2005

AN EXAMINATION OF DIETARY AMENDMENTS TO AFFECT PHOSPHORUS UTILIZATION IN GROWING PIGS

Jorge Hernan Agudelo-Trujillo
University of Kentucky

ABSTRACT OF DISSERTATION

Jorge Hernan Agudelo-Trujillo

The Graduate School

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A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture
at the University of Kentucky

By
Jorge Hernan Agudelo-Trujillo

Lexington, Kentucky

Director: Dr. Merlin D. Lindemann, Professor of Animal Science

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For economical and ecological reasons, efficiency and profitability of swine production relies heavily on the way pigs utilize key nutrients such as P, which is considered a potential pollutant of water ecosystems. Although cereal grains and oilseed meals contain enough P to fulfill the biological needs of pigs, most of this P is tightly bound as phytate. As pigs do not have enough phytase (PHY) to cleave P from phytate, it is excreted in the feces. To prevent a deficiency, diets have traditionally been supplemented with highly available inorganic sources of P. Today, an environmentally-friendly alternative is to supplement diets with PHY.

Growth promoting antibiotics are also used to enhance the utilization of dietary components such as energy and N. It has been suggested that the antibiotic virginiamycin (VIR) could also improve phytate-P utilization by pigs.

Eight experiments evaluated the effects of VIR and/or PHY amendments on digestibility, retention, excretion, growth, bone characteristics, meat traits, and ileal microflora populations of growing pigs fed corn–soybean meal (SBM) diets (seven experiments) or corn-SBM-rice bran diets (one experiment). Additionally, a comparison between two digestibility procedures was conducted for two of the experiments.

On average, VIR improved P digestibility and total P excretion by 5.0%, and P retention as a percent of absorption by 1.0%. Phytase amendments improved P digestibility between 14 and 27%, and P retention (as a % of absorption) between 0.7 and 2.5%. In the growth trial, VIR supplementation was associated with numerical differences favoring bone mineralization and ileal phytate-utilizing bacteria populations. These observations demonstrate additional

research is warranted with this antibiotic under conditions of higher stress and bacterial load in the environment.

According to the comparisons between digestibility methods, a single grab fecal collection was not reliable. Further, a cumulative grab collection for five days was not as good an option as the total collection method.

It is concluded that VIR does improve P utilization in pigs fed corn-SBM diets not supplemented with inorganic P. Similar effects, but of greater magnitude, were confirmed for PHY-amended diets with either normal or high levels of phytate P.

KEYWORDS: Pigs, Virginiamycin, Phytase, Phosphorus utilization, Phosphorus excretion

Jorge H. Agudelo-Trujillo

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By

Jorge Hernan Agudelo-Trujillo

Merlin D. Lindemann
Director of Dissertation

David L. Harmon
Director of Graduate Studies

September 6, 2005

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CHAPTER 1

INTRODUCTION

The increasing demand for feedstuffs by the swine industry, along with various environmental issues resulting from high density swine operations, are situations addressed by swine nutritionists worldwide. Feed resources have to be used in the most efficient way in order to keep the industry competitive and also to decrease the excretion of potential environmental pollutants. Feed efficiency and economic profitability depend heavily on how well pigs (*Sus scrofa*) digest, absorb and transform feed nutrients into pork. Profitability and success of pig production are tied to the digestibility of costly and potentially polluting nutrients.

The digestibility value of a nutrient is the percent apparently absorbed from the gastrointestinal tract. Those digestibility values can be assessed through different methods during a digestion trial. Some of the methods can be applied only under specific conditions and each has its advantages and disadvantages.

Among the nutrients commonly investigated in digestibility studies, P has been at the center of attention for some time, both from a biological and also from an environmental perspective. Although it is recognized as a key nutrient required for important physiologic and metabolic processes, including a significant role as a structural element in bone, it is also considered a potential pollutant of water ecosystems.

Common cereal grains (e.g., corn – *Zea maiz*) and oilseed meals (e.g., soybean - *Glycine max*) contain enough P to fulfill the requirements of pigs, but most of it is incorporated in phytic acid, which is poorly available to monogastric animals. For this reason, swine feeds are usually supplemented with highly available inorganic sources of P such as monocalcium phosphate. As pigs can not use most of the organic P in the feed, it is excreted and may end up polluting bodies of water, producing eutrophication. The swine industry has attempted

different approaches to prevent this risk. The most effective solution currently available is adding microbial phytases to the diet in order to increase the availability of phytic P to the pig, thus decreasing its excretion. Another alternative, currently under research, is to feed genetically modified (GM) grains low in phytate. A more futuristic approach would be having GM pigs that secrete high amounts of phytase in saliva.

The industry faces yet another challenge, which is the possible phasing out or banning of antibiotics as growth promoters. Fed at subtherapeutic levels, they have played a major role in swine production since the early 1950s. Although their mode of action is not totally understood, it is known that they increase protein and energy utilization in several species and improve mineral utilization in some of them. The limited research available on this latter topic indicates that the antibiotic virginiamycin may improve the digestibility of P for pigs. The effects of antibiotics on P digestibility and excretion deserve attention in modern times, when the industry simultaneously faces increasing pressure for antibiotic banning and for decreasing P pollution.

During recent years, when the opening of markets and the globalization of formerly closed economies seems to be irreversibly increasing, many countries, including Colombia in South America, face not only the challenge of increasing the feed efficiency of their swine industries, but also the need to research potential alternative local feedstuffs as cheaper sources of nutrients. The effects of diet amendments intended to increase nutrient utilization should be addressed not only in traditional corn-soybean meal diets but also in alternative feed materials.

The experiments presented in this dissertation examine several aspects of nutrient utilization in growing pigs fed practical diets amended with virginiamycin and/or phytase. They also assess the methodology of digestibility measures. The research covers a variety of macro and micronutrients, focusing on phosphorus as one of the elements representing the most common pollution issues in modern swine production.

CHAPTER 2

LITERATURE REVIEW

Feed Utilization by Swine

Pork is the meat of choice worldwide, with a 40 to 43% share of the global meat protein market (Hollis and Curtis, 2001; Pond and Lei, 2001). Swine production has to keep pace with human population growth and the resultant increased demand for pork. As a result of this ever-growing demand, the modern swine industry is facing important challenges in several areas. Most of those issues are related to inputs such as feed as well as outputs such as manure. In economic terms, feed is considered the major input to the swine production system, representing over 65% of all production expenses (Hollis and Curtis, 2001). For the growing-finishing stage of swine production, generally encompassing the weight range from 30 to 120 kg, or the time between removal from the nursery until market, feed represents 50 to 60% of the total cost of pork production (Cline and Richert, 2001). The impact of this figure on business sustainability depends, in part, on the efficiency by which feed is used, and on the cost of the feedstuffs utilized. Although improving the efficiency of feed utilization by the pig is not a new issue for nutritionists, it becomes more relevant in times when the human population increasingly competes with animal production for the use of cereals such as corn (Close, 1993). As described by Pinstrup-Andersen et al. (1997), “Humanity is entering an era of volatility in the world food situation” due to diverse factors including low grain stocks, growing scarcity of water, and drastic weather fluctuations. Furthermore, feed is not the only resource becoming compromised as the human population grows and expands into rural areas. Clean water and even clean air in the areas influenced by densely populated swine farms are also central issues. Swine nutritionists face the challenge of increasing the fraction of nutrients that are effectively

digested and metabolized into pork, in order to gain efficiency of feed utilization and to decrease the excretion of potential pollutants. For many countries this goes along with the necessity of including locally available nontraditional materials as alternative feedstuffs.

The efficiency of feed utilization, expressed as the Feed Conversion Ratio (FCR), measures the ratio of amount of feed consumed to the body weight (BW) gained by the animal during a limited period of time. FCR is one of the most important traits measured in commercial farms for growing pigs at the various stages from weaning to market weight and is usually highly correlated with the profitability of a given lot of animals. It gives a general idea of how well feed was utilized and transformed into pork. But the FCR calculation assumes that all the feed delivered to the feeders was ingested by the pigs. It does not account for the portion of feed actually wasted by the pigs. The wasted feed ends up mixed with the feces and urine excreted. So, FCR is just a general reference on feed utilization. It does not tell how well particular nutrients of interest (i.e., potential environmental pollutants such as phosphorus or nitrogen) were used by the animal.

Digestion and balance assessments

A more precise way to account for feed utilization is to measure the digestibility of each nutrient in the whole diet or in each of the feedstuffs used. Digestion may be defined as the preparation of food for absorption into the body from the GI tract, while digestibility is taken to mean disappearance of food from the GI tract. This broader definition of digestibility includes digestion as well as absorption (Schneider and Flatt, 1975). The expression ‘apparent digestibility’ (or ‘apparent absorption’) represents the difference between intake and excretion of a nutrient in the feces, or its disappearance from the gastrointestinal tract (or percent digested and absorbed out of the total intake). This result is not corrected for the endogenous portion resulting either from sloughed mucosal cells or from excretion of the element back into the gastrointestinal tract (Ammerman, 1995). Digestibility is generally reported as ‘apparent’, since it is difficult to devise

appropriate corrections for the amount of digestive secretions, sloughed cells, and other waste products mixed in the undigested feed. ‘True’ digestibility (or true absorption), on the other hand, involves the correction for the amount of nutrient excreted back in the gastrointestinal tract (as digestive secretions) and sloughed intestinal cells that end up excreted with the feces (Kidder and Manners, 1978; McDowell, 2003). For that reason, true digestibility is a more valid estimate of the amount that is available for physiological purposes.

McDowell (2003) points out that absorption studies measuring intake and fecal excretion have been carried out with Ca, P, and Mg, but very few studies have been done with micro-elements due to the large errors resulting from the slightest sample contamination and unknown endogenous sources. Cole and Haresign (1985) agree that mineral balance experiments are notoriously difficult, because pigs confined in metabolism crates can go into negative mineral balance because of lack of exercise which can be associated with demineralization of the skeleton.

Digestibility data are extensively used in nutrition research to evaluate feedstuffs or study nutrient utilization. Nevertheless, once calculated it does not become a constant value. Several factors may alter digestibility values, including: the level of feed intake, digestive disturbances, feeding frequency, feed processing, and possible associative and interactive effects between feedstuffs (Pond et al., 1995). Furthermore, Whittemore (1998) indicates that the digestibility of some nutrients can be negatively affected by the level of fiber in the diet and also by the particle size (a matter of amount of surface area exposed to enzymatic attack).

While the digestibility result, also called the digestibility index or digestion coefficient, shows how much of the ingested nutrient was apparently digested and absorbed, the subtraction of this number from 100 tells how much of it was excreted in the feces without being used. It is noteworthy that, in some cases, a relatively small improvement in digestibility can signify an important reduction in nutrient excretion. For instance, when increasing N digestibility from 90 to 92%, the excretion of fecal N will be reduced 20% (from 10 to 8%). Obviously, the

impact on excretion will be more significant the more digestible the nutrient is (e.g., if we assume normal P digestibility is as low as 30%, another 2 percentage point increase, from 30 to 32%, represents a 2.85% decrease in fecal P excretion, a number much lower than that of the N example).

Digestibility of nutrients in complete diets or in specific feedstuffs is usually assessed through digestion studies which may involve quantifying nutrient intake, collecting and quantifying fecal nutrient output, and calculating the difference between intake and output expressed as a percent of the total input. The output can also be measured at the end of the small intestine (ileum), by means of a surgically fitted canula. Results are then referred to as ileal digestibility. A more complete study, the balance trial, involves not only collecting feces but also quantifying nutrient concentration in urine, gases, milk, or conception products (Adeola, 2001).

Apparent digestibility can be assessed in vivo by several methods. In all cases the animals should spend a previous period of time consuming the diet of interest in order to adapt the gastrointestinal system to it. During this pre-experimental period, animals are expected to adapt not only to the composition and amount of feed that will be provided, but also to the confinement conditions of the trial (i.e., digestion or metabolism crates). All the digestibility methods involve the assessment of the nutrient concentration in feed and feces. According to McDowell (2003), the apparent digestibility is of limited value for those elements excreted mostly via feces (e.g., Ca, P, Zn, Mn, and Cu).

Digestibility methods

Adeola (2001) explains that the digestibility of a nutrient can be assessed by using either ‘direct’ or ‘indirect’ approaches. Accordingly, he classifies two digestibility methods, the so called total collection and the index methods as direct approaches, and three others as indirect or ‘digestibility by difference’ methods. Either of the direct methods can be used when the diet is formulated in such a way that all the nutrient of interest is supplied only by the feedstuff under test. Both methods can also be used to find the digestibility of the nutrient in the

total diet when several dietary feedstuffs provide the nutrient. On the other hand, the difference approaches are used only when the diet includes other feedstuffs that supply the nutrient in addition to the feedstuff under test. The 'digestibility by difference' methods assume that there are not associative effects among the feedstuffs supplying the nutrient under analysis.

In regard to the results, it should be mentioned that for any type of digestion trial it is possible to obtain negative digestibility coefficients. This is true particularly for low digestible nutrients (Schneider and Flatt, 1975). In their review of digestibility methods, the authors state that this can also happen when the nutrient concentration is very low in the feedstuff researched. They explain: "in the study of the variation of digestion coefficients that are close to zero, it is only to be expected that some might be negative". This can happen when the variation in the nutrient contents among samples is in excess of the mean concentration of the nutrient.

The total collection method

Different researchers have named the total collection method differently. Years ago, it was referred to as the 'bag' method (Barnicoat, 1945). It has also been called the 'direct' method, 'quantitative collection', 'classic', 'conventional', or 'marker to marker' collection (Schurch et al., 1950; Irwin and Crampton, 1951; Schurch et al., 1952; Clawson et al., 1955; Kohler et al., 1990; Bakker and Jongbloed, 1994). Some researchers still refer to this as the 'direct' method because the calculations are based directly on the total amounts of the nutrient present in feed and feces (Schneider and Flatt, 1975).

This method involves the meticulous collection of the total amount of feces produced during the digestion trial. It also requires keeping accurate records on the total amount of feed actually ingested by the animals (feed offered minus feed rejected). In modern times, to facilitate the collection of feces, animals are individually confined in a special crate were they can lie down and get up, but not turn around. This crate is designed to prevent coprophagy and to separate feces from urine. According to Schneider and Flatt (1975), the early digestibility

experiments were conducted having attendants or watchmen on duty, by shifts, 24 hours per day, behind the animals in the field to catch the feces. The same author credits Dr. W. P. Garrigus from the University of Kentucky as the designer of one of the first feces bag and harness ever attached to a steer to collect the feces without the need of watchmen.

To visually separate the feces resulting from the feed consumed during the collection period, easily distinguishable substances or markers are added to the feed. The most widely used substances for this purpose are Indigo carmine (a blue substance), ferric oxide and chromic oxide. Other substances have also been tried (purple, green, and yellow cellophane; barium sulfate; copper sulfate; bismuth subcarbonate; lampblack (soot, carbon); methylene blue). Schneider and Flatt (1975) described the ideal substance as one that sharply demarcates the feces without diffusing and does not have any physiological effects on the animal. The low diffusion is a characteristic of very insoluble substances. Carmine (a red substance) is commonly used, although Schneider and Flatt (1975) argue that it does not sharply demarcate the feces because it is very soluble, and tends to be laxative with some individuals.

To calculate apparent digestibility by the total collection method, the formula by Adeola (2001) can be used:

$$\text{Digestibility, \%} = 100 \times \left[\frac{\text{Amount of component consumed} - \text{Amount of component in feces}}{\text{Amount of component consumed}} \right]$$

The index method

The index method is sometimes referred to as the ‘indicator’, ‘indirect’, ‘marker’, ‘clue’, ‘reference’, ‘inert reference substance’, ‘tracer’ method, or the ‘ratio technique’ (Kane et al.,1952; Balch et al., 1957; Moore, 1957; Kotb and Luckey, 1972; McCarthy et al., 1974; Schneider and Flat, 1975; Miller et al., 1978). Sometimes researchers refer to this method as the ‘indirect’ method when they want to compare it to the total collection method (Ly et al., 2002). As pointed out by Schneider and Flat (1975), the index method has been named the indirect method, although this last term has also been applied to a totally different methodology known as ‘digestibility by difference’.

The basic difference between the total collection method and the index method is that the total collection method calculates the digestibility coefficients based on the total amount of the nutrient measured in feed and feces, while the index method calculates them based on the relative concentrations of the nutrient and an indicator or marker substance in both feed and feces.

The method uses an indigestible indicator such as a metal oxide (e.g., chromic oxide, ferric oxide, titanium oxide, etc), a dye (e.g., anthraquinone violet, monastral blue, sudan III, etc), a mineral salt (e.g., barium sulfate, cuprous thiocyanate, etc), or even a water-soluble indicator (e.g., polyethylene glycol, Cr-EDTA, etc). The indicator is added to the feed and then its concentration in feed and feces is determined in order to calculate the percent of the nutrients digested and absorbed. In order to calculate the digestibility of a nutrient by the index method, the indicator:nutrient ratio is determined in feed and in feces. So, to determine the apparent digestibility of a nutrient by this method it is only required to know the concentrations of the indicator and the nutrient in both the diet and in the feces (Kotb and Luckey, 1972).

The following example, adapted from Barnicoat (1945), explains the logic behind the calculations:

Fed: 100 parts dry matter (DM):1 part of inert reference substance.

Recovered in feces: 10 parts DM:1 part of indicator.

1 part of indicator accompanies 100 parts of the DM fed, and since it is insoluble and indigested, all of it should be excreted. As there are only 10 parts of DM for 1 part of indicator in feces, 90 of the total 100 parts of the DM fed have been digested – i.e., the apparent digestibility of the DM is 90%.

