario Pork” on “Determination of Optimal True Digestible Calcium to True Digestible Phosphorus Ratio in Post-Weaned Pigs for Minimizing Phosphorus Excretion.” Ontario Pork Project # 04-13; University of Guelph Project # 046629.

Fan, M. Z., Y. Shen, Y. L. Yin, Z. R. Wang, Z. Y. Wang, T. J. Li, T. C. Rideout, R. L. Huang, T. Archbold, C. B. Yang, and J. Wang. 2008. Methodological considerations for measuring phosphorus utilization in pigs. In: “Mathematical Modelling in Animal Nutrition”. J. France and E. Kebreab (Eds.). CABI International, Wallingford, Oxon, UK, pp. 370 – 397.

Fang, R. J., Y. L. Yin, K. N. Wang, J. H He, Q. H. Chen, and M. Z. Fan. 2007. Comparison of the regression analysis technique and the substitution method for the determination of true phosphorus digestibility and faecal endogenous phosphorus losses associated with feed ingredients for growing pigs. Livest. Sci. 109: 251-254.

Flipot, P. M., and G. Ouellet. 1988. Mineral and nitrate content of swine drinking-water in four Quebec regions. Can. J. Anim. Sci. **68:** 997-1000.

Golovan, S., R. D. Meidinger, A. Ajakaiye, M. Cottrill, M. Z. Weiderkehr, C. Plante, J. Pollard, M. Z. Fan, A. Hayes, A. C. Jesper Laursen, J. P. Hjorth, R. R. Hacker, D. Barney, J. P. Phillips, and C. Forsberg. 2001. Enhanced phosphorus digestion and reduced pollution potential by pigs with salivary phytase. Nature-Biochem. 19: 741-745.

Hall, D. D., G. L. Cromwell, and T. S. Stahly. 1991. Effects of dietary calcium, phosphorus, calcium: phosphorus ratio and vitamin K on performance, bone strength and blood clotting status of pigs. J. Anim. Sci. 69: 646-655.

Han, Y. K., and P. A. Thacker. 2006. Effects of the calcium and phosphorus ratio in high zinc diets on performance and nutrient digestibility in weanling pigs. J. Anim. Vet. Adv. 5: 5-9.

Hanni, S. M., M. D. Tokach, R. D. Goodband, S. S. Dritz, and J. M. Derouchey. 2005. Effects of increasing calcium-to-phosphorus ratio in diets containing phytase on finishing pig growth performance. The Professional Anim. Scientist, 21: 59-65.

Hollis, G. R., and S. E. Curtis. 2001. General Characteristics of the U. S. Swine Industry. In: A. J. Lewis and L. L. Southern (ed.) Swine Nutrition. 2nd Ed. P. 19. CRC Press. New York, NY.

Hu, Q., L. Yang, J. Fang, S. X. Wang, X. G. Shu, Z. Y. Deng, G. Liu, M. Z. Fan, and Z. Ruan. 2010. Estimating an optimal ratio of true digestible Ca: P in corn-rough-soybean meal-based diets for 20-50 kg growing pigs. J. Food, Agric. & Environ. 8 (3&4): 556-562.

Jongbloed, A. W., H. Everts, and P. A. Kemme. 1991. Phosphorus availability and requirements in pigs. Recent advances in Animal Nutrition. Pp. 65-80. Butterworth, Heinemann Eds. London, UK.

Jongbloed, A. W., and H. Everts. 1992. Apparent digestible phosphorus in the feeding of pigs in relation to availability, requirement and environment. 2. The requirement of digestible phosphorus for piglets, growing-finishing pigs and breeding sows. Netherl. J. Agric. Sci. 40: 123-136.

Jongbloed, A. W. 1987. Phosphorus in the feeding of pigs: Effect of diet on the absorption and retention of phosphorus by growing pigs. Ph.D. thesis, Landbouwuniveriteit Wageningen, Rapport IVVO-DLO no. 179, Lelystad, The Netherlands, 343 pp.

Jongbloed, A. W., H. Everts, P. A. Kemme, and Z. Mroz. 1999. Quantification of absorbability and requirements of macroelements. Pp. 275-298. In: Quantitative Biology of the Pig. I. Kyriazakis (Ed.). CABI publ., Wallingford, UK.

Jongbloed, A. W., and C. H. Henkens. 1996. Environmental Concerns of Using Animal Manure – The Dutch Case. In: E. T. Kornegay (ed.) Nutrient Management of Food Animals to Enhance and Protect the Environment. Pp. 315-333. CRC Press, Boca Raton, Fl.