According to Kidder and Manners (1978), digestibility can be calculated by the index method as:

$$\text{Digestibility, \%} = \frac{\text{Ratio of indicator to nutrient in feces} - \text{Ratio of indicator to nutrient in feed}}{\text{Ratio of indicator to nutrient in feces}} \times 100$$

Or as expressed by Adeola (2001):

$$\text{Digestibility, \%} = 100 - \left[100 \times \frac{\text{\% indicator in feed}}{\text{\% indicator in feces}} \times \frac{\text{\% nutrient in feces}}{\text{\% nutrient in feed}}\right]$$

When the indicator is a substance that is added to the feed, as in the examples already mentioned, the substance is referred to as an external indicator. Other type of indicators can be used instead of external indicators. These are the indigestible substances naturally present in feeds. Examples of these are: lignin, acid insoluble ash, silica, and chromogen - a naturally occurring plant pigment (Kotb and Luckey, 1972; Schneider and Flatt, 1975). As the substance is not added to the diet but rather is a component of it, these are regarded as internal indicators (Kotb and Luckey, 1972). According to Schneider and Flatt (1975), the index method was first used in 1874 when silica naturally present in roughages was used as an internal indicator.

According to Kotb and Luckey (1972), an effective nutritional indicator should meet all the following criteria: be inert, non-toxic, not having physiological or psychological effects, non-absorbed (therefore, be fully recovered in feces),

have no appreciable bulk, mix well with the feed and remain uniformly distributed in the digesta, and have no influence on digestive secretions, absorption or motility of the gastrointestinal tract, or in its microflora. The characteristics of major concern in the search for ideal indicators are its lack of digestibility, its complete recovery and ease of measurement. Adeola (2001) adds other attributes to the indicator: it should be non-essential for the animal and regularly and completely voided in the feces.

Chromic oxide (chromium sesquioxide: Cr_2O_3), sometimes called chrome green (Schneider and Flatt, 1975), is regarded as the most frequently used index material (McCarthy et al., 1974; Fenton and Fenton, 1979; Saha and Gilbreath, 1991). According to Barnicoat (1945), it is one of the least soluble substances known. It is a light to dark-green substance insoluble in water, alcohol or acetone, but slightly soluble in acids and alkalis. It has been widely used in studies of feed and food utilization. According to Kotb and Luckey (1972), it was first used as an indicator in 1918. In studies with pigs, Cr_2O_3 has been added to the feed at levels as low as 0.05% (Kohler et al., 1990; Apgar and Kornegay, 1996) and as high as 1% of the diet (Barnicoat, 1945; Schurch et al., 1952; Everts and Smits, 1987).

Total collection versus the index method

Before choosing to use one or the other method, it is important to consider their advantages, disadvantages and any particular factors relative to the method that can impact the digestibility results.

Advantages and disadvantages. The main advantage of the index method over the total collection method is that there is no need to collect and process all the feces produced during the trial, thus reducing the time, labor and costs of the digestion trial. Besides this, it also avoids the need for keeping quantitative records of feed consumption, and the housing of pigs in digestion crates is not required, so trials can be conducted in ordinary, less expensive pens. In the index method the total quantitative collection is replaced by the random sampling

of feces, which is sometimes referred to as ‘grab’ sampling (Kane et al., 1952; Kotb and Luckey, 1972; Schneider and Flatt, 1975; Adeola, 2001; Kavanagh et al., 2001). Grab sampling is a technique where a sample is taken directly from the rectum or from a recent fecal pat (Kotb and Luckey, 1972). There is not a unique procedure for grab sampling, nor agreement on the minimum number of grab samples or collection days required to obtain a representative composited sample. The length of the collection period used by researchers to obtain a composited sample for the index method (Cr_2O_3) fluctuates widely (Barnicoat, 1945; Schurch et al., 1950; Schurch et al., 1952; Everts and Smits, 1987; Dellaert et al., 1990; Moughan et al., 1991; Kavanagh et al., 2001).

Coprophagy in regular pens. One of the problems observed with the index method, when done in regular pens instead of metabolism crates, is that pigs could eat some feces. This would change the concentration of the indicator excreted, affecting the digestibility results.

Kemme et al. (1997) investigated the effects of coprophagy and digestibility calculation method (Cr_2O_3 vs. total collection for 10 days) on DM, P, and Ca apparent total tract digestibility in finishing barrows (95 to 120 kg BW). They offered pigs 40 g of fresh pig feces daily/kg of diet. The feces fed to one of the groups contained Cr from Cr_2O_3. Even though no significant effects on Ca (P = 0.217), and total P (P = 0.103) digestibility were found by the feces consumption, numerical improvements of 1.7 and 4.1 percentage units were seen in Ca and total P digestibility, respectively, when feces were supplemented to the diet. The authors concluded that coprophagy may have improved Ca and total P digestibility, primarily because feces are a material that has highly available Ca and P. According to their calculations, the altered nutrient to indicator ratio was of minor importance for the change observed in digestibility.

Metabolism crates versus regular pens for balance trials. Besides the problem of coprophagy found in regular pens, it is more difficult to collect the urine excreted using this type of confinement. Urine collection is facilitated by the

use of metabolism crates. Collecting urine is required in order to assess the extent of nutrient retention after absorption. In this case the study is referred to as a balance study. This implies collecting and measuring the total volume of urine excreted during the period of the trial and keeping a representative sample of that volume for nutrient concentration analysis. Nutrient retention results from subtracting the total intake minus the total fecal and urinary excretion of the nutrient.

Physical exercise in regular pens. The type of housing might also influence the estimation of nutrient digestibility. In the previously cited study by Kemme et al. (1997), the effect of pig movement on apparent total tract digestibility of DM, P, and Ca was also investigated. Pigs housed in pens, and thereby having free movement, had higher Ca and total P digestibility, but lower DM digestibility, than pigs housed in metabolism crates. Nevertheless, results were inconclusive because it was not possible to separate the effects of coprophagy from the effects of housing. The researchers indicate that both free movement in ordinary pens as well as coprophagy play a role in increasing the digestibility of Ca and total P of animals housed in pens, even though the particular effect of movement on the enhancement of Ca and P digestibility could not be demonstrated in their study.

Collection length. Several factors determine the optimum length of time pigs can be scheduled for the total collection method. Having the pig restrained in a digestion crate or in a metabolism crate for a long collection period increases the risk of accidents, animal sickness, feed refusal or other circumstances that may affect results. On the other hand, a very short collection period may give rise to inaccuracies due to the inability to collect material representative in quantity and chemical composition. Adeola (2001) recommends an adaptation period of 3 to 7 days followed by a collection period of 4 to 6 days.

In testing the suitability of Cr_2O_3 as an indicator in pig trials, researchers have compared different grab sampling procedures to the total collection method.

In some of those experiments grab sampling consisted in collecting feces once or twice daily during a period of four to seven days, and then compositing all the collected samples prior to assessing nutrients and Cr concentration (Schurch et al., 1952; McCarthy et al., 1974; Moughan et al., 1991; Bakker and Jongbloed, 1994). In the search for a more practical (shorter) grab sampling method for group-housed pigs, Kavanagh et al. (2001) reported no difference for DM and energy digestibility between total collection (5 days collection using individually penned pigs) and a 1-day grab sampling for the index method (Cr_2O_3). The grab sampling procedure consisted of collecting feces from at least eight pigs out of 13/14 total pigs housed per pen, and then compositing them into a single sample prior to analysis. To facilitate the fecal collection, this was done by taking the pigs out of the pens to be individually weighed in a scale. It is not clear for how long researchers fed Cr_2O_3 before collection.

One of the first studies that reported the use of Cr_2O_3 in pigs was done by Barnicoat in 1945. He found, in agreement with previous research in other species, that the rate of fecal elimination of Cr_2O_3 was uneven or irregular through the collection period, being more irregular at the beginning and at the end of the period. He noticed that Cr_2O_3 excretion became approximately constant - i.e., in equilibrium with the intake - between two to four days after Cr_2O_3 started to be fed. Taking into account these variations, the researcher concluded that a considerable number of samples of excreta should be taken in order to obtain a truly representative sample for analysis. His comparison of protein digestibility by this method with the total collection gave similar results (87.1 vs. 86.8%, respectively). Nevertheless, it should be noted that he used two different diets and only one pig per diet for the Cr_2O_3 method, repeating the collection twice. Despite the poor replication of this early experiment, it brought some light to the question of when to start collecting. Obviously, the appropriate time to start collecting should be once the fecal indicator comes to equilibrium with the indicator in the feed consumed. At that point in time, total fecal recovery of the Cr fed is assumed. An experiment by Clawson et al. (1955) indicated that Cr equilibrium comes one day later than what Barnicoat (1945) suggested. The

data by Clawson et al. (1955) showed that equilibrium was reached between the third and fourth days counted after the initial feeding of the indicator.

Digestibility results by both methods. Clawson et al. (1955) found that digestion coefficients for DM, crude protein (CP), ether extract (EE), crude fiber (CF), and nitrogen-free extract (NFE) calculated from the mean concentrations of Cr in compounded fecal samples taken rectally twice a day (6:00 a.m. and 5:00 p.m.) during three or four consecutive days were lower than those obtained by a total collection method over seven days. Averaging the data for the ten lots (8 pigs each) used by these researchers, the digestibility results obtained for the index method vs. the total collection were, respectively, DM: 73.8 vs. 75.7, CP: 72.6 vs. 74.8, EE: 46.9 vs. 50.1, CF: 18.3 vs. 24.9, and NFE: 86.0 vs. 87.3.

Other researchers have also found a tendency for lower digestibility results using the index method as compared to the total collection method, which is usually explained by the Cr recovery in feces, which is generally lower than 100%. Mroz et al. (1996) found that the overall mean total tract digestibility calculated by the index method (Cr_2O_3) was lower than that calculated by total collection, regardless of the nutrient assayed. The difference was equal to three percentage units for organic matter (OM) ($P < 0.05$), nine percentage units for ash ($P < 0.001$), three percentage units for CP ($P < 0.01$), five percentage units for EE ($P < 0.001$), and nine percentage units for CF.

Everts and Smits (1987), working with sows, also reported lower digestibility for DM and CP using Cr_2O_3, compared with the total collection method (79.0 vs. 80.2, and 82.4 vs. 83.1, respectively). Kavanagh et al. (2001) obtained lower digestibility coefficients for energy and DM in growing pigs for the Cr_2O_3 method as compared to the total collection method (84.8 vs. 85.8, and 85.9 vs. 86.5, respectively). Apgar and Kornegay (1996) also found DM digestibility in finishing pigs to be slightly lower for the indirect method (87.0% by the indirect method vs. 87.6% by total collection).

Although some research has been reported comparing the digestibility of DM, OM, energy, CP, EE, NFE, CF, and macro-minerals between the total

collection and the Cr_2O_3 method, the literature is very scarce in regard to comparing the digestibility of micro-minerals by both methods.

The study by Apgar and Kornegay (1996) consisted of evaluating the mineral balance and DM digestibility in finishing pigs fed elevated levels of Cu in the presence of excess amounts of otherwise 'trace' minerals (Fe, Cu, Mn, and I) and measuring the digestibility coefficients by both methods. After correcting for Cr recovery by utilizing Cr absorption values to correct fecal Cr concentrations for zero absorptive loss, the researchers found that the percent estimates of Cu, Zn, and Fe absorption by the index method were lower and within one percentage unit of the estimates obtained using the total collection method (11.3 vs. 11.9 for Cu; 18.6 vs. 19.4 for Zn; and 4.4 vs. 4.9 for Fe, respectively). The authors agreed that estimating DM digestibility by the index method resulted in values relatively similar to those obtained by the total collection method. However, they concluded that the index determination of Cu, Fe, and Zn absorption was not feasible for estimating their availability. The researchers concluded that the use of chromic oxide did not seem to be a reliable method with which to estimate trace mineral absorption.

Indirect methods

In many instances it is desirable to evaluate the digestibility of a feedstuff when it is fed in a mixture with one or more other feeds. Examples include protein supplements or feedstuffs that are normally not used as a complete diet by themselves.

This requires first to measure nutrient digestibility (by either the total collection or the index method) in two or more related diets, and then digestibility is calculated indirectly for one of the feedstuffs by using those results. The diets are said to be related in the sense that one of them is a basal or 'balancer' diet, while the other(s) consist of the basal replaced with known proportions of the feedstuff under test. Digestibility in the test feedstuff is calculated by difference, considering the amount of the nutrient present in both the basal and the other diet. The difference between the two estimates gives the contribution of the test

ingredient. It seems logical to name these ‘indirect’ methods because they require a further step after having assayed the nutrient digested by either the total collection or the index method. As mentioned before, the indirect methods are used when the nutrient of interest is supplied by more than one feedstuff in the diet and we are interested in finding the digestibility in only one of the feedstuffs used (Schneider and Flatt, 1975; Whittemore, 1998; Adeola, 2001).

This method of calculating ‘digestibility by difference’ sometimes yields digestion coefficients greater than 100, and sometimes even negative values are obtained. Some researchers consider these as absurd coefficients, while others argue that such values represent phenomena observable in nature and should be treated as reasonable possibilities (Schneider and Flatt, 1975). According to Whittemore (1998), this method is more prone to error the lower the proportion of the nutrient contributed by the test ingredient. This means that a small error in the determination of the nutrient digestibility in the basal or balancer diet, or in the mixed diet, can have a large effect upon the digestibility result calculated for the nutrient in the test ingredient.

According to Adeola (2001), one variation of the indirect method consists of feeding a basal diet to one group of pigs and determine nutrient digestibility in the whole diet (by either the total collection or index method). Simultaneously, a different group of pigs is fed the basal diet added with a known amount of the feedstuff under test, and the nutrient digestibility of this mixture is determined. To calculate the digestibility in the test ingredient, the formula used is:

$$\text{Digestibility, \%} = 100 \times [(T \times t) - (B \times b)] / a$$

Where:

T = Digestibility (%) of the nutrient in the total diet (basal plus the test feedstuff), calculated by either the total collection or the index method.

t = Amount of the nutrient ingested in the total diet.

B = Digestibility (%) of the nutrient in the basal diet, calculated by either total collection or index method.

b = Amount of the nutrient ingested in the basal diet.

a = Amount of the nutrient in the test feedstuff added to the basal diet

t = b + a

Another indirect method is the regression method. It consists in feeding a basal diet to a group of pigs and simultaneously feeding different groups of pigs diets that have at least two proportions of the nutrient in the basal diet replaced by the test feedstuff. The digestibility of the nutrient in the test feedstuff is determined by regressing the digestibility of the nutrient against the proportions of the nutrient replaced, and extrapolating to 100% replacement. Again, this method requires feeding more than two diets; a basal diet and at least two other diets with different proportions of the basal replaced by the test feedstuff. To estimate digestibility of the nutrient in the test feedstuff, the digestibility of the nutrient in the different diets is linearly regressed against the proportions of the test nutrient replaced. Then extrapolation to 100% replacement is done to estimate the digestibility of the nutrient in the feedstuff by itself. When doing digestibility by regression it is important to plot the proper variables: percent of digestibility (Y axis) observed at the different levels of inclusion of the tested feedstuff, versus the *percent of the nutrient* (X axis) that comes from the tested feedstuff in each diet. The regression equation is different and has a different coefficient of correlation (R^2) if the X axis is mistakenly assumed as *the percent of the tested feedstuff* in each diet. It is also important, for any type of digestibility trial, that while changing the levels of the nutrient under test, the levels of the rest of nutrients are kept constant in order to avoid changes in digestibility that could be due to the fluctuation of other nutrients instead of the nutrient under test.

Utilization of Alternative Feedstuffs in Modern Swine Nutrition

Alternative feedstuffs are the crop residues or food industry byproducts not consumed by humans but which are suitable for feeding pigs, which transform them into pork - a human-edible product. These are edible waste

products or co-products from agriculture or the food processing, food preparation or food service industries. Example industries include grain milling, brewing and distillation; baking; fruit and vegetable processing; meat, milk and egg processing; seafood processing; prepared food manufacturing; and retail food outlets. Other alternative feedstuffs include those not regularly fed to pigs but fed during times of low prices and/or surpluses, or during shortages of traditional feedstuffs. Alternative feedstuffs may include materials available locally that can be economical substitutes for expensive or not readily available traditional feedstuffs (Myer and Hall, 2004). Miller et al. (1994) group some potential byproducts for swine according to their primary product origin: animal (milk, meat and egg byproducts), grain (milling, baking, brewing, and distilling byproducts), sugar and starch production (cane, beet and corn molasses, and salvage candy), and vegetable materials (cull beans, roots and potato byproducts).

The suitability of an alternative feedstuff for a particular age or physiological stage of the pig depends, among many factors, on its legality of use, availability in the local market, cost (including transportation, storage, processing and labor), palatability, consistency, nutrient composition and availability, presence of potential health hazards (toxic or disease factors) or anti-nutritional factors, and potential effects on pork quality and perishability – including spoilage and rancidity (Miller et al., 1994; Myer and Brendemuhl, 2001; Myer and Hall, 2004).

Justification for using alternative feedstuffs in swine production

According to FAO (2004), global cereal production has been stagnating since 1996. Global cereal utilization, on the other hand, has been continuing on an upward trend and has been exceeding production by significant amounts continuously since 2000.

Several reports affirm that swine production will compete in the future directly with humans for cereal grains and high quality protein supplements (Pond, 1987; Dierick et al., 1989; Close, 1993). This is already a reality in most countries of the developing world (Oke, 1990) where the human population

obtain 58% of their total caloric intake from cereal grains as compared to 28% for the developed world (Ensminger et al., 1990).

Among cereals, corn is an important food for the fast-growing human population. Currently in the western hemisphere only four countries (U.S., Argentina, Paraguay and Bolivia) produce enough corn to fulfill their needs and export the excess, while nineteen other American countries show a permanent deficit in corn production (Ministerio de Agricultura y Desarrollo Rural de Colombia, 2004). Among those is Colombia, a tropical country located in the north of South America, where the demand for corn for both human and swine populations during the last decade chronically exceeded local production of the cereal. Colombian imports of corn started to climb in 1993 when the country opened its markets to free global trade. Since that year, the country has been steadily increasing its imports of yellow corn (Figure 2.1), which is used for animal production, particularly to feed poultry and swine.

Corn imports by Colombia now account for 50.1% of the total corn consumed in the country. Since 1996, among all the commodities imported by this country, corn ranks first in terms of total cost and volume. During 2002, Colombia imported 2,098,679 metric tons of corn (yellow and white) for a total cost of 250,166,000 U.S.D., turning the country from a no-net importer ten years before, into the 9th largest world importer of the cereal (FAO, 2004). This is interesting, taking into account that the country ranks 28th in terms of human population (42 million), has a low consumption of animal protein, and is not a meat or egg exporter. In this country, which is not particularly strong in animal production, the figure reflects a total dependency on foreign corn prices. The reason for the decline in local production and subsequent dependency on foreign corn is that local production is not competitive under the new global trade scheme. The magnitude of the corn deficit is similar for most Andean countries. In most Central American countries this deficit is even greater, exceeding 90% (FAO, 2004; Ministerio de Agricultura y Desarrollo Rural de Colombia, 2004). This situation calls swine nutritionists to consider partial replacement alternatives

such as grain derivatives and other materials that are in poor demand as direct food sources by the human population.

In general, another reason for using the alternative feedstuffs that are locally available is the need for proper disposal of these materials. In many countries alternative feedstuffs for pigs represent not only an economic option to lower production costs, but also an environmentally-friendly approach to the disposal of the enormous amounts of these organic materials in times of increasing byproduct generation and landfill shortages. According to Fadel (1999), during 1993 the calculated total world dry matter tonnage for about twenty types of by-product feedstuffs was almost 1 billion metric tons of which about 65% were crop residues. These per capita tonnage will likely increase in developed countries and remain the same or increase slightly in developing countries over the next 20 years, suggesting that by-products will become an increasing waste problem. Thus, the role of pigs in recycling and "adding value" to many of these byproducts and wastes is becoming increasingly interesting as a viable waste management option.

Alternative energy feedstuffs

In most swine diet formulations, those ingredients that provide most of the diet's energy (i.e., corn) usually represent the highest cost among all the ingredients. This is because of the high proportion accounted for by energy ingredients. In a typical corn-soybean meal (corn-SBM) diet for growing-finishing pigs, the total cost of corn is about 50% higher than the total cost of the soybean meal used. To illustrate this, a typical corn-SBM diet for growing-finishing pigs contains between 70 to 85% corn (ave. 77.5%) and 14 to 25% soybean meal (ave. 19.5%). Using the U.S. prices reported on July 22-2004 (Feedstuffs, 2004) for both feedstuffs: $2.24/bushel of corn (1 bushel: 54 lb for U.S. No.2 grade corn, equivalent to $0.042/lb), and $239/ton of soybean meal ($0.12/lb), we have the corn component cost at 3.3 cents/lb of the corn-SBM mix, while the SBM costs 2.3 cents/lb of the mix. So, for reasons of total cost, alternative energy feedstuffs should be considered first when thinking about partially replacing

swine diets. Table 2.1 presents the nutrient composition of corn, compared to several alternative feedstuffs commonly available in tropical countries. According to Myer and Brendemuhl (2001), the main disadvantages of the byproducts listed in Table 2.1 are:

- Rice (*Oryza sativa*) byproducts: rice bran is a bulky, fibrous material with high potential for rancidity depending on the fat level. Rice polishings are not as bulky and fibrous as rice bran, but also have the problem of potential rancidity. Broken rice has a high energy contents, but is low in lysine. Paddy rice is highly fibrous and has a higher risk of aflatoxins contamination.
- Bananas have high moisture content. Whole, green bananas have large concentration of tannins, which lower its palatability for pigs.
- Fresh cassava also has high moisture content, while some varieties of the root may contain large residual concentrations of toxic HCN in the meal.
- The juice of sugar cane has high moisture content. The stalks have the same problem and are also a bulky material.
- Raw potatoes are high in moisture. In general, cooking improves potato byproducts utilization.
- Restaurant food waste is not only high in moisture, but also highly variable in nutritional value.

As can be seen in Table 2.1, the main limitations presented by these materials, in the form generated by the industry, are either high water content or high fiber content, or both.

In general, most of the common energy feedstuffs available in tropical countries make no significant protein contribution to the diet and require further processing to increase their dry matter content, which increases their cost. Many of these materials, in their non-processed form, are inexpensive because they are basically waste byproducts. It is usually the cost of transport and drying which limits their potential as economical alternatives. Drying is sometimes recommended to facilitate handling and incorporation in dry diets. It also

concentrates the nutrients, which is necessary when dealing with animals having limited gastrointestinal capacity.

As mentioned, digestibility of the dietary nutrients present in feedstuffs varies according to different factors, including the amount and type of fiber present in the diet (Wisemann and Cole, 1985; Noblet and Le Goff, 2001). Crude fiber is comprised of three major fractions: cellulose, hemicellulose and lignin. These components are measured by detergent fiber analysis, which determines neutral detergent fiber (NDF) and acid detergent fiber (ADF). The NDF is the residue insoluble in a neutral detergent solution after eliminating the plant cell contents. It represents the cell wall constituents or cellulose, hemicellulose and lignin. The ADF is the residue comprised of cellulose and lignin. The difference obtained when subtracting NDF and ADF is the hemicellulose fraction of fiber.

The NDF is partially fermented in the large intestine to volatile fatty acids (VFA) such as acetic, propionic and butyric, also producing CO_2, H_2, CH_4, urea and heat. It is known that regardless of its source, an increase in NDF decreases energy availability and may increase fecal loss of nitrogen (Sauber and Owens, 2001). Both the old CF analysis, as well as the newer detergent methods of analysis, underestimate the amount of total fiber due to their inability to recover soluble fiber components such as pectin, mucilage, gums and B-glucans (Grieshop et al., 2001).

In growing pigs, digestibility coefficients for dietary fiber are lower than coefficients for other nutrients. According to Noblet and Le Goff (2001), average fiber digestibility is 40 to 50%, ranging from around zero in high lignin sources (e.g., wheat straw) to between 80 and 90% in high pectin sources (e.g., sugar beet pulp and soybean hulls). This shows how the different fractions vary in digestibility: lignin is indigestible, while pectin is almost completely digested. Also, hemicellulose is more digestible than cellulose, but both are just partially digested. It is also known that digestibility increases with body weight, so adult sows can utilize fiber better, but this also depends on the botanical origin of the fiber (Noblet and Le Goff, 2001).

Thus, for a more complete understanding of the nutritional value of feedstuffs, the information obtained by the proximate analysis is complemented with digestion and balance trials to assess how well those nutrients are utilized by the pig. In Table 2.2, digestible and metabolizable energy, as well as digestible protein results, are shown for several of the feedstuffs listed in Table 2.1.

Availability of alternative energy feedstuffs

Continuing with the Colombian situation; despite a lack of corn, it is interesting to notice that the country is an important producer and net exporter of several potential alternative feedstuffs or derivatives. In 2003, Colombia was ranked 1st in the world as an exporter of plantains and sugar cane, 4th in the world as exporter of bananas, among the top 10 exporters of fresh fruits, roots and tubers; and among the top 20 exporters of paddy rice (FAO, 2004). This means not only that the country produces more of these staples than required to fulfill its internal demand, but also that there is a competitive production infrastructure that is generating important amounts of materials potentially suitable for feeding swine. Table 2.3 illustrates Colombian production and world rank among the top 20 producers of several feedstuffs.

From the feedstuffs listed in Table 2.3, all but coffee are generally considered energy sources. Nutritional information is available for most of the feedstuffs listed. 'Coffee grounds' is a material for which limited information is available. According to Ensminger et al. (1990), on as fed basis, it has 10.2% CP, equivalent to 6.1% digestible protein for pigs. It also has 74% DM, 9.3% EE, 1.2% ash, 0.09% Ca, 0.06% P, and 21.5% CF.

Rice bran

Rice bran is an alternative feedstuff that could be used to partially substitute for corn in temperate as well as tropical zones. It is abundant and inexpensive in the USA and many other countries. According to the NRC (1998), It contains 13.0% EE, 13.3% CP, 1.61% total P, and 2,040 kcal/kg of net energy,

which is not too different from corn, which has a net energy value of 2,395 kcal/kg.

Although richer in fat (13%) compared to corn (3.9%), it is also richer in fiber (23.7% NDF and 13.9% ADF for rice bran versus 9.6% NDF and 2.8% ADF for corn) which makes it poorly digestible by the young pig. Because of the high fat content, it often turns rancid during storage. According to Cunha (1977), it has about 90 to 95% the feeding value of corn if used at a level of not more than 20 to 30% of the diet. When used at higher levels, its relative feeding value decreases and it tends to produce 'soft pork' (soft carcass fat due to high concentration of unsaturated fat) which negatively affects pig market price. Cunha (1977) also indicates that the material should be used fresh in order to prevent rancidity because this rancidity decreases palatability. As a substitute, he proposes the use of de-fatted or solvent–extracted rice bran, which has about the same feeding value as corn when fed at levels no higher than 30% of the diet, and does not produce soft pork.

Besides of its low cost, rice bran is also interesting because of its high concentration of total P. Total P in rice bran is about five times higher than in corn, and three times higher than in soybean meal, but most of it is present in the form of phytic P. About 75% of the P in rice bran is bound as phytic acid (Cromwell and Coffey, 1991). That form of P is not available and is almost completely excreted by the pig, creating an environmental concern. The low availability of P in rice bran is due to its low digestibility. In a series of trials Jongbloed et al. (1999a) reported 14% P digestibility for rice bran (range: 9 to 20%), 19% for corn (range: 12 to 26%), and 39% for soybean meal (range: 33 to 46%). Table 2.4 shows rice bran and several traditional energy feedstuffs and byproducts ranked by phytate P concentration. Among these feedstuffs, rice bran has the highest content of unavailable P, thus it has the greatest pollution potential.

Phosphorus, a Nutrient under Scrutiny

The digestibility and excretion of several nutrients is of key importance for swine producers not only from nutritional but also from environmental points of view. Excretion of nutrients such as N and P raise concerns with regard to their role in eutrophication of water resources. Both Cu and Zn are toxic to plants when present in high concentrations in the soil solution (Sterritt and Lester, 1980; Agarwal et al., 1999; Athar and Ahmad, 2002). Among all these nutrients, P captures most of the attention because of its paramount biological importance and increasing concern about its polluting potential in modern swine production (Jongbloed et al., 1999a).

Biological importance of phosphorus

Of the 118 elements in the periodic table, only a small number seem to play some role in biological systems. Life, as we know it, is based on carbon which combined with hydrogen, oxygen and nitrogen, creates complex nutrients such as carbohydrates, lipids, amino acids and vitamins. Besides those compounds, and often as an active part of them, cell processes require a few mineral elements. Only seven minerals (Ca, P, K, Na, Cl, Mg, and S) are required in the diet of swine at levels greater than 100 mg/kg (ppm) of diet. They are referred to as essential macro-minerals. Eight other minerals (Zn, Fe, Cu, Mn, Se, I, Co and Cr) are required in smaller amounts, and are referred to as essential micro-minerals or trace minerals (NRC, 1998). Some other elements (including As, B, Br, F, Mo, Ni, Si, Sn and V) are required by other species and may also be required by swine in very small amounts, but this has not yet been established (Underwood, 1977).

Among the minerals, calcium and phosphorus are the most abundant in the body. Phosphorus is the second most abundant mineral and is required for a variety of physiological functions (Underwood and Suttle, 1999; McDowell, 2003).

The word ‘phosphorus’ means ‘carrier of light’, because the element ignites when exposed to air, and also glows in the dark in a process known as

chemiluminescence. It was first discovered in urine around 1677 and in bones in 1770 (Matheja and Degens, 1971).

Phosphorus plays a key role in vital biochemical reactions such as in energy-dependent processes and also in the regulation of acid-base equilibrium. It is the major intracellular buffer in the body. Furthermore, P is an important component of nucleic acids and membrane lipids (Zubay, 1998). Phosphorus also plays an important role as a structural element in bone, were it is stored along with Ca in the form of hard and poorly soluble hydroxyapatite crystals. In this chemical form Ca and P give bones strength and rigidity (McDowell, 2003). The reason for its activity in many biological processes is that the P atom has an electron configuration where there is one electron in excess for its various functional or structural work assignments, so P can not form very rigid networks. Instead, phosphates can readily adapt to changes in the biological environment simply by shifting the fifth electron around. This flexibility in functional and structural behavior makes P a key element in living processes (Matheja and Degens, 1971).

Phosphorus distribution in the body

Approximately 1% of the mature body weight of the pig is P (Peo, 1991). Also, approximately 80% of the total body P and 99% of the total Ca is located in the skeleton and teeth (Cromwell and Coffey, 1991). Both minerals are present in bone, mostly in the form of an apatite salt composed of calcium phosphate [$Ca_3(PO_4)_2$] and hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] (Hays, 1976; McDowell, 2003).

The Ca:P ratio in bone is very constant, being close to 2:1 (Lloyd et al., 1978; McDowell, 2003). This ratio does not change during dietary deficiency of Ca, P, vitamin D, or during significant changes in nutrient intake. The same can not be said for the total amount of ash accumulated in bones, which varies according to the animal's nutrient status (Hays, 1976).

Crenshaw (2001) points out that there is a large variation in the P percentage of skeletal tissue (60 to 80%) in pigs, which is due to the change in proportions of skeletal and soft tissues as pigs grow. According to Georgievskii et

al. (1982), these differences seem to be caused by different amounts of fat deposition because only small differences are found when the P contents are calculated on defatted tissue.

Phosphorus absorption and excretion

Phosphorus enters the digestive tract of the pig in organic and inorganic forms. Its absorption occurs after it is hydrolyzed by brush border phosphatases in the enterocytes. It is also absorbed as a part of phospholipids (Crenshaw, 2001). In general, mineral elements in the intestine must become available in ionic form (cations and anions) in order to be absorbed. Monovalent elements (e.g., Na, K, Cl) are very soluble at neutral pH, so they are more readily absorbed and transported than the polyvalent minerals. Phosphorus valence is 3 or 5. In general, mineral absorption in pigs has been studied by intestinal cannulation and perfusion of mineral solutions and markers into surgically isolated loops of intestine. The marker is used as an indicator of net water movement (Partridge, 1979). Although net absorption of several minerals has been demonstrated in both the distal small intestine and in the large intestine, the small intestine is recognized as the principal site of Ca and P absorption (McDowell, 2003). The poor role of the large intestine in exogenous P absorption was found by Fan et al. (2001). During assessment of true P digestibility in pigs, Fan et al. (2001) found no difference between true ileal and fecal P digestibility values, concluding that most P absorption occurs in the small intestine. According to Crenshaw (2001), the major site of Ca and P absorption is the upper part of the small intestine. In the case of P, the absorption is four times higher in jejunum, as compared with the duodenum, and very little absorption occurs in the ileum.

In regard to the absorption process, both Ca and P absorption have active and passive components. In general, the active transport system is saturable, and is enhanced by dietary insufficiency. This active transport occurs in the duodenum and jejunum, while the passive transport system is nonsaturable and occurs mostly in the ileum. Passive Ca absorption includes unregulated

paracellular transport, which becomes more important in Ca rich diets (Crenshaw, 2001).

Absorption of both minerals is closely related to their demand, according to the physiological state of the animal (McDowell, 2003). The amount of P absorbed is dependent upon several conditions; including the level of P in the diet, the source of P, the amount of Ca, the Ca:P ratio, the intestinal pH, and the presence of other minerals that are antagonistic to the absorption and utilization of P (Peo, 1991). Jongbloed (1999) points out that dietary Ca content should be standardized when assessing P digestibility in feeds, due to the interactions occurring between Ca and P in the digestive tract. In regard to the level of P, it is known that a deficient dietary intake of the mineral stimulates absorptive processes, particularly the active absorption, and also renal reabsorption which minimizes excretion of P in urine (Combs, 1998; Crenshaw, 2001).

The active absorption of Ca and P is stimulated by vitamin D, which is activated from 25–OHD_3 into 1,25–$(OH)_2D_3$ by the enzyme 1α – hydroxylase in the kidneys, and targets the enterocytes to increase the synthesis of calcium binding proteins (or calbindins). The 1α–hydroxylase activity is stimulated by parathyroid hormone (PTH), which is released into circulation from the parathyroid gland immediately after Ca plasma concentration decreases. Besides that indirect role of PTH on the stimulation of mineral absorption, this hormone directly increases plasma Ca concentration by inhibiting bone osteoblasts activity while promoting osteoclastic resorption. It also inhibits renal P reabsorption, increasing its urinary loss (Georgievskii et al., 1982; Crenshaw, 2001; McDowell, 2003). When serum Ca is elevated, the thyroid gland secretes the hormone calcitonin which enhances uptake of Ca by tissues, increases Ca renal excretion and inhibits bone resorption (Georgievskii et al., 1982; Combs, 1998; Crenshaw, 2001). Summarizing, P homeostasis depends on several factors, including intestinal absorption, bone catabolism and regulation of excretion through the kidneys (Combs, 1998; Crenshaw, 2001).

In regard to the excretion pathway, it is known that feces are the primary path for P excretion in herbivores, while urine is the principal path of P excretion

for humans and carnivores (McDowell, 2003). During periods of P depletion, the kidneys respond by reducing excretion virtually to zero, thus conserving body phosphate (McDowell, 2003). In all species, feces are the primary path for Ca excretion. Urinary loss of Ca is minimal, owing to efficient reabsorption by the kidneys (McDowell, 2003).

Phosphorus bioavailability and bone mineralization

It is known that growing and finishing pigs do not require maximal bone mineralization in order to reach maximum growth performance. Higher dietary Ca and P levels may be desirable over prolonged reproduction periods, but it is not necessary for swine destined to market (Cromwell et al., 1972; McDowell, 2003).