Jongbloed, A. W., J. Th. M. van Diepen, P. A. Kemme, and J. Broz. 2004. Efficacy of microbial phytase on mineral digestibility in diets for gestating and lactating sows. Livest. Prod. Sci. 91: 143-155.

Kaune, R. 1996. Mechanisms of intestinal calcium absorption and availability of dietary calcium in pigs. Dtsch. Tierarztl. Wschr. 103: 215-218.

Ketaren, P. P., E. S. Batterham, E White, D. J. Farrel, and B. K. Milthorpe. 1993. Phosphorus studies in pigs. Brit. J. Nutr. 70: 249-268.

Knowlton, K. F., J. S. Radcliffe, C. L. Novak, and D. A. Emmerson. 2004. Animal management to reduce phosphorus losses to the environment. J. Anim. Sci. 82 (E. Suppl.): E173-E195.

Kornegay, E. T., B. G. Diggs, O. M. Hale, D. L. Handlin, J. P. Hitchcock, and R. A. Barczwski. 1984. Reproductive performance of sows fed elevated calcium and phosphorus levels during growth and development. A cooperative study. Report S-145 of the Committee on Nutritional Systems for Swine to Increase Reproductive Efficiency. J. Anim. Sci. 59(Suppl. 1): 253 (Abstr.).

Kornegay, E. T. 1985. Calcium and phosphorus in Animal Nutrition. West Des Moines, IA: National Feed Ingredients Association.

Kornegay, E. T. 2001. Digestion of phosphorus and other nutrients: the role of phytases and factors influencing their activity. In: Enzymes in farm animal nutrition. (Eds.) Bedford, M. R. and Partridge, G. G. pp. 237-271. CABI publ. Marlborough, UK.

Lei, X. G., and J. M. Porres. 2003. Phytase enzymology, applications and biotechnology. Biotechnology Letters, 25: 1787-1794.

Li, Y. Z., L. Chenard, S. P. Lemay, and H. W. Gonyou. 2005. Water intake and wastage at nipple drinkers by growing-finishing pigs. J. Anim. Sci. 83: 1413-1422.

Liu, J., D. W. Bollinger, D. R. Ledoux, and T. L. Veum. 2000. Effects of dietary calcium: phosphorus ratios on apparent absorption of calcium and phosphorus in the small intestine, cecum, and colon of pigs. J. Anim. Sci. 78: 106-109.

Liu, J., D. W. Bollinger, D. R. Ledoux, and T. L. Veum. 1998. Lowering the dietary calcium to total phosphorus ratio increases phosphorus utilization in low-phosphorus corn-soybean meal diets supplemented with microbial phytase for growing-finishing pigs. J. Anim. Sci. 76: 808-813.

Lo, G. S., S. L. Settle, F. H. Steinke, and D. T. Hopkins. 1981. Effect of phytate: zinc molar ratio and isolated soyabean protein on zinc availability. J. Nutr. 111: 2223 – 2225.

Low, A. G. 1985. The role of dietary fibre in digestion, absorption and metabolism. In: 3rd International Seminar on Digestive Physiology in Pig. National Institute of Animal Science, Copenhagen, Denmark. Landhusholdningsselskabets Forlag, Copenhagen, Beretning Fra Statens Husdyrbrugsforsog, No. 580: 157-179.

Maga, J. A. 1982. Phytate: Its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. J. Agric. Food Chem. 30: 1-9.

Mallin, M. A. 2000. Impacts of Industrial Animal Production on Rivers and Estuaries. Am. Sci. 88 (1): 26-37.

Matsuzaki, H., K. Arai, M. Uehara, K. Suzuki, S. Sato, Y. Kanke, and S. Goto. 1997. Increasing magnesium intake prevents high phosphorus diet-induced kidney damage in young rats. Nutr. Res. 17: 325-337.

Miura, T., H. Matsuzaki, K. Suzuki, and S. Goto. 1999. Long-term high intake of calcium reduces magnesium utilization in rats. Nutr. Res. 19: 1363-1369.

Montminy, M. P. L., C. Jondreville, P. Lescoat, F. Meschy, C. Pomar, J. F. Bernier, and D. Sauvant. 2007. First step of a model of calcium and phosphorus metabolism in growing pigs: Fate of ingested phosphorus in the stomach. Livest. Sci. 109: 63-65.

Mroz, Z., A. W. Jongbloed, N. P. Lenis, and K. Vreman. 1995. Water in pig nutrition: Physiology, allowances and environmental implications. Nutr. Res. Rev. 8: 137-164.

NRC, 1998. Nutrient Requirements of Swine. 10th Ed. Natl. Acad. Press, Washington, DC.