According to Peo (1991), adequate Ca and P nutrition for all classes of swine depends upon three main factors: (1) adequate provision of each element in available form in the diet; (2) a suitable ratio of available Ca and P in the diet; and (3) the presence of adequate levels of vitamin D. Given those conditions, proper nutrition is expected to be reflected in adequate bone mineralization.

A way to assess the degree of mineralization of the skeleton is by measuring the ash content in the dry defatted bone (Georgievskii et al., 1982). It is known that bone mineralization is an accurate estimate of the biological availability of P, provided there are adequate levels of all other nutrients (Hays, 1976). Bioavailability of a mineral can be defined as the amount of the mineral that is absorbed and later utilized by an animal, or the fraction that is retained in the body (feed – (feces+urine)) (Cromwell, 1999b; Jongbloed et al., 1999a). The term is also defined as the degree to which an element ingested from a particular feed source is available for use in metabolism by the animal in question (Ammerman et al., 1986). The value for availability is usually expressed as a percentage.

McDowell (2003) defines net retention for a given mineral as the total intake minus the total excretion (fecal plus urinary) of the mineral. The same author indicates that net retention may be useful in interpreting results, but in many situations it probably has limited value in determining the bioavailability of

minerals. This is because urinary excretion represents a portion of the minerals that were potentially effective from a nutritional stand point, and that were either involved or available for use in metabolism.

The bioavailability of Ca and P from a particular source or feedstuff can be assessed by measuring the total body retention of the mineral, or also by the assessment of their incorporation into bone. Bone characteristics, such as its ash percentage and its breaking strength, are good criteria to evaluate the bioavailability of those minerals. Bone breaking strength or shear strength is regarded as one of the most dependable and sensitive tests in determining bone mineralization in pigs (Underwood and Suttle, 1999). Bone breaking strength, which is a more sensitive indicator of bioavailability than percent of ash, is the measure of the amount of force, applied to the center of the bone, required to break the bone when it is horizontally placed on two supports (Cromwell, 1999b).

In regard to the methods used for determining bioavailability of minerals for pigs, much of the early research was done through digestibility trials and balance trials. True digestibility, which could be interpreted as a measure of bioavailability, can be obtained in the mentioned types of trials by correcting the observed apparent digestibility for the endogenous excretion of the mineral under study. In this case, the amount of available mineral is the difference between the amounts consumed and excreted, corrected for the endogenous portion. Cromwell (1999) indicates that this procedure is not generally accepted for measuring the availability of minerals because the corrected retention obtained in balance trials, which also considers urinary excretion, does not necessarily represents the bioavailability of the mineral in terms of its digestibility and absorbability from the tract. Besides this, McDowell (2003) argues that digestion and balance trials have the disadvantage that bioavailability of minerals can not be obtained for specific feedstuffs or mineral supplements, but only for the entire diet. Taking this into account, this author indicates that a more accurate and acceptable method for estimating bioavailability of a specific mineral is by obtaining a relative bioavailability value. This value expresses the availability of the mineral in the test source relative to its availability in a standard form of the

mineral, which is given a score of 100. Currently, the most commonly used method for determining relative mineral bioavailability in a particular feedstuff for growing pigs is the slope-ratio procedure. In this method, graded levels of the feedstuff under test are added to a basal diet which does not contain the mineral of interest. As an example, if the mineral is P, and the feedstuff under test is rich in protein, then the basal diet can have starch, sucrose or dextrose as a source of energy (to balance energy and protein). It can also have casein as a source of protein when the test feedstuff is high in energy (although casein contains P, it is considered 100% available). The responses obtained (e.g., bone breaking strength or bone ash) are regressed on the dietary level of the mineral or on its absolute intake. Similarly, graded levels of the mineral in a highly available form (a standard, e.g., monosodium phosphate) are added to the basal diet in order to produce a linear response which is also regressed in the same way done for the test diets. The slope ratio is the slope of the regression line calculated for the test feedstuff divided by the slope of the standard line and multiplied by 100. This ratio represents the bioavailability of the mineral in the test feedstuff relative to the standard used (Cromwell, 1999b).

Phosphorus dietary requirements and supplementation

Being abundant in the body, Ca and P are required in relatively high amounts in swine diets. A phosphorus deficiency causes a reduction in bone mineralization and thereby impairs calcium metabolism which can lead to rickets in the young animal or osteomalacia in the adult. Some signs of these conditions are stiffness of the gait, enlarged and painful joints, deformities of the long bones (bent, bowed, broken), humped back or the camel back syndrome, and posterior paralysis or downer sow syndrome (Peo, 1991; Soares, 1995).

Calcium requirements are between 0.45 and 0.90% of the diet, while total P requirements are between 0.40 and 0.70% (NRC, 1998). The requirement varies according to body weight and physiological stage of the pig. These proportions indicate that the Ca requirement is approximately 1.2 times that for total P. The term ‘total P’ refers to all the P present in the diet, including the

unavailable fraction that is bound within the phytate complex or *myo*-inositol 1,2,3,5/4,6-hexakis (dihydrogen phosphate). This unavailable fraction is called phytic P, and has the structure of a fully phosphorylated *myo*-inositol ring (Maenz, 2000). Figure 2.2 depicts a model of the phytin complex, represented by a phytate molecule chelating various cations, protein, and starch residues.

Due to the fact that the proportion of phytic P varies greatly in different feedstuffs (Coehlo, 1999; Harland and Overleas, 1999; Nys et al., 1999; Ravindran, 1999), P requirements for pigs are better expressed in terms of available P rather than in terms of total P. Availability is defined as that proportion of the nutrient provided that can be extracted, absorbed and utilized by the animal to meet its net requirements at a stated level of inclusion and level of feeding (Mudd and Stranks, 1985). The net requirement for P comprises the inevitable losses of the mineral via feces and urine, its retention in the form of both body weight gain and conception products, and its secretion via milk (Rodehutscord et al., 1998).

Current requirements for available P are estimated to be between 0.15 and 0.55% of the diet (NRC, 1998). Research has shown that the requirement for Ca and P should be considered not only in terms of the ideal proportion of each in the diet, but also in terms of the best ratio between dietary Ca and P. For grain-soybean meal diets, the Ca:total P ratio should be between 1:1 and 1.25:1. The currently accepted Ca:available P ratio is between 2:1 to 3:1. Wider Ca:P ratios tend to lower P absorption, resulting in reduced growth and bone calcification, particularly when the diet is low in P (NRC, 1998).

Regular grains and oilseeds provide pigs with the total amount of P they require. However, as an important portion of this P is not available to the animals, common diets have to be supplemented with highly available inorganic sources of P, such as defluorinated rock phosphate, and mono and dicalcium phosphates. Phosphorus bioavailability from monosodium and monocalcium phosphate is close to 100%, while P availability from dicalcium phosphate is about 95% (NRC, 1998; Cromwell, 1999a). Feed grade dicalcium phosphate, which consists of about two-thirds dicalcium phosphate and one-third

monocalcium phosphate, is the most commonly used phosphorus supplement in pig feeds (Cromwell and Lindemann, 2002). Most modern swine operations supplement diets with one of these sources to fulfill the pig's requirement for available P.

Phytate phosphorus

Although phytic acid is the acid form of the phytate anion, both terms are used interchangeably in most of the literature and also in this dissertation.

The phytic acid complex is the most important form of P storage in plants, serving different functions during seed dormancy and germination such as initiation of dormancy, antioxidant protection during dormancy and storage of P, high energy phosphoril groups and cations for use during germination (Gibson and Ullah, 1990; Ravindran, 1999).

Phytic acid biosynthesis starts with D-Glucose 6-P, which undergoes irreversible cyclization to myo-inositol-3-P and posterior phosphorylations into the six phosphate-containing phytate molecule, which is deposited in the cells in the form of phytin granules (Loewus and Murphy, 2000). Phytin is the name given to phytate when it is chelated to cations, proteins and/or starch (Angel et al., 2002). Phytin is a complex salt with counterions that include K^+, Mg^{2+}, Ca^{2+}, the protein matrix in which it is embedded, and several minor cations (Loewus, 1990). Phytin is capable of strongly binding divalent and trivalent cations at all pH values normally encountered in feeds, because it carries a strong negative charge (Angel et al., 2002). According to Maenz et al. (1999), among all the cations, Zn has the highest affinity for chelating with phytin, followed by Fe, Mn, Ca and Mg.

Phytate is hydrolyzed by endogenous phytases during seed germination and early seedling growth, providing the seedling with *myo*-inositol, P, and several mineral cations (Hegeman et al., 2001; Raboy et al., 2001; Raboy, 2001). Phytate concentration varies among the different seeds. Phytic acid comprises from 55 to 85% of the total P present in grains such as corn and oilseeds such as soybean (Nelson et al., 1968; Raboy, 1990; Raboy et al., 2001). According to the review by Ravindran (1999), phytate P, as a percentage of the total P, is slightly

higher in corn (68%; Range: 61 to 85%) than it is in soybean meal (60%; Range: 46 to 65%).

The amount of phytate varies not only among seeds, but also in the different parts of the kernel. In corn, about 90% of the phytate is concentrated in the germ portion of the kernel. The rest is mostly located in the aleurone tissues (O'Dell et al., 1972). In soybean and other dicotyledonous seeds, phytate is distributed throughout the kernel including cotyledon, endosperm, and embryonic axis (Raboy, 1990). In rice, it is mostly concentrated in the aleurone layers. This makes rice bran a feedstuff particularly rich in phytate P. Rice bran contains about 1.38% (1.02 to 1.81%) phytate P, which corresponds to about 85% of the total P present in this byproduct (O'Dell et al., 1972; DeBoland et al., 1975; Raboy, 1990; Ravindran, 1999). Additionally, phytate is present in other plant tissues, such as roots, tubers, pollen and vegetative tissues (Graf and Eaton, 1990; Raboy, 2001).

Besides binding P, phytate has also been reported to bind Ca, Zn, Mg, Mn, Cu, Fe, Ni, Se and Co (Harland and Overleas, 1999; Overleas and Harland, 1999; Ravindran, 1999). Minor amounts of minerals such as sodium and barium have also been reported to be bound by phytate (Gibson and Ullah, 1990). The binding of cations by the negatively charged phytate molecule in the intestinal lumen of monogastrics renders them unavailable to the animal (O'Dell et al., 1972; Hegeman et al., 2001). Phytate also binds feed proteins and decreases their solubility, affecting the functionality of pepsins (Kornegay et al., 1999; McKnight, 1999; Ravindran et al., 1999).

Endogenous phytases

As stated by Kornegay and Yi (1999), the presence of endogenous phytase activity is negligible for improving the availability of phytate P in nonruminants. Phytate P is not available to pigs, because they do not have enough phytase enzyme in the digestive tract to cleave P from the phytate complex. Cromwell (1999b) further explains that although hind gut microbes

produce phytase, this phytate degradation is of little value because of the poor absorption of the liberated P that occurs in the large intestine.

The phytase activity observed in the digestive tract of pigs not supplemented with the enzyme in the diet has four possible sources: intestinal phytase present in digestive secretions, endogenous phytase present in some feed ingredients, phytase originated from resident bacteria, and phytase produced by exogenous microorganisms (Kornegay and Yi, 1999). The activity present in the extract of small intestinal mucosa of pigs is minimal (Pointillart et al., 1987; Nys et al., 1999). The phytase activity from resident bacteria is probably negligible (Kornegay and Yi, 1999). There is higher activity in the hind gut, compared to the small intestine, but it is of little use to the host, because, as already mentioned, most P absorption occurs in the small intestine.

In regard to feed ingredients, there is variable amount of phytase present in seeds (Gibson and Ullah, 1990). Its function is to convert P from the stored form as phytate P into readily available inorganic phosphate during seed germination (Maenz, 2000). Phytase activity of seeds differs greatly among species of plants (Reddy et al., 1982; Maenz, 2000). With the exception of wheat, rye, and their hybrid triticale, most seeds contain very low levels of phytase activity. Activity is low in corn, sorghum, oil meals and legume seeds (Kornegay and Yi, 1999; Nys et al., 1999). Phytase activity is somewhat higher in rice bran (122 U/kg) than soybean meal (40 U/kg) and corn (15 U/kg). But phytase concentration in rice bran is much lower than it is in wheat (1193 U/kg), wheat bran (2857 U/kg), rye (5130 U/kg), barley (582 U/kg), or triticale (1688 U/kg), according to a review by Eeckhout and De Paepe (1994).

The phytase activity present in wheat bran is so high that the inclusion of 10 to 20% of this feedstuff in growing and finishing diets, respectively, can completely replace inorganic P supplementation in Landrace x Hampshire x Meishan crossbreds (Han et al., 1997). Nevertheless, it has to be noted that these are not commercial crosses of pigs and they have low genetic growth potential. The inability of pigs to digest phytate P means that an important fraction of dietary P is not used, and is excreted in the feces.

Phosphorus and the environment

Providing animals with the required amounts of nutrients for growth and production is an important objective of animal nutritionists. But modern animal production also demands serious consideration of the environment.

Phosphates are natural, nonrenewable and highly valued resources (Abelson, 1999; Vance et al., 2003). Not surprisingly, P is the most expensive mineral supplemented to swine (McDowell, 2003). Its cost per kilogram of diet exceeds the cost of the rest of the supplemented minerals altogether. According to Stevermer et al. (1994), the supplemental phosphorus source is the third major contributing factor to the total cost of the diet.

Even more important than the cost factor are the growing concerns regarding P excretion. It is well known that although the number of swine farms in the U.S. has been decreasing constantly during the last decades, the inventory of pigs per farm is vigorously increasing. The magnitude of these changes can be easily illustrated. Swine operations with more than 5,000 head accounted for 75% of the pig crop in 2001 as compared with only 27% in 1994. Conversely, operations with less than 5,000 head accounted for 73% of the U.S. pig crop in 1994 and only 25% in 2001. Meanwhile, the number of hog operations with more than 5,000 head has grown from just under 1,000 in 1993 to slightly over 2,200 in 2001. The number of operations with less than 5,000 head has declined from 217,000 to less than 79,000 during the same period (National Agricultural Statistics Service, NASS 2004).

As the density of pigs in an operation increases, the amount of manure P to be spread in the field increases. If there is not a corresponding increase in the rate of P extraction by crops grown in these fields, the mineral may result in a threat to the bodies of water in the farm's areas of influence (Hollis and Curtis, 2001; Strak, 2003; Cheeke, 2004). According to Miner (1999), application of P in excess of crop utilization is common in much of the United States. DeLaune et al. (2000) agree, explaining that the reason for this excessive application of P is that manure is usually applied according to the crop's need for N, and animal manures generally exhibit a low N:P ratio.

Water pollution from P may lead to eutrophication. This is a process whereby water bodies, such as lakes, estuaries, or slow-moving streams receive excess nutrients that stimulate excessive plant growth (algae, periphyton attached algae, and nuisance weeds). This enhanced plant growth, also called algal bloom, reduces the concentration of dissolved oxygen in the water when dead plant material decomposes, causing fish and other organisms to die (Sweeten, 1991; Henry, 1996; Jongbloed and Lenis, 1998). In most fresh water ecosystems P is the nutrient limiting eutrophication (Sharpley et al., 1994; Correll, 1998; Correll, 1999; McDowell, 2003). According to Correll (1999), an excessive concentration of P is the most common cause of eutrophication in freshwater lakes, reservoirs, streams, and in the headwaters of estuaries. The same author indicated that for most of those bodies of water, concentrations of 100 µg total P/L were unacceptably high, and even concentrations as low as 20 µg P/L were often a problem. Van Horn et al. (1996) added that in regions near critical lakes and streams where P in surface runoff is believed to accelerate excessive algae growth, total farm P balance is considered more critical than that for N.

The potential for pollution derived from the increasing animal densities in modern pig farming is leading to rising governmental interventions that tend to control and restrict the growth of the swine business in the U.S. and in many other countries (Jongbloed and Lenis, 1998).

The arguments regarding dietary cost and environmental concerns are both good reasons to search for suitable alternatives to inorganic P supplementation.

Minerals other than phosphorus as potential pollutants

Besides P, dietary minerals such as Cu and Zn raise concerns in regard to environmental pollution. Essential trace minerals for plants, including Cu and Zn, are generally considered to be toxic when accumulated in high concentrations in the soil, and many environmental researchers refer to these two minerals as ‘heavy metals’ (Athar and Ahmad, 2002; Stoyanova and Doncheva, 2002).

According to some modeling scenarios, at the present rate of manure derived Cu and Zn application, agricultural land use may not be sustainable due to accumulation of these elements (Keller et al., 2002). Nevertheless, studies disagree with regard to the polluting potential of specific minerals, such as Cu (Martens et al., 1993).

It is noteworthy to mention that the expression 'heavy metal', frequently used to categorize all metals having densities above 5 g/cm^3 can be misleading (e.g., silver is not recognized as a toxic 'heavy metal' despite its high density). According to Hawkes (1997), however, the 'heavy metal' designation has little to do with density. It is concerned more with the particular chemical properties of these elements. Thus, the use of this term with recognized nutrients like Cu and Zn can be misleading and should be avoided.

Copper, a relevant mineral found in pig feces, is also an essential element for plants. The role of Cu in many physiological processes is well known, especially photosynthesis, respiration, carbohydrate distribution, N reduction and fixation, protein metabolism, reproduction and disease resistance (Bussler, 1981). Nevertheless, according to Alloway (1990), Cu is considered to be one of the most important pollutants of the air and is also a very significant pollutant of agricultural soils. Morphological and physiological alterations in chloroplasts are among the observed toxic effects of excessive Bioavailable soil Cu (Panou-Filotheou et al., 2001).

Zinc, another essential mineral nutrient for plants, plays an important role in several metabolic processes, including activation of enzymes and participation in protein synthesis and carbohydrate, nucleic acid and lipid metabolism (Marschner, 1986; Pahlsson, 1989). When Zn is accumulated in excess in plant tissues, it causes alterations of vital growth processes such as photosynthesis and chlorophyll biosynthesis (Doncheva et al., 2001), affects membrane integrity (De Vos et al., 1991) and has been reported to have negative effects on mineral nutrition (Chaoui et al., 1997).

Copper is commonly added at levels of 10 to 20 ppm in most diets, but can be at 100 to 250 ppm in pig starter diets in order to stimulate feed intake and

growth rate after weaning, and some times is also included at 100 to 150 ppm in grower and finisher diets (Coffey et al., 1994; Cromwell, 1999a; McDowell, 2003). Zinc, as Zn oxide, is sometimes fed at 2,000 to 3,000 ppm to pigs during the first 7 to 10 days after weaning to prevent post weaning diarrhea and to improve feed intake and growth (Cromwell, 1999a). When these minerals are fed at high doses (about 50 to 100 times the requirement), they also exert pharmacological effects, especially in piglets, such as prevention of diarrhea (McDowell, 2003). The Zn and Cu fed at these high levels is almost completely excreted in the feces (Cromwell, 1999a). Because the mobility of Zn and Cu in the soil is extremely low, these minerals may progressively accumulate in areas heavily fertilized with pig manure (Cromwell, 1999a).

It is known that some metals can be transferred and biomagnified in plants grown on contaminated soil. These metals have damaging effects on plants and may represent a health hazard to man and animals. Above certain concentrations, they also adversely affect natural soil microbial populations, leading to disruption of vital ecological processes (Sterritt and Lester, 1980). Stress originated from the accumulation of these metals negatively affects processes associated with biomass production and grain yield in almost all major crops (Agarwal et al., 1999). Athar and Ahmad (2002) illustrate the negative effects of toxic metals, concluding that soils contaminated with them exhibit a marked depletion of non-symbiotic nitrogen fixing bacteria and interference with nitrogen uptake mechanisms in plants, which probably leads to substantial losses in dry matter and grain yield. These researchers studied the effects of high levels of several metals, including Cu and Zn, on growth and grain yield of wheat plants in pots. They added 368 to 1,461 mg Cu/kg soil and 2,559 to 10,235 mg Zn/kg soil. They found that plants treated once with Cu, Zn and other metals added to the soil singly and in combination as chloride salts, exhibited decreased dry matter and grain yield, reduced plant tissue nitrogen content, and lowered protein content in grains. The order of toxicity found was: Cadmium > Copper > Nickel > Zinc > Lead > Chromium. Nevertheless, it has to be noted that metals are not normally present in animal manure as chloride salts. Thus, their solubility in the

soil and potential for absorption could be different than that in the experiment by Athar and Ahmad (2002).

On the other hand, a series of short-term field studies by Martens et al. (1993) at the Virginia Polytechnic Institute and State University evaluating the response of corn to the application of large amounts of Cu, did not find decreased corn yields nor increased Cu concentration in grains after 15 years of continuous manure application. Those studies were conducted on three soils having wide differences in texture and cation exchange capacity. The 15 annual applications of manure from pigs fed high levels of Cu supplied from 380 to 390 kg Cu per hectare (ha). The total amount of copper-enriched wet manure added was 1300 metric ton/ha over the 15 years, obtained from pigs fed an average of 260 mg Cu/kg of feed. The manure contained 1320 mg Cu/kg on a dry weight basis. Soil from these field studies was later used in greenhouse experiments to evaluate soybean and wheat response to high levels of Cu application. The researchers did not find negative effects of these high levels of Cu on the growth of the plants. One more study by the same group evaluated the application of excessive amounts of Cu and/or Zn (540 kg Cu/ha and 1180 kg Zn/ha) as sulfates, over a period of 26 years. Corn yield was relatively high during the last season (26^{th} year) and was not affected by the mineral additions. Also, the Cu concentration in grain was not increased by the Cu treatment. No toxicity was reported from the Cu or Zn additions.

Targeting phosphorus excretion

Different approaches have been proposed to diminish P excretion by swine. In general, those approaches are intended to increase P digestibility and utilization by the pig. It is known that digestibility of nutrients varies according to different factors, including level of the nutrient fed, presence of other constituents in the diet, age of the animal and genetics of the pigs used (Kidder and Manners 1978). In regards to the level of the nutrient fed, it is important not to over-feed P, in order to limit its excretion. High levels of P in the diet should be avoided, allowing only a moderate amount of excess for nutritional safety purposes

(Cromwell, 1999a). To reduce P excretion, diets should also be formulated on an available P basis, instead of on a total P basis, using highly available sources of the mineral (Cromwell and Coffey, 1994; Cromwell, 1999b). The moderate amount of overage mentioned before, intended for proper bone development, should only be about 0.05% in excess of the available P requirement. The excess should not be more than 0.10% (Cromwell and Lindemann, 2002).

When moderately low P diets (0.1% below the requirement) are offered during the last two weeks before slaughtering of terminal-cross pigs destined to market, a reduction in the dietary Ca level is also recommended. This is to avoid depressed performance, because a high Ca:P ratio negatively affects growth even in diets moderately deficient in P (Cromwell et al., 1995b). In regard to age, it is known that the ability of pigs to digest phytic P increases as the animals grow (Calvert et al., 1978). This should be considered when deciding the feedstuffs to be used during the different stages of growth.

Exogenous phytases

Currently, the most effective method for increasing the digestibility of P from plant feedstuffs at the commercial level is the addition of microbial phytases (*myo*-inositol hexaphosphate phosphohydrolases) to the feed (Henry, 1996; Sheppy, 2000; Cline and Richert, 2001). Two classes of phytases (3-phytase and 6-phytase) have been identified. The 3-phytase initially removes orthophosphate from the 3-position of phytic acid, whereas 6-phytase catalyzes the removal of orthophosphate from the 6-position of phytic acid. Successive dephosphorylations result in intermediates from inositol mono to tetra phosphates and free *myo*-inositol. The seeds of higher plants typically contain 6-phytases, while 3-phytases are found in microorganisms and filamentous fungi (Gibson and Ullah, 1990).

Although it was demonstrated three decades ago that phytase improved P utilization in chicks (Nelson et al., 1971)**,** microbial phytase was not commercially available until recent years. Microbial phytase research in pigs increased in the late 1980s and early 1990s when enzyme production systems were optimized to

make it an economically sound alternative (Cromwell, 2002). Different microorganisms are currently used to produce phytases: recombinant A*spergillus niger* (Natuphos® G from BASF), non recombinant *Aspergillus niger*, (Allzyme Phytase™, by Alltech Inc), *Peniophora lycii* (Ronozyme® P, by DSM Nutritional Products) and even bacteria such as *Escherichia coli* (EcoPhos™, by Phytex, not yet approved for commercial use).

The product from BASF is a 3-phytase, and it has two pH optima: one at pH 2.5 and the other at pH 5.5. Natuphos® G is produced by recombinant DNA techniques involving the cloning and isolation of a specific complimentary DNA (cDNA) encoding for phytase from *Aspergillus niger* var Van Tieghem. Then, the cDNA is transferred into the production organism, which is *Aspergillus ficuum,* also called *Aspergillus niger* (Kies, 1999; Nys et al., 1999). BASF defines one phytase unit (PU) as the amount of enzyme which liberates 1 micromole inorganic phosphorus per minute from 0.0051 mol/L sodium phytate at 37.0°C and at pH 5.50 (BASF, 2005).

In general, 250 to 750 phytase units/kg (i.e. U/kg) of diet DM can fully replace inorganic P supplements at all stages of pig production, from nursery through growing to finishing, feeding diverse cereals such as corn, sorghum and pearl millet (Underwood and Suttle, 1999).

In a review of 82 experiments using pigs since 1990 to 2002, Johansen and Poulsen (2003) concluded that phytase supplementation to corn-based diets can increase P digestibility to a maximum of 65 to 70%. The experiments they reviewed tested the effects of different addition levels (250, 500, 750, 1000, 1250 and 1500 U/kg diet). Based on their review, they predicted a mean increase in digestible P of 0.65 g/kg of diet when 500 U/kg of diet are added.

Phytase not only improves P digestion and absorption, but also increases its bioavailability to the animal tissues. Cromwell et al. (1993) demonstrated that phytase can increase threefold P bioavailability for growing-finishing pigs, from 15% to 45%, measured at the bone level. In a series of experiments with growing pigs, Xavier (2003) observed a 34 to 63% increase in P availability when normal corn-soybean meal diets were supplemented with 750 U/kg.

It is known that phytate-P digestibility depends not only on the amount of phytase but also on the amount of dietary Ca. It is presumed that increasing the concentration of a multivalent cation such as Ca will increase the formation of insoluble mineral-bound phytin crystals, which may be resistant to hydrolysis by phytase. Described in several species, including swine, this negative effect of cations on phytate hydrolysis is consistent with the model of pH-dependent mineral-phytate interaction. In this model the protonation of weak acid phosphate groups displaces minerals, turning phytase-resistant mineral bonds into phytase-susceptible forms (Maenz, 2000).

Low phytate grains

Biotechnology makes possible yet another strategy to tackle the problem of P excretion. Genetically modified low phytate grains and oil seeds have been developed, tested in pigs, and may be commercially released soon. Growing pigs fed low phytate corn and low phytate soybean meal at the University of Kentucky have shown an increase of about 35% in P digestibility (from 58.2 to 78.6%) and about 32% reduction in P excretion. Simultaneous use of phytase and low phytate feedstuffs has decreased total P excretion in pigs by about 70%, when compared to regular grain diets with no added phytase (Xavier, 2003).

Genetically modified pigs

A different approach to tackle the problem of P excretion was attempted by Golovan et al. (2001). They developed a genetically modified (GM) transgenic pig carrying a phytase-coding gene from *Escherichia coli*, that expresses abundantly in the salivary glands. According to the authors, these pigs produced between 2,000 and 3,000 U/mL of saliva, which would represent the delivery of about 200,000 PU during the consumption of one kg of feed. These first 'phytase-pigs' – both weanling and growing-finishing - showed a true P digestibility close to 100%, compared to 50% for non-transgenic animals. The fecal P excretion was reduced by as much as 75% when compared to non-GM

pigs. According to Ward (2001), this is a significant step toward improved agricultural productivity and enhanced environmental protection.

Growth Promoting Antibiotics

Taking into account that the antibiotics commonly used as growth promoters may also have a role on mineral excretion, this section focuses on that subject, describing their modes of action and their effects on the digestibility of several nutrients, including minerals such as P. Then, it continues with a specific review of virginiamycin as a growth promoting antibiotic commonly used in the swine industry, and finishes with some comments on the future availability of antibiotics as growth promoters.

Antibiotics can be defined as chemical substances produced or derived from various microorganisms which exert an inhibitory effect, when used in small concentrations, on the growth of other microorganisms (Joklik et al., 1980).

The use of low levels of antibiotics as feed additives has been an effective approach to improve growth promotion, feed utilization, mortality and reproductive efficiency in farm animals. Not long ago, the subtherapeutic usage of antibiotics accounted for about 88% of the total amount of antibiotics used in animals (Hays, 1969; Whittemore, 1998; Cromwell, 2001; Gaskins et al., 2002). Aureomycin, which was the first of the tetracyclines discovered (Jukes, 1977), was the first antibiotic found to have growth promoting effects in animals. This happened one year after aureomycin was discovered when Stokstad et al. (1949) reported that chickens fed a fermented “mash” produced with *Streptomyces aureofaciens* showed increased gain compared with the control diet. The mash contained vitamin B_{12}, which was expected to increase the growth rate of the chicks, but the results were much higher than what could be expected from the vitamin alone. The fermentation product was found to contain aureomycin which produced the response in growth.

A variety of antibiotics have been fed to pigs at subtherapeutic levels for the last five decades, showing consistent improvement in growth parameters,

particularly in younger pigs (Cline and Richert, 2001; Gaskins et al., 2002). Cromwell (2001) pointed out that hundreds of experiments, involving thousands of pigs, at universities and other research stations have used subtherapeutic levels of antibiotics, improving average daily gain about 16.4% in weanling pigs, 10.6% in growing pigs and 4.2% during the whole growing-finishing stage. Feed/gain improvements have been about 6.9% in weanling pigs, 4.5% in growing pigs, and 2.2% in growing-finishing pigs. At the farm level, where stress is higher due to greater microbial loads, the improvements obtained in gain and feed efficiency are even more remarkable. Under farm conditions, antibiotics may improve growth in weanling pigs by as much as 25 to 30%, and feed efficiency by 12 to 15% (Cromwell, 2001).

Another review by Baynes and Varley (2001) summarized the typical responses of growing pigs to antibiotics as 3 to 8% improvement in average daily gain and 2 to 4% improvement in the feed conversion ratio. For finishing pigs, the improvement is generally reduced to 0 to 3% in average daily gain, and 0 to 2% in feed conversion ratio. Cromwell (2001) gives similar figures for the whole growing-finishing stage, mentioning that improvements in growth rate and feed efficiency are around 8 to 10%, and 4 to 5%, respectively.

The higher stress at the farm level, due to buildup of microorganisms in the facilities, seems to be responsible for a sub-clinical disease level in the pig. It is very difficult, and sometimes impossible, to duplicate in the university labs those stress conditions which occur in the field (Cunha, 1977).

Modes of action and effects of antibiotics

The mechanism of growth promotion by antibiotics is still speculative (Francois, 1961; Hays, 1969; Corpet, 2000; Mathew and Ebner, 2004). Francois (1961) and Hays (1969) reviewed several possible modes of action that have been postulated to explain the growth promoting effect of feeding low levels of antibiotics. Among those, three mechanisms have received most of the attention: the metabolic effect, the nutrient-sparing effect, and the disease control effect. Besides those, various steps in the nutrition process, such as digestion and

absorption, appear to be affected by antibiotics. These different hypothesis are not incompatible with each other, and may even be complementary (Francois, 1961). Currently it is believed that growth promotion is more likely due to modifications of the gut flora, since antibiotics do not promote the growth of germfree animals (Corpet, 2000).

Antibiotics and metabolism

The 'metabolic effect' hypothesis suggests that antibiotics directly influence the metabolism rate of the animal, affecting energy, nitrogen, nucleic acid, fat, carbohydrate, vitamin and mineral metabolism. There is also evidence that antibiotics have an effect on the metabolism of gastrointestinal flora.

Antibiotic effects on energy metabolism. The effects of antibiotics on energy metabolism have been studied in pigs and other animal species. According to the review by Francois (1961), pigs fed antibiotics have higher carbon retention, consume less oxygen, and have less loss of energy in the form of heat. Catron et al. (1953) reported an increased rate of glucose absorption in pigs fed antibiotics, providing evidence for the improved nutrient utilization resulting from feeding antibiotics. Similar results have been observed in other animal species. In sheep fed chlortetracycline, Tillman and MacVicar (1953) found lower rectal temperature, which would indicate reduced heat loss. In rats, Knoebel and Black (1952) also found that several antibiotics (aureomycin, terramycin and streptomycin) lowered heat production and improved energy utilization. Brody et al. (1954) reported that tetracycline inhibited fatty acid oxidation in the mitochondria of rat liver homogenates.

Based on these and similar results by other researchers, it has been concluded that antibiotics have an "energy sparing effect" that is reflected in a reduced feed consumption index or feed conversion ratio (Francois, 1961). It is also known that this effect is enhanced when energy requirements are increased, for instance when there is a decrease in the environmental temperature (King, 1960).

Antibiotic effects on nitrogen metabolism. It is known that antibiotics not only affect energy metabolism, but also nitrogen metabolism in different ways. Tetracyclines, for instance, inhibit protein synthesis in bacteria cells (Hash et al., 1964; Cocito et al., 1997), while carbadox increases protein synthesis in pig muscle cells (Moser et al., 1980). According to some researchers, the growth responses observed in pigs are associated with improved nitrogen metabolism, including an increase in apparent nitrogen digestibility (3.0%), increased nitrogen retention (5.8%), and reduced nitrogen excretion (10%) in animals fed the antibiotic tylosin (Gaskins et al, 2002).

It has been shown that feeding chlortetracycline affects water and nitrogen excretion in pigs, suggesting that it may affect the metabolic rate (Braude and Johnson, 1953). By means of nitrogen balance trials, several researchers have found improvements in nitrogen retention by feeding antibiotics, particularly when pigs are fed low protein diets (Catron et al., 1952; Burnside et al., 1954; Russo et al., 1954). It is known that the inclusion of antibiotics in the feed of pigs, rats, chickens and turkeys generally permits to lower the protein level of the diet. This has been referred to as the protein sparing effect of antibiotics (Francois, 1961).

More recent experiments have shown that antibiotics inhibit the deaminating and decarboxylating actions of intestinal flora. In an in vitro experiment by Dierick et al. (1986a), ileal contents from donor pigs were incubated with several free amino acids, observing 20 to 30% degradation of the amino acids by the flora. This degradation occurred either by deamination with formation of ammonia, or by decarboxylation with formation of amines. The most important amine found was cadaverine, the decarboxylation product of lysine. Researchers found both processes were severely reduced when antibiotics, including virginiamycin (50 ppm), were present in the ileal contents. They also reported that E. coli was the main producer of amines in the small intestine of pigs. These results were confirmed in vivo (Dierick et al., 1986b). They found that low levels (20 ppm) of virginiamycin and spiramycin greatly reduced deamination and decarboxylation processes in the small and large intestines of pigs. From the analysis of the N and urea contents of the urine they concluded that both

antibiotics enhanced N retention. Their digestibility experiments with cannulated pigs revealed that virginiamycin (50 ppm) increased the apparent ileal digestibility of N (+2.1%), lysine (+1.4%), glycine (+4.8%), valine (+2.2%) and methionine (+3.3%). Their absorption experiments, based on perfusion of an isolated loop of the terminal small intestine, demonstrated that virginiamycin enhanced net absorption of free amino acids by about 9%. These researchers concluded that growth promotion by antibiotics can be explained, in part, by the altered digestion, absorption and retention of N in pigs.

Antibiotic effects on mineral metabolism. Although less studied, it is also known that, at least for some animal species, there is an effect of some antibiotics on mineral metabolism. Hartsook (1956) pointed out that there are species differences with regard to their growth response to antibiotics.