NRC, 2005. Mineral Tolerance of Animals. National Research Council (NRC), 2nd revised edition. National Academy Press, Washington, DC.

O'Dell, B. L. 1997. Mineral-Ion Interaction as Assessed by Bioavailability and Ion Channel Function. Pp. 641 – 659. In: handbook of nutritionally essential mineral elements (O'Dell, B. L. and Sunde, R. A. Ed.) Marcel Dekker, Inc. New York.

Pallauf, J. and Rimbach, G. 1995. Recent results on phytic acid and phytase. Proceedings of 5th Forum Animal Nutrition. May 4-5. BASF, p. 43.

Patience, J. F., P. A. Thacker, and C. F. M. de Lange. 1995. Swine Nutrition Guide (2nd Ed.). pp. 253 - 259. Prairie Swine Centre, Saskatoon, SK, Canada.

Patterson, J. K., X. G. Lei, and D. D. Miller. 2008. The pig as an experimental model for elucidating the mechanisms governing dietary influence on mineral absorption. Exp. Biol. Med. 233: 651-664.

Peo, E. R. 1991. Calcium, phosphorus, and vitamin D in swine nutrition. Pp. 165-182 in Swine Nutrition, E. R. Miller, D. E. Ullrey, and A. J. Lewis, eds. Stoneham, ME: Butterworth-Heinemann Publishing.

Petersen, S. T. 2010. The potential ability of swine nutrition to influence environmental factors positively. J. Anim. Sci. 88(E. Suppl.): E95-E101.

Petersen, G. I., and H. H. Stein. 2006. Novel procedure for estimating endogenous losses and measurement of apparent and true digestibility of phosphorus by growing pigs. J. Anim. Sci. 84: 2126-2132.

Pettey, L. A., G. L. Cromwell, and M. D. Lindemann. 2006. Estimation of endogenous phosphorus loss in growing and finishing pigs fed semi-purified diets. J. Anim. Sci. 84: 618-626.

Pointillart, A., N. Fontaine, and M. Thomasset. 1984. Phytate phosphorus utilization and intestinal phosphatises in pigs fed low phosphorus: wheat or corn diets. Nutr. Rep. Int. 29: 473-483.

Pond, W. G., and X. G. Lei. 2001. Of Pigs and People. In: A. J. Lewis and L. L. Southern (Ed.) Swine Nutrition. 2nd Ed. p 3. CRC Press, New York, NY.

Poulsen, H. D., A. W. Jongbloed, P. Latimier, and J. A. Fernandez. 1999. Phosphorus consumption, utilisation and losses in pig production in France, the Netherlands and Denmark. Livest. Prod. Sci. 58: 251-259.

Poulsen, H. D. 2000. Phosphorus utilization and excretion in pig production. J. Environ. Qual. 29: 24-27.

Prairie Swine Centre Inc. 2000. Pork production reference guide. p. 19.

Qian, H., E. T. Kornegay, and D. E. Conner. 1996a. Adverse effects of wide calcium: phosphorus ratios on supplemental phytase efficacy for weanling pigs fed two dietary phosphorus levels. J. Anim. Sci. 74: 1288-1297.

Qian, H., E. T. Kornegay, and D. M. Denbow. 1996b. Phosphorus equivalence of microbial phytase in turkey diets as influenced by calcium to phosphorus ratios and phosphorus levels. Poult. Sci. 75: 69-81.

Reinhart, G. A., and D. C. Mahan. 1986. Effect of various calcium: phosphorus ratios at low and high dietary phosphorus for starter, grower and finishing swine. J. Anim. Sci. 63: 457-466.

Rodehutscord, M., M. Faust, and E. Pfeffer. 1999. The course of phosphorus excretion in growing pigs fed continuously increasing phosphorus concentrations after a phosphorus depletion. Archives of Anim. Nutr. 52: 323-334.

Rodehutscord, M., R. Haverkamp, and E. Pfeffer. 1998. Inevitable losses of phosphorus in pigs, estimated from balance data using diets deficient in phosphorus. Arch. Anim. Nutr. 51: 27-38.

Rotter, B. A., M. Neskar, W. Guenter, and R. R. Marquardt. 1989. Effect of enzyme supplementation on the nutritive value of hulless barley in chicken diets. Anim. Feed Sci. Technol. 24: 233-245.

Rude, R. K. 1998. Magnesium Deficiency: A Cause of Heterogonous Disease in Humans. J. Bone and Miner. Res. 13(4): 749-758.

Sands, J. S., D. Ragland, B. C. Baxter, B. C. Joern, T. E. Sauber, and O. Adeola. 2001. Phosphorus bioavailability, growth performance, and nutrient balance in pigs fed high available phosphorus corn phytase. J. Anim. Sci. 79: 2134-2142.