Most of the research on the effects of antibiotics on mineral metabolism has been done with Ca in poultry. Migicovsky et al. (1951) demonstrated that 1-day old chicks supplemented with penicillin for two weeks had increased absorption of ^{45}Ca, measured at the tibia level. Ross and Yacowitz (1954) also found that dietary penicillin significantly increased the bone ash of chicks fed the antibiotic during a three week study. Nevertheless, the same researchers also reported no increase in bone ash for chicks fed diets with no or very low levels of vitamin D. Lindblad et al. (1954) found that the positive growth effect of aureomycin in chicks and poults is greater when the diet is more inadequate (deficient) in Ca and P. Brown (1957) also found that penicillin increased Ca retention between 4.5 and 7.7% in chicks fed low Ca rations. The same researcher suggested that the increased retention was probably the result of increased absorption, instead of increased utilization, supported by the fact that parenterally administered penicillin did not have any effect on calcium metabolism in pullets. On the other hand, Pepper et al. (1952) found reduced bone ash in aureomycin-fed chicks. Nevertheless, they also reported that this antibiotic reduced the incidence of perosis in Mn-deficient diets, suggesting that aureomycin may have a Mn-sparing effect. In research on laying hens, Gabuten

and Schaffner (1954) found better shell strength in the eggs of pullets fed penicillin during a three week study. They also found higher Ca levels in the blood of the antibiotic treated animals. This same antibiotic, as well as tetracycline and bacitracin, increased Ca levels in the blood of male chicks fed a diet containing 2.6% Ca. In Ca-deficient diets for chicks, penicillin also increased plasma Ca, but did not have any effect on plasma P (Bogdonoff and Shaffner, 1954).

Balance experiments in rats have given mixed results with regard to the effect of specific antibiotics on Ca absorption and retention. Researching in rats, Hartsook (1956) did not find any effect of aureomycin on Ca retention after supplementing the animals with the antibiotic for 35 days. Heggeness (1959) reported that neomycin improved Ca and Mg absorption in rats, although the increased Ca absorption did not persist for extended periods of time. This investigator reported that the antibiotic effect on Ca was only observed for the first period (7 days) of several successive balance trials. There was no effect for the following two to three week-long balance periods and there was no significant increase in the femur ash of the rats fed neomycin.

Antibiotics and the gastrointestinal flora

The gastrointestinal tract of the pig harbors a numerically dense and metabolically active microbiota comprised mostly by bacteria (Gaskins, 2001). It is known that antibiotic growth promoters exert no benefits on the performance of germ-free animals, which suggests that their effects on growth are due to their antimicrobial function rather than being caused by direct interaction with the physiology of the animal (Muramatsu et al., 1994).

An important mode of action of antibiotics in nonruminant animals is to reduce the numbers of potentially harmful microorganisms in the digestive tract, which not only reduces the amount of disease, but also reduces the amount of toxins, ammonia and amines normally produced in the gut (Buttery, 1993).

The nutrient sparing effect theory, in which antibiotics reduce the dietary requirement for certain nutrients by modifying the intestinal flora, has also

considerable research support. This mode of action implies that antibiotics stimulate the growth of desirable organisms (e.g., coliforms) that synthesize essential nutrients for the host, while depressing the populations of bacteria that compete with the host for nutrients (e.g., lactobacilli). This nutrient sparing effect also suggests that those changes in the intestinal flora lead to increased availability of nutrients via chelation mechanisms, and improved absorptive capacity of the intestine (Hays, 1969).

It appears that feeding low levels of antibiotics mostly affects the flora in the small intestine, having little or no effect on the flora of the rest of the digestive tract (Sieburth et al., 1954). Nevertheless, determining precisely how the flora is affected by antibiotics is not an easy task. Gaskins (2002) indicated that traditional methods for quantifying and typifying intestinal bacteria may not be reliable because the selective media used for different types of bacteria impose a bias on the types of bacteria that can be enumerated. Further, only 20 to 40% of bacterial species from the mammalian gastro-intestinal tract can be cultured and identified using current cultivation techniques. In other words, we may be overlooking 60 to 80% of intestinal bacterial species.

Although probably not very accurately typified, it is generally accepted that the small intestinal microflora consists predominantly of gram-positive bacteria (Stewart, 1997). It is also known that most growth promoting antibiotics target gram-positive organisms. This explains the fact that, in several species, including the pig, antibiotics decrease the number of lactobacilli, which favors the growth of coliform bacteria, and sometimes also increase the number of *Shigella, Proteus* and staphylococci (Rhodes et al., 1954; Scaletti et al., 1955).

As the small intestine is the principal site of nutrient and energy absorption, bacterial activity in this region is likely to have the greatest influence on growth efficiency. The proximal small intestine has a high rate of digesta flow. Because of this, the rate of bacterial washout exceeds the maximal growth rate of most bacterial species, which explains why this area is usually colonized by bacteria that adhere to the mucus layer or to the epithelial cell surface. As lactobacilli and streptococci are acid tolerant, they predominate in this area. On

the other hand, the ileum has a more diverse microflora and higher bacterial numbers than the upper intestine (Gaskins, 2002).

Small intestinal bacteria compete with the host for energy and amino acids. As much as 6% of the net energy in pig diets can be lost due to bacterial utilization of glucose in this organ (Vervaeke et al., 1979), producing lactic acid, which also enhances peristalsis, thus increasing the passage rate of nutrients through the intestine (Saunders and Sillery, 1982). Bacteria also degrade amino acids, which not only decreases their availability to the pig, but also generates toxic metabolites such as amines, ammonia, phenols and indoles (Macfarlane and Macfarlane, 1995).

Intestinal bacteria also have mucolytic activities that compromise the mucosal barrier. This mucolytic activity indirectly affects growth efficiency via stimulation of additional mucus production, which requires energy, negatively affecting animal growth (Gaskins, 2002).

It is interesting to note that lactobacillus and enterococcus, which apparently have a negative effect on growth, are also used as probiotic organisms for enhancing health and promoting growth in livestock. But the growth-promoting effect of probiotics is less consistent than that observed with antibiotic supplementation (Johnsson and Conway, 1992). According to Gaskins (2002), probiotics apparently promote growth under situations in which certain pathogens are present. However, the same organisms used on animals growing in a cleaner facility may suppress growth via the mechanisms mentioned.

The study by Vervaeke et al. (1979) found increased pH in incubated ileal contents (5.5 to 7.13) four hours after having added 50 ppm of virginiamycin. That change in pH was correlated with the observed reduction in volatile fatty acids (VFA) and lactic acid production, which was probably caused by the observed decrease in lactobacilli counts. When comparing the amount of energy lost from the fermentation flask during the period previous to adding the antibiotics with the post-antibiotic period, the authors found that virginiamycin addition produced a saving in dietary energy equivalent to 2.68% of the net energy for growth. They concluded that this saving was the result of an 80%

inhibition in lactic acid production and 47% reduction in CO_2 production, which resulted from a decrease in carbohydrate fermentation.

Besides the effect on the bacterial populations in the intestine, antibiotics in general may also affect the metabolism of microorganisms. It has been reported that antibiotics decrease the energy and nitrogen metabolism of bacteria. Some researchers have suggested that the change in the carbohydrate metabolism observed in the intestinal bacteria of growing pigs fed antibiotics was nutritionally more important for the animal than the observed change in bacterial numbers (Visek, 1978; Vervaeke et al., 1979). Intestinal bacteria partially deaminate and decarboxylate dietary amino acids (e.g., arginine, cystine and methionine), rendering them unavailable for the host animal (Carrol et al., 1953). It is known that growth promoting antibiotics inhibit in vitro and in vivo amino acid deamination and decarboxylation, and decrease the fermentation of carbohydrates and the decomposition of bile salts. This produces a double positive effect for the host by increasing the available energy and nutrients for absorption (sparing effect), and at the same time diminishing the formation of toxic molecules like ammonia or amines in the gut, leading to a reduced turnover in the gut epithelium (Francois, 1961; Corpet, 2000).

Antibiotics may also affect, in different ways, the rate of passage of digesta through the gastrointestinal tract. Penicillin and aureomycin were found to speed up digestive transit in chickens and turkeys, probably by stimulating intestinal peristalsis (Hillermann et al., 1953; Jukes et al., 1956). On the other hand, the antibiotic virginiamycin seems to produce the contrary effect, by decreasing lactic acid producing bacteria in the gut (Hedde et al., 1981).

Besides the effects mentioned, a relatively recent study showed that feeding an antibiotic ‘cocktail’ containing chlortetracycline, sulfamethazine and penicillin (22.7, 22.7, and 11.4 ppm, respectively) to weaning pigs for five weeks increased the serum levels of insulin-like growth factor I (IGF-I), which is a potent mitogen for myogenic cells. It was suggested that assumed changes in the gastrointestinal flora (not specified in that study) would be responsible for the increased production of this positive growth factor (Hathaway et al., 1996).

In regard to the environmental conditions where experiments take place, it is important to mention that the effect of antibiotics on growth depends on the bacterial load in the environment. In general, the more 'infected' or 'dirty' the environment, the bigger the effect of antibiotics on growth (Lillie et al., 1953).

Antibiotics and carcass composition

With regard to the possible effects of antibiotics on meat quality and carcass composition of animals, it appears that those characteristics are not affected by these substances. According to Francois (1961), at equal weights the body composition of control and antibiotic treated animals was found to be identical. A later review by Buttery (1993) also concluded that carcass characteristics such as back-fat thickness and dressing percentage were not affected by pigs fed tylosin, olaquindox, salinomycin or virginiamycin at growth promoting levels.

The antibiotic virginiamycin

Virginiamycin is produced by *Streptomyces virginiae.* It is used both in topical preparations for human and veterinary medicine and as a growth promoter in animal feed. It was first approved for use in feed for food-producing animals in the U.S. in 1975, and it is currently approved for use in chickens, turkeys, swine, and cattle (Claycamp and Hooberman, 2004).

Description and mode of action of virginiamycin

Virginiamycin belongs to the class of the streptogramins. This antibiotic is insoluble in water and is poorly absorbed because of its large molecular weight (Vervaeke et al., 1979).

Streptogramin antibiotics have a narrow spectrum of activity which includes mostly gram-positive bacteria (mainly staphylococci, streptococci, and enterococci) and some gram-negative cocci. Most gram-negative bacteria are naturally resistant to virginiamycin due to the impermeability of their cell wall (Cocito et al., 1997; Butaye et al., 2003).

Streptogramins always consist of two components which work synergistically. Because of this, streptogramins are included in a class called synergistines. The two components of virginiamycin are named factors M and S. Factor M is a polyunsaturated cyclic peptolide ($C_{28}H_{35}N_3O_7$. Molecular weight: 525), while factor S is a cyclic hexadepsipeptide ($C_{43}H_{19}N_7O_{10}$. Molecular weight: 823). Both factors M and S pass through the cell membrane of gram-positive bacteria (gram-negative bacteria are generally impermeable to factor M). Once in the cytoplasm, one molecule of M and one of S bind to the bacterial 23S rRNA of the 50S ribosomal subunit to form a stable virginiamycin M-ribosome-virginiamycin S complex, which interferes with peptidyltransferase activity irreversibly inhibiting protein synthesis, resulting in bacterial cell death (Parfait and Cocito, 1980; Cocito et al., 1997).

Despite the different structure of both factors (Figure 2.3), they act synergistically to provide greatly enhanced levels of antibacterial activity. It is known that individually the M and S components have a bacteriostatic effect while a mixture of the two components is usually bactericidal (Cocito, 1979). According to Claycamp and Hooberman (2004), factor M comprises about 75% of Stafac® (the commercial product), while factor S comprises about 5% of the product.

Virginiamycin regulation by the FDA

The FDA permits the use of virginiamycin at therapeutic levels in the feed for the treatment of swine dysentery (100 g/ton for 14 days followed by 50 g/ton up to 120-pound pigs and 100 g/ton for 14 days for non-breeding stock larger than 120 pounds). For stimulating growth and feed efficiency, it is permitted to be continuously fed from weaning to 120 lb at 10 ppm, or at 5 to 10 ppm from weaning to market weight (Food and Drug Administration's Center for Veterinary Medicine, 2004). This antibiotic requires no withdrawal period as it does not leave residues in edible animal products, because it is not absorbed from the alimentary tract (NRC, 1999).

Effects of virginiamycin on growth

The first study published in the Journal of Animal Science that demonstrated the growth promoting effects of virginiamycin on pigs was done by University of Kentucky researchers. The study, published as an abstract, consisted of four growth trials involving 195 weanling pigs fed a corn-soybean meal diet supplemented with either aureomycin or virginiamycin at 10, 20, 40 and 80 g/ton (Barnhart et al., 1960).

One year later, a team of researchers at the Tennessee Agricultural Experimental Station published an abstract in the same journal comparing virginiamycin with aureomycin and other growth promoting antibiotics, such as tylosin and bacitracin, as well as the antimicrobial thiofuradene. This trial found a significant difference in growth of pigs fed virginiamycin between 44 and 100 lb body weight. No difference was found from 44 to 200 lb (Griffin et al., 1961).

The first full manuscript in the Journal of Animal science evaluating virginiamycin as a growth promoter for weanling pigs was published in 1963. In that study researchers compared the effect of several levels of the antibiotic and/or lysine singly or in combination in a corn-soybean meal diet. The study, consisting of four growth trials, found that added at 44 ppm, virginiamycin significantly improved the average daily gain of the pigs (Jones and Pond, 1963).

In a study by Miller et al. (1972), virginiamycin improved ADG in both sexes of Hampshire, Yorkshire and crossbred weaned pigs fed a corn-soybean meal diet. After eleven weeks on trial, researchers found a similar response at 11 mg virginiamycin/kg of diet as at 44 mg/kg.

A later analysis of four studies conducted in different geographical locations in the U.S. by Miller and Landis (1973) showed that either 10 or 40 g of virginiamycin/ton of diet significantly improved weight gain and feed efficiency in young growing swine. The combined analysis of these studies when the pigs reached slaughter weight showed a statistically significant improvement over controls in weight gain and feed conversion at both 10 and 40 g/ton levels. In this analysis the response to 40 g/ton was significantly better than to 10 g/ton. These

researchers also stated that because virginiamycin was not used in human medicine, it should be a safe drug that did not pose any danger to humans.

Cromwell et al. (1976) found that virginiamycin, added at 40 g/ton to growing-finishing pigs resulted in better gains (784 vs. 734 g/d) and feed/gain (2.86 vs. 3.00) than control pigs. Results were better at the end of the first 6 weeks, and at the end of the first 11 weeks of the trial.

Evaluating the rate of inclusion in the diet, researchers at the University of Kentucky (Hays et al., 1973) conducted three experiments to test the effects of virginiamycin on growth performance of pigs. In their first trial they found that daily gain and feed/gain from 19 to 57 kg body weight were significantly improved by increasing levels of the antibiotic (0, 22 or 88 mg of virginiamycin/kg of diet). The response for rate of gain was maintained to market weight, although there was no difference in feed/gain at slaughter weight. The second trial tested the antibiotic at a level of 88 mg/kg of diet from 19 to 95 kg of body weight, finding not a significant but only a numerical difference in gain favoring virginiamycin. The feed/gain was significantly reduced by the antibiotic.

Krider et al. (1975) conducted a large study involving 288 weanling crossbred pigs to test different levels of virginiamycin (0, 5.5 and 11 ppm) fed in different combinations during two growth phases: Phase I (from 11.4 to 54.6 kg), and Phase II (from 54.6 to 91 kg). During Phase I, levels of 0 or 11 ppm were compared, resulting in better daily gain for the pigs fed virginiamycin (0.65 vs. 0.70 kg, respectively), and also better feed/gain (2.56 vs. 2.39, respectively). During Phase II, ADG was 0.75, 0.77 and 0.78, for 0, 5.5 and 11ppm virginiamycin, respectively. The feed/gain ratios in this second stage were 3.51, 3.52 and 3.46, respectively. Given that during the second phase the three levels of virginiamycin were fed to both groups in Phase I (0 and 11 ppm), some pigs consumed the antibiotic during both phases, while other pigs were fed the drug during Phase II only. It was not clear if the pigs that consumed virginiamycin only during Phase II performed differently from the ones that consumed the antibiotic during both phases.

It is worth noting that the positive effect of virginiamycin on growth is lost if the antibiotic is withdrawn from the diet. This was demonstrated in two experiments by Pelura III et al. (1980) who found improved gains by supplementing virginiamycin to a starter diet fed for 42 days. When virginiamycin feeding was stopped at day 42, and pigs continued without antibiotic for the next 35 days, it resulted in pig performance similar to that of the control pigs fed no antibiotic. In one of those experiments the main effect of virginiamycin was significant for feed/gain both at 42 and 77 days.

Veum et al. (1980), reported a significant positive effect of virginiamycin in the ADG of pigs fed moderately low protein diets, particularly during the starting phase. Other researchers found improved gain for protein-deficient pigs treated with virginiamycin during all the period from weaning to finishing (Kennedy et al., 1980).

Stahly et al. (1980) reported that the growth-promoting effects of virginiamycin and high levels of dietary Cu are additive in nature. These researchers investigated the effects of 0 or 250 ppm dietary additions of Cu (as copper sulfate) with and without 27.5 ppm virginiamycin on growth traits of weaned pigs for four weeks. They found that virginiamycin alone improved the daily gain 17%, and feed/gain 8.2% as compared to the unsupplemented diets. The inclusion of both Cu and virginiamycin further improved daily gains between 9 and 10%, and feed/gain between 1 and 4% as compared to single additions of either of the two antimicrobial agents. On the other hand, a study by Riveiro et al (1981) did not find additivity between the effects of 27 ppm virginiamycin and 250 ppm Cu (as copper sulfate) when fed to growing-finishing swine.

Moser et al (1985) reported that the addition of 11 mg of virginiamycin/kg of diet was not effective at overcoming the decrease in performance of growing-finishing pigs caused by crowded conditions. In their studies they allowed 0.37, 0.33 or 0.28 m^2/pig during the growing phase, and 0.74, 0.66, or 0.56 m^2/pig during the finishing phase. This was done by varying the pen size and keeping the feeder space per pig constant. Daily gain and feed conversion deteriorated

during both stages of growth as floor space allowance was decreased. The addition of virginiamycin to the diet had no effect on pig performance.

Effects of virginiamycin on DM, N, and mineral nutrition

According to the calculations by Vervaeke et al. (1979), using in vivo and in vitro data, virginiamycin increases the net energy for the small intestinal digestion by 2.68%, which could explain the commonly observed growth promoting effects of this antibiotic in pigs.

Buresh et al. (1985) reported that virginiamycin not only increased gain and feed efficiency but also improved P utilization in one day old chicks fed a corn-soybean meal diet supplemented with different levels of dicalcium phosphate. In their 3 week-long trial, four diets with graded levels of total P (0.40, 0.47, 0.54, and 0.61%) without and with virginiamycin (22 ppm) were compared for their response in terms of bone ash and bone P. The greatest response from virginiamycin in both characteristics was obtained for the diet containing 0.47% total phosphorus. At this P level, the virginiamycin-added diet resulted in a 4.6% increase ($P < 0.05$) in tibia ash, and a 3.3% increase in tibia P compared to the same diet without virginiamycin.

It is known that high fiber diets decrease mineral absorption. This effect could have several causes, including an increased rate of passage, a greater volume of digesta (which reduces mineral concentration at the mucosa level), an increase in the intestinal secretion of minerals, and reduced availability of the minerals present in fiber (Jongbloed, 1987). Ravindran et al. (1984) studied the effects of two levels of dietary fiber (13.5 and 20.2%) and virginiamycin (11 ppm) on mineral absorption and retention in growing pigs (35 kg BW). In the high fiber diet, virginiamycin improved DM, energy and fiber digestibility. It also decreased fecal N excretion and improved the absorption and retention of P, Ca, Mg, Cu, Fe, Zn and Mn when added to the high fiber diet, but had little or no effect when added to the low fiber diet.

Effects of virginiamycin on carcass composition

According to some studies, virginiamycin may have an effect on some carcass traits. Kennedy et al. (1981) reported that purebred Yorkshire barrows fed 10 ppm virginiamycin for 84 days exhibited an increase ($P < 0.05$) in hot carcass percent muscle (4.4%) compared with the non-antibiotic fed controls. Virginiamycin-fed pigs in the study also had less back fat than the control pigs (13.6%; $P < 0.05$). Other researchers (Veum et al., 1980) fed growing-finishing pigs (28 to 105 kg) virginiamycin (27.5 mg/kg of diet) and two levels of dietary protein (14 or 16%). They found that virginiamycin increased ($P < 0.05$) the longissimus muscle area of the pigs in the 14% protein diet, but decreased it ($P < 0.05$) in the 16% protein diet.

Effects of virginiamycin on the gastrointestinal flora

Studies using pigs have shown that virginiamycin effectively reduces the populations of organisms that compete with the pig for nutrients (e.g., streptococci), although the growth stimulating effect on coliforms, which are regarded as desirable organisms (Hays, 1969), is not always found.

Hays et al. (1973) conducted an experiment in which growing pigs were fed 88 mg of virginiamycin/kg of diet to study the effects on fecal flora patterns. These researchers found reduced streptococcal counts (amounts not reported) during the time the pigs were fed the antibiotic. The counts returned to normal after withdrawal of the drug. No other bacterial types were affected by virginiamycin. In agreement with those findings, in their in vitro evaluation of virginiamycin influences on the metabolism of ileal bacteria, Vervaeke et al. (1979) demonstrated that this antibiotic reduced lactic acid bacteria numbers, particularly streptococci, without influencing coliforms. After two hours of anaerobic in vitro incubation of ileal contents from growing pigs fed no antibiotics, the researchers found increased populations of coliforms, streptococci and lactobacilli (7.10 to 7.80, 6.95 to 7.90, and 7.85 to 8.10 log/g, respectively) which corresponded with a drastic drop in the pH of the cultured media (7.74 to 6.37)

caused by the intense metabolic activity of these bacteria. These cultures with 50 ppm virginiamycin added and incubated for the same length of time, showed no significant change in coliforms when compared with the non-treated media (7.80 vs. 7.75 log/g, respectively), but a decrease was observed in streptococci (7.90 vs. 6.03 log/g, respectively) and lactobacilli (8.10 vs. 7.38 log/g, respectively), and an increase in pH (6.37 to 7.23) probably due to the negative effect of virginiamycin on the acid-producing bacterial populations.

On the other hand, Cromwell et al. (1976) did find a significant increase in the log counts of fecal coliforms (6.74 vs. 6.06, $P < 0.01$) for growing pigs fed 40 g/ton of virginiamycin. Nevertheless, it should be noted that their results were not based on ileal contents. There is now evidence that the bacterial populations in pigs vary across the gastrointestinal tract (Simpson et al., 1999), so that fecal samples do not necessarily reflect other parts of the tract (Zoetental et al., 2004).

Effects of virginiamycin on rate of passage

It is known that lactic acid exerts a stimulatory effect on intestinal motility (Yokokura, 1977). Lactic acid and other short chain acids raise the osmolality, delay fluid reabsorption and decrease transit time (increase passage rate) of the contents of the colon. As lactic acid has been found to comprise about 80% of the total fermentation products at the ileum level (Hedde et al., 1981), it would not be surprising if virginiamycin could decrease the rate of feed passage through the alimentary canal, considering that it decreases the populations of lactic acid producing bacteria. In the study by Hedde et al. (1981), growing-finishing pigs (30 to 120 kg) fed 10 ppm virginiamycin took longer ($P < 0.05$) to excrete feces marked with chromic oxide during both the growing and finishing stages. During the grower stage, the mean 50% excretion times (time at which 50% of the indicator was excreted) for virginiamycin and control were 29.6 and 28.0 hr, respectively. During the finishing stage, the means were 34.0 and 33.2 hr for virginiamycin and control, respectively. This is equivalent to a decrease in passage rate of 5.7 and 2.4%, respectively.

In the experiments by Ravindran et al. (1984) on the effects of virginiamycin (11 ppm) on two levels of dietary fiber (13.5 and 20.2%) in growing pigs (35 kg BW), they also measured the rate of passage by observing the time required for a change in feces color after the addition of 0.5% Cr_2O_3 to the diet. The difference found was even greater than that reported by Hedde et al. (1981). Virginiamycin supplementation slowed the rate of passage at both levels of fiber from 20.6 to 26.7 hr, equivalent to a 29.6% decrease.

Growth-promoting antibiotics and human health

The future availability of antibiotics for growth promoting purposes is under question (Acar et al., 2000). Sectors of public opinion have been advocating the phasing out or even banning the use of antibiotics as growth promoters (Jukes, 1977; Lyons, 1988), fearing that bacterial resistance developed to an antimicrobial used for growth promotion could result in cross-resistance to other antimicrobials of the same chemical group that are used in human medicine. Succumbing to this pressure, Sweden banned all food animal growth-promoting antibiotics in 1986. Following Sweden, the European Union banned avoparcin in 1997 and then virginiamycin, tylosin, bacitracin, and spiramycin in 1999 (Acar et al., 2000; Casewell et al., 2003). Currently, the American Medical Association and several other health, consumer, environmental, agricultural, and humane organizations are promoting the “Preservation of Antibiotics for Medical Treatment Act” (S. 1460/H.R. 2932) that, if approved, would phase out the practice of feeding growth-promoting antibiotics to farm animals in the U.S. (Union of Concerned Scientists, 2000).

It is not clear if feed grade antibiotics are an important factor contributing to the increased resistance to therapeutic antibiotics observed in certain human pathogenic bacteria. Major reports issued on antibiotic drug use in food production over the last 35 years have been inconclusive. For one or another reason, the question of the health consequences of antibiotic use in food animal production is still not answered with certainty (NRC, 1999).

These concerns are not new. They started long ago, soon after growth-promoting antibiotics were discovered. Microbial resistance to feed grade antibiotics was observed in farm animals as early as 1951 (Starr and Reynolds, 1951).

It has been argued that feeding antibiotics at low doses might increase the levels of resistant bacteria in the animal population and subsequently increase the probability of resistant bacteria being consumed by humans. Virginiamycin is at the center of this debate. Dissemination of genes encoding virginiamycin acetyltransferases, enzymes that confer resistance to streptogramins, threatens to limit the medical utility of the related drug Synercid®, a new semi-synthetic streptogramin-derived antibiotic containing quinupristin and dalfopristin (QD). Synercid, approved in 1999, is used as a last resource therapy in the treatment of life-threatening infections caused by glycopeptide-resistant *Enterococcus faecium* and some other bacterial pathogens (Aarestrup et al., 2000; Witte, 2001; Kehoe, 2003; Claycamp and Hooberman, 2004). The structural similarity between the components of virginiamycin and Sinercid relates to similarities in their bactericidal activity and a high degree of cross-resistance between both drugs (Claycamp and Hooberman, 2004). The medical relevance of Synercid is in the fact that enterococci are the second to third most important bacterial genus involved in hospital infections and especially as *E. faecium* possesses a broad spectrum of natural and acquired antibiotic resistance (Klare et al., 2003).

Recent research has found chickens testing positive for QD-resistant *E. faecium*, raising concerns that virginiamycin use in chickens might compromise QD effectiveness against virginiamycin resistant *E. faecium* infections by promoting the development of QD-resistant strains that can be transferred to human patients (Butaye et al., 2000; Cox and Popken, 2004). Pointing to the fact that resistance to Synercid is rare in isolates of staphylococci and *E. faecium* from humans, but is found in isolates recovered from food animals, some researchers think that the use of virginiamycin as a feed additive is responsible for the resistance transmission (Zervos, 2004). This is supported by studies showing a significant decline in resistance to virginiamycin observed among

E. faecium from broilers and broiler meat in Denmark after the banning of antimicrobial growth promoters in 1998 (Aarestrup et al., 2001; Emborg et al., 2003). Some risk assessment models support this idea (Smith et al., 2003).

The concern that on-farm use of virginiamycin could cause the development of resistance to Synercid in humans and the required science-based decision making regarding a possible ban of virginiamycin prompted the Center for Veterinary Medicine to start a virginiamycin risk assessment in 2000. A first draft on this assessment was released for comments in November 2004 (Claycamp and Hooberman, 2004). The assessment concluded that the prevalence of streptogramin-resistant *E. faecium* appears to be related to the usage of virginiamycin on poultry and swine farms, with poultry studies showing a greater extent of resistance than swine studies. Nevertheless, because of the current incomplete knowledge of the genetic basis of streptogramin resistance, the authors did not conclude to what extent, if any, the use of streptogramins in food animals contributes to the occurrence of streptogramin-resistant *E. faecium* infections in humans via a food-borne pathway. They emphasize that the transfer of streptogramin resistance determinants from animal *E. faecium* to human *E. faecium* through the food-borne pathway is biologically plausible, but the extent of such transfer in vivo cannot be currently determined. Their draft provides two scenarios. The first one assumes that 10% of the risk of acquiring resistant streptogramin-resistant *E. faecium* in hospitals is due to a food pathway. In this case, they estimate that the risk to a hospitalized American patient ranges from 6 to 120 chances in 100 million per year, which is equivalent to 0.7 to 14 chances in 100 million per year for a member of the general US population. The second scenario assumes that all existing resistance among the human population originates from food animal uses of virginiamycin. In this case, the risk is 10-fold greater than under the previous scenario, or 60 to 1,200 chances in 100 million persons per year among the hospitalized population and 7 to 140 chances in 100 million persons per year for the general US population. Just to put those risk numbers in a more familiar context, the risk of drowning in a bathtub is 125 in 100 million, according to Ropeik and Gray (2002) using data from the National

Safety Council and from the National Center for Health Statistics.

As a conclusion of recent research (Donabediana et al., 2003; Claycamp and Hooberman, 2004), at the present time there is no agreement among scientists whether the transfer of antibiotic resistance from animal to human bacteria, assuming it exists, is as important as other factors not related to animal production. In the case of virginiamycin, there is no agreement in regard to the potential this antibiotic has, fed as growth promoter to pigs and chicks, to contribute to the development of QD resistant bacteria.

Besides the old struggles over banning the growth promoting antibiotics, or probably as a result of them, another element has recently appeared. Strong market forces have started to demand pork produced without growth promoting antibiotics. This is exemplified by the McDonald's corporation, one of the world's biggest purchasers of meat, which asked its meat suppliers to phase out the use of growth promoting antibiotics. The company announced that its "Global Policy on Antibiotic Use in Food Animals" became effective as of January 1, 2005. The program requires covered suppliers to certify that they are "not using, for growth promoting purposes, antibiotics that belong to classes of compounds approved for use in human medicine" (Gill and Best, 2004; www.mcdonalds.com. 2005).

Conclusions

The development of the modern swine production system has been the direct answer to the demand for high quality protein to satisfy an ever growing human population. To meet the always increasing demand for pork, the swine industry has been constantly developing ways to optimize the use of the costly resources required, particularly feed and construction space.

To optimize the utilization of feed, an understanding of the flow of nutrients through the animal is required. This flow is measured in digestibility and balance trials. These methods allow researchers to determine how much of the ingested nutrient was apparently absorbed, retained, and excreted. Different

methodologies have been used to determine digestibility in feedstuffs. Each method has advantages and disadvantages, and even though the methods themselves have been both used and examined for a long time, there is still no agreement among researchers on sample collection procedures, which raises questions about the reliability of some of the methodological variations still in use. More research is required for establishing practical, but at the same time reliable, collection procedures to be used in digestibility assessments.

Another way to approach the issue of feed cost in local markets is to include alternative feedstuffs in the diets. There are many suitable materials and byproducts all around the world that can be supplemented to pigs in order to lower production costs. Nevertheless, most of the cheapest and more readily available byproducts have disadvantages in terms of nutrient composition or digestibility that limit their level of inclusion in the diet. Among many byproducts rice bran is an interesting option. It is cheap and high in energy, but it is also particularly high in phosphorus, a key biological nutrient that is usually supplemented to pigs in expensive inorganic compounds due to its low digestibility in plant feedstuffs. As P is considered an important pollutant of water ecosystems, its increased excretion by pigs fed rice bran is an undesirable outcome that has not been addressed.

Supplementing diets with phytase is the most practical solution currently available to the problem of low digestibility and consequent excretion of P from plant materials. Phytase supplementation has proven to be an effective way to increase P digestibility in conventional diets, and it could be an interesting solution for rice bran-based diets.

A related subject that also requires understanding is the possible effect of antibiotics used as growth promoters on P utilization. It is recognized that the dietary inclusion of low levels of antibiotics has facilitated the industry's ability to cope with pork demand by improving pig gain and feed utilization under conditions of heavy bacterial loads, common in densely populated farms. Antibiotic effects on growth have been related to increased nutrient utilization,

particularly energy and protein, but effects on mineral nutrition have not been sufficiently addressed in swine. For the aforementioned reasons, the study of the effects of dietary amendments such as antibiotics and phytase on P utilization in common and non-traditional diets for pigs is of interest.

Table 2.1. Nutrient composition of corn and some alternative feedstuffs (adapted from Myer and Brendemuhl, 2001)

		Nutritional value for pigs (as fed basis)								RFV[a] vs.
Feedstuff	Description	DM (%)	ME[b] (kcal/kg)	Protein (%)	Lysine (%)	Fat (%)	Fiber (%)	Ca (%)	P (%)	Corn (%)
Corn	**Grain**	**89**	**3400**	**8.3**	**0.26**	**3.9**	**2.2**	**0.03**	**0.25**	**100**
Rice	Bran, full fat	90	3000	12.5	0.60	12.0	11	0.05	1.70	70-100
	Bran, fat extracted	91	2600	14.0	0.65	1.5	13	0.10	1.40	60-80
	Polishings	90	3300	13.0	0.50	13.0	2	0.10	1.20	95-100
	Broken	89	3300	8.0	0.30	0.6	0.6	0.04	0.18	95-100
	Paddy	89	2800	9.0	0.30	2.0	10	0.05	0.25	70-80
Bananas	Ripe, whole	25	750	1.0	<0.10	0.1	0.5	0.01	0.03	20-25
	Green, whole	26	700	1.0	<0.10	0.1	0.5	0.01	0.03	15-20
Cassava	Meal	89	3300	3.0	0.10	0.5	5	0.12	0.15	95-100
	Fresh	35	1200	1.0	<0.10	0.2	1.5	0.04	0.05	30-40
Sugar cane	Molasses	80	2200	3.0	<0.10	0.1	0	0.70	0.08	60-70
	Juice	18	700	<1.0	<0.10	<0.1	2	0.20	0.05	15-25
	Stalks	25	500	1.0	<0.10	0.5	8	0.10	0.05	10-20
Potatoes	Chips or fries	90	4400	6.0	0.20	30.0	1	0.10	0.20	120-150
	Cooked flakes	92	3500	8.0	0.40	0.5	2	0.10	0.20	100
	Pulp, dried	88	2200	6.0	0.20	0.3	9	0.10	0.20	60-70
	Boiled	22	700	2.4	0.10	0.1	0.5	0.02	0.05	15-25
	Raw	20	500	2.0	0.10	0.1	0.5	0.02	0.05	10-15
Resturant food waste	Non-dried	20	800	5.0	0.20	5.0	1	0.10	0.10	15-25

[a] RFV (relative feed value): nutritional value relative to corn.
[b] Metabolizable energy.

Table 2.2. Digestible energy and protein for pigs in selected feedstuffs (as fed basis) (adapted from Ensminger et al., 1990)

Feedstuff	Description	Digestible Energy (kcal/kg)	Metabolizable Energy (kcal/kg)	Crude Protein (%)	Digestible Protein (%)
Corn	Grain, yellow	3338	3246	9.9	7.3
Rice	Bran, with germs	3250	2971	13	9.5
	Polishings	3713	3428	12	10.1
	Paddy, ground	3290	3107	7.5	5.1
Bananas	Fruit, dehydrated	3535	3355	3.5	1.6
	Peelings, dehydrated	3425	3240	8.6	6
Cassava	Meal, dehydrated	2923	2748	2.2	1.6
	Fresh	1127	1063	1.2	0.5
Sugarcane	Molasses, dehydrated	2663	2485	9.7	7
Potatoes	Tubers, fresh	878	830	2.2	0.7
	Tubers, boiled	918	869	2.2	1.5
	Tubers, dehydrated	3450	3261	8.1	5.6
	Peelings, fresh	882	835	2.1	1.5
Restaurant food waste	Boiled, wet	1105	1053	3.6	2.8
	Boiled, dehydrated, ground	4293	4090	16.1	12.8

Table 2.3. Colombian production of several potential alternative feedstuffs during 2003 (FAO, 2004)

Product	Colombian production (Metric ton/year)	World Rank
Coffee, green	695,000	2
Plantains	2,925,000	2
Avocados	141,638	5
Tropical fruits	1,120,000	5
Palm kernels	125,000	5
Sugar cane	36,600,000	7
Bananas	1,450,000	11
Cocoa beans	47,000	11
Pineapples	353,000	11
Papayas	105,000	12
Roots and tubers	75,000	12
Cassava	1,850,000	18
Potatoes	2,850,000	20

Table 2.4. Energy feed sources and their unavailable phosphorus contents (Adapted from NRC, 1998)

	Phosphorus, %			
Feedstuff	Total	Bioavailable	Non available	Total unavailable
Rice bran	1.61	25	75	1.21
Wheat bran	1.20	29	71	0.85
Wheat middlings, < 9.5%fiber	0.93	41	59	0.55
Corn grits (hominy Feed)	0.43	14	86	0.37
Oat groats	0.41	13	87	0.36
Barley, six row	0.36	30	70	0.25
Oats	0.31	22	78	0.24
Corn	0.28	14	86	0.24
Sorghum	0.29	20	80	0.23
Wheat, soft red winter	0.39	50	50	0.20
Wheat, hard red winter	0.37	50	50	0.19
Whey, dried	0.72	97	30	0.02

Figure 2.1. Colombian imports of yellow corn (adapted from Martinez and Acevedo, 2004)

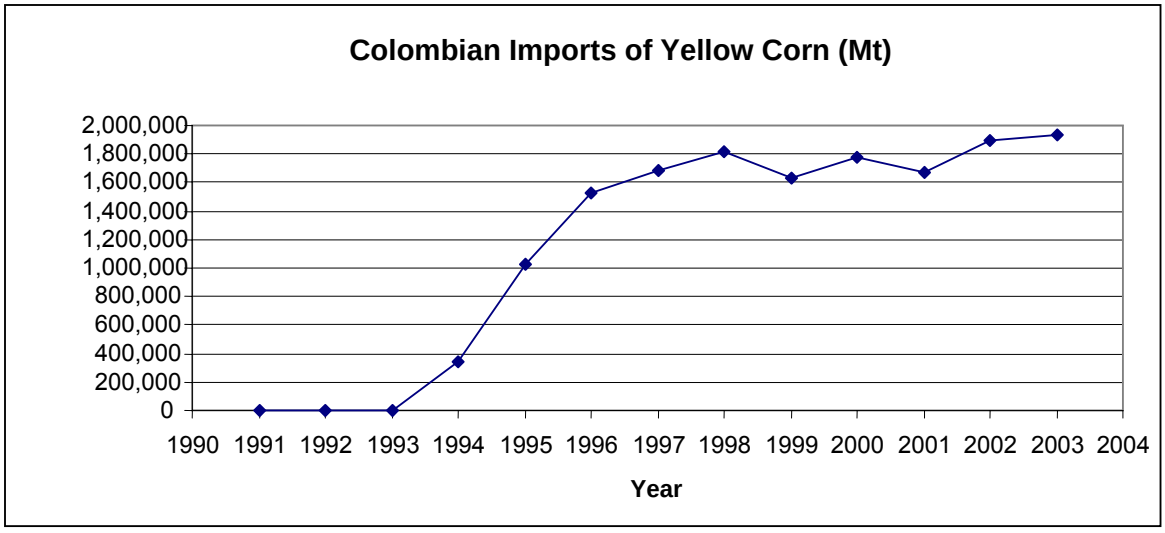


Figure 2.2. Model of phytate complex (phytin) chelating different nutrients (Sutton et al., 2004)

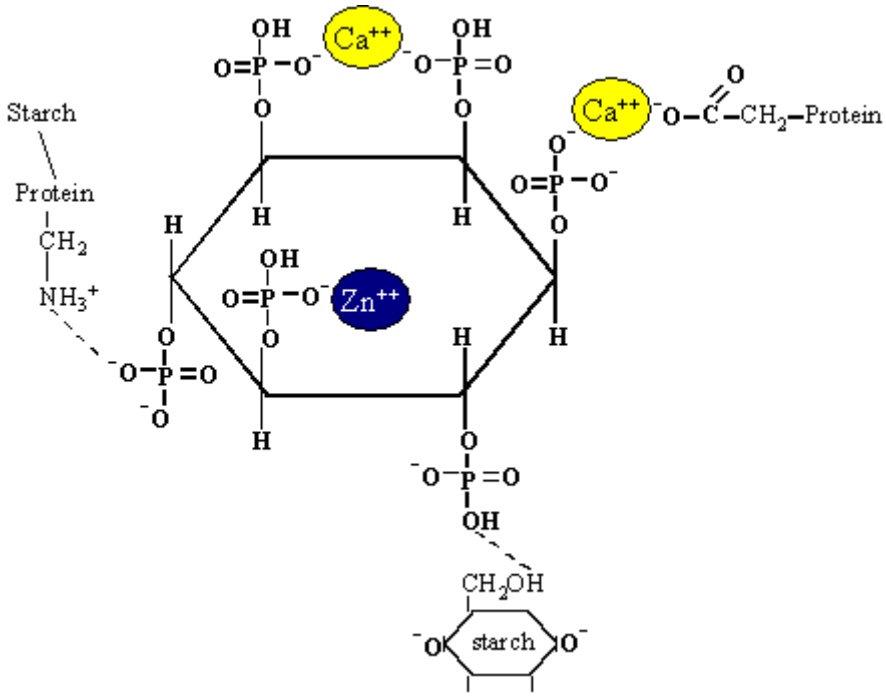
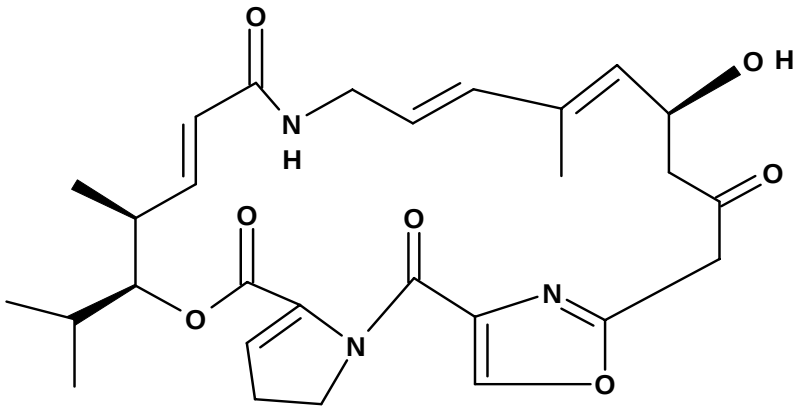
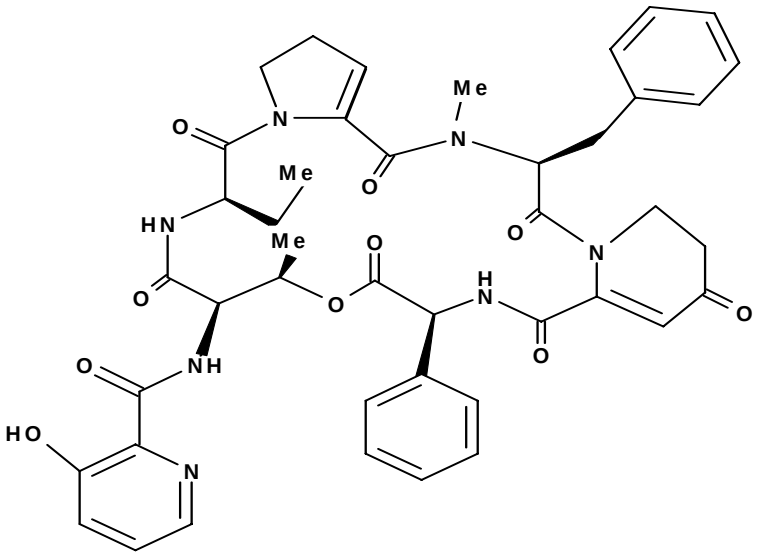


Figure 2.3. Virginiamycin structures M and S (Adapted from Lee et al., 1996)



Virginiamycin M1



Virginiamycin S, Me, Methyl

CHAPTER 3

GENERAL METHODOLOGY

General Objectives

The primary objective of the research presented here was to evaluate the effects of virginiamycin (VIR) and phytase (PHY) amendments on the digestibility, retention, and excretion of nutrients, particularly P, by growing pigs fed a P-deficient corn-soybean meal diet. An additional objective was to make a preliminary nutritional assessment of a high phytate feedstuff with and without phytase supplementation in a balance study.

Specific Objectives

The first experiment (Experiment 1 or UK0201) was conducted to determine possible differences on mineral digestibility, retention, and excretion when VIR was included in a P-deficient diet. A secondary objective was to test the reliability of a digestibility assessment based on a single grab collection of fecal samples analyzed by the index method (chromic oxide) in comparison to the standard total collection method.

Subsequently, three experiments (Experiment 2 or UK0301, Experiment 3 or UK0309, and Experiment 4 or UK0402) were conducted to compare the effects of VIR and PHY on P digestibility, retention, and excretion by pigs fed the same diets. One of these experiments (Experiment 2) was also intended to assess nutrient digestibility by the index method, using a different fecal grab sampling strategy than the one used in the first experiment (Experiment 1).

The effects of VIR on growth performance, bone traits, and ileal microbial populations were assessed in two nursery (short-term growth) experiments (Experiment 7 or UK0210, and Experiment 8 or UK0311).

Based on results from the first experiment (Experiment 1) and on one of the short-term growth experiments (Experiment 7), a full-term growing-finishing experiment (Experiment 5 or UK0312) was conducted to assess the effect of a partial dietary P deletion in VIR-supplemented diets upon growth performance, bone traits, ileal microflora populations, carcass traits, and meat characteristics.

A final balance experiment (Experiment 6 or UK0407) was conducted to assess nutrient digestibility, retention and excretion in a commercial rice bran product using the regression method, to evaluate the effect of different levels of inclusion of the product, and also to assess the effect of PHY on the same traits.

Methods

Methodologies common to all the experiments or to a particular set of them are described next. A list of abbreviations is provided in Table 3.1.

Animals

All the animals used were either nursery or growing-finishing crossbred pigs (Hampsire or Duroc by Yorkshire x Landrace) born and raised in University of Kentucky swine facilities, either in the Coldstream Swine Research Farm at Fayette County or in the Swine Unit of the Animal Research Center (ARC) at Woodford County.

Animals were selected according to several criteria in order to decrease the variation in each experimental group. Only barrows were used in the balance experiments - to facilitate urine and feces separation during collection - and in the nursery experiments – to reduce the problem of urination into the feeders. Each group of pigs used in the experiments was selected from intermediate sized animals taken from larger groups. When possible, litters with at least as many

full-siblings as the number of dietary treatments to be tested were chosen. When the number of full-siblings available was not enough to cover all the treatments, half-siblings by sire were preferred over totally unrelated pigs. Animals with health problems or visible conformation conditions (e.g., leg problems) were not used. The selected pigs were assigned to replicate groups by ancestry, then randomly allotted to the dietary treatments and then to the pens or crates. After having started the experiments, the few animals that presented health or feed intake problems were removed from the experiments. All the experiments were conducted under protocols approved by the University of Kentucky Institutional Animal Care and Use Committee (IACUC).

General management and facilities

All the experiments were conducted in totally confined conditions in temperature-controlled rooms at the Animal Laboratory of the Animal and Food Sciences building (W. P. Garrigus Building) located on the University of Kentucky Campus. During the experiments, the rooms were cleaned daily. Room temperature, water availability, and animal well being were checked at least twice per day.

Common methods for the balance trials

All the balance experiments were conducted under similar environmental and animal handling conditions. Most lab methods were common as well. The common methods are described next. Particularities of each experiment are reported in the corresponding, appropriate, chapter.

Housing conditions for the balance trials

For these experiments, described in Chapters 4 and 6, pigs were individually confined in metabolism crates. Crates were made of stainless steel and had plastic-coated expanded-metal flooring and stainless steel feeders. Crates also had a window in each side panel, near the feeder, to allow visual contact between pigs in adjacent crates. Underneath the floor of the crates a

sliding aluminum screen was set as a feces/urine separator, along with a stainless steel funneled-pan used to direct the urine into a 10 L plastic bucket. The interior space of the crates was adjusted to restrain pigs from turning around, preventing defecation into the feeder but leaving enough room for the pig to stand up and lie down. Room temperature was kept in the thermo-neutral range at all times.

Adaptation and collection procedures for the balance trials

Pigs were fed a basal, low-P diet ad libitum for 5 to 10 days before starting the adaptation periods in each experiment in order to standardize gastrointestinal (GI) tract conditions among the animals. Then, pigs were weighed, allotted to the experimental diets and confined in the crates for 7 days to adapt to the crates and the level of feed offered, which corresponded to 3% of body weight. Movement was restricted during the first 24 h by adjusting the sides and top of the crates, preventing pigs from turning around. As pigs became accustomed to the crates, they were gradually allowed more space. At the end of each adaptation period pigs were weighed again to determine the feed allowance for the collection period.

During the collection periods, pigs were also fed at the equivalent of 3% of their body weight per day. The daily feed allowance was split into two equal meals, fed at 8:00 am and 4:00 pm. At meal times, feed was added with a volume of water equivalent to the feed weight (e.g., 1 L water was added per 1000 g of feed). Rejected feed was dried in a forced-air oven at 55ºC, air-equilibrated, weighed, and discounted from the amount initially offered. Water was supplied ad libitum in the feeder during non-feeding times. Indigo carmine (Aldrich Chemical Company Inc, Milwaukee, WI), a blue dye, was mixed with two meals of the experimental diets at a 0.5% inclusion rate. Indigo-marked meals were given at the beginning and at the end of the collection periods. All the feces produced during the period between excretion of the initial and final marker were collected daily and kept frozen in labeled plastic bags. Care was taken to include

in the collected material all marked feces at the beginning of the collection period, as well as to exclude any marked feces at the end of the period.

Feed intake during the 5-day collection periods was recorded as feed allowance minus feed rejection. Urine collections were simultaneous with feces collections. For each pig, the total amount of urine excreted was measured and individual urine samples were collected. Urine collection started at 9:00 am after pigs were fed the indigo dye, and finished when five 24-h collections were completed. Fiber-glass wool was placed in the stainless steel funneled-pans during urine collections to prevent urine contamination with feed or fecal particles. Urine was collected in 10 L plastic buckets containing 150 mL of 3 N HCl to limit microbial growth and reduce loss of ammonia. Every day, after measuring the total amount excreted, urine was stirred, and a 100 mL urine sample was taken and stored frozen in labeled, capped, plastic containers, while the rest of the collected urine was discarded.

Digestibility and retention calculations

Nutrient digestibility and retention (DM basis) by total collection were calculated using the formulae:

Apparent digestibility, % =

$$\left[\frac{\text{Amount of component consumed} - \text{Amount of component in feces}}{\text{Amount of component consumed}}\right] \times 100$$

Apparent retention per day, g =
Nutrient intake/d – Total nutrient excretion (fecal + urinary)/d

Retention as a percent of intake, % =

$$\left[\frac{\text{Nutrient retained per day}}{\text{Nutrient intake per day}} \right] \times 100$$

Retention as a percent of absorption, % =

$$\left[\frac{\text{Nutrient retained per day}}{\text{Nutrient intake per day} - \text{Nutrient in feces per day}} \right] \times 100$$

For the index method, apparent digestibility was calculated using the formula:

$$\text{Digestibility, \%} = 100 - \left[100 \times \frac{\text{\% Cr in feed}}{\text{\% Cr in feces}} \times \frac{\text{\% Nutrient in feces}}{\text{\% Nutrient in feed}} \right]$$

Dietary ingredients

Conventional yellow corn and soybean meal (dehulled) were used in all the experiments.

Ingredients common to all experiments

Ground corn and soybean meal (SBM) were provided for the experiments by the University of Kentucky Feed Mill. The limestone used was Franklin High Calcium Limestone (Franklin Industrial Minerals, Nashville, TN) containing approximately 38.5% Ca (approximately 97% $CaCO_3$).

The vitamin and mineral mixes were also provided by the University of Kentucky Feed Mill. In regard to the vitamins, 0.075% of the vitamin premix in the diet supplied: 4,950 IU vitamin A, 660 IU vitamin D_3, 33 IU vitamin E, 4.8 mg

vitamin K (as menadione sodium bisulfite complex), 6.6 mg riboflavin, 16.5 mg pantothenic acid, 33.0 mg niacin, 0.99 mg folic acid, 0.165 mg d-biotin, 24.5 µg vitamin B_{12}, and 3.3 mg vitamin B_6 per kilogram of diet (calculated).

For the minerals, 0.075% of the mineral premix in the diet supplied,: 135 mg Fe (iron sulfate monohydrate), 135 mg Zn (zinc oxide), 45 mg Mn (manganous oxide), 13 mg