Schenker, M., D. Christiani, and Y. Cormier. 1998. Respiratory health hazards in agriculture. Am. J. Respiratory Critical Care Med. 158, suppl., S1-S76.

Shen, Y., M. Z. Fan, A. Ajakaiye, and T. Archbold. 2002. True phosphorus digestibility and the endogenous phosphorus loss associated with corn for growing-finishing pigs are determined with the regression analysis technique. J. Nutr. 132: 1199-1206.

Shen, Y., M. Z. Fan, T. Archbold, B. W. McBride, and R. R. Hacker. 2003. True phosphorus digestibility and the endogenous phosphorus outputs associated with conventional corn for weanling pigs. The 9th International Symposium on Digestive Physiology in Pigs, R. Ball, Ed. Pp. 395-397. University of Alberta, Banff, Alberta, Canada.

Shoback, D. M., and D. E. Sellmeyer. 2010. Disorders of the Parathyroids and Calcium and Phosphorus Metabolism. Pp. 465 -496 in Pathophysiology of Disease 6th Ed. (McPhee, S. J. and Hammer, G. D. Ed.) McGraw-Hill Companies Inc.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59: 215-221.

Simons, P. C. M., H. A. J. Versteegh, A. W. Jongbloed, P. A. Kemme, P. Slump, K. D. Box, M. G. E. Wolters, R. F. Beudeker, and . G. J. Verschoor. 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Brit. J. Nutr. 64: 525-529.

Smith, J. W., M. D. Tokach, P. R. O'Quinn, J. L. Nelssen, and R. D. Goodband. 1999. Effects of dietary energy density and lysine: calorie ratio on growth performance and carcass characteristics of growing-finishing pigs. J. Anim. Sci. 77: 3007-3015.

Snedeker, S. M., S. A. Smith, and J. L. Greger. 1982. Effect of dietary calcium and phosphorus levels on the utilization of iron, copper, and zinc by adult males. J. Nutr. 112: 136-143.

Spencer, J. D., G. L. Allee, and T. E. Sauber. 2000. Phosphorus bioavailability and digestibility of normal and genetically modified low-phytate corn for pigs. J. Anim. Sci. 78: 675-681.

Stein, H. H., O. Adeola, G. L. Cromwell, S. W. Kim, D. C. Mahan, and P. S. Miller. 2011. Concentration of dietary calcium supplied by calcium carbonate does not affect the apparent total tract digestibility of calcium, but decreases digestibility of phosphorus by growing pigs. J. Anim. Sci. 89: 2139-2144.

Summers, J. D. 1997. Precision phosphorus nutrition. J. Appl. Poult. Sci. 6: 495-500.

Tamminga, S. 2003. Pollution due to nutrient losses and its control in European and production. Livest. Prod. Sci. 84: 101-111.

Traylor, S. L., G. L. Cromwell, M. D. Lindemann, and D. A. Knabe. 2001. Effects of level of supplemental phytase on ileal digestibility of amino acids, calcium, and phosphorus in dehulled soybean meal for growing pigs. J. Anim. Sci. 79: 2634-2642.

Veum, T. L., D. R. Ledoux, D. W. Bollinger, V. Raboy, and A. Cook. 2002. Low-phytic acid barley improves calcium and phosphorus utilization and growth performance in growing pigs. J. Anim. Sci. 80: 2663-2670.

Wang, F., M. Zhang, Q. Chen, P. Liu, and M. Xu. 2001. Effect of dietary phosphorus and the ratio between calcium and phosphorus on the activities of alkaline phosphatise and serum calcium and phosphorus in miniature pigs (Xiang pig). Acta Zoonutrimenta Sinica, 13: 36-42.

Widmer, M. R., L. M. McGinnis, and H. H. Stein. 2007. Energy, phosphorus, and amino acid digestibility of high-protein distillers dried grains and corn germ fed to growing pigs. J. Anim. Sci. 85: 2994-3003.

Wood, R. J., and J. J. Zheng. 1997. High dietary calcium intakes reduce zinc absorption and balance in humans. Am. J. Clin. Nutr. 65: 1803-1809.

Woyengo, T. A., A. J. Cowieson, O. Adeola, and C. M. Nyachoti. 2009. Ileal digestibility and endogenous flow of minerals and amino acids: responses to dietary phytic acid in piglets. Brit. J. Nutr. **102:** 428-433.

Wright, P. L., and M. C. Bell. 1966. Comparative metabolism of selenium and tellurium in sheep and swine. Am. J. Physiol. 211 (1): 6-10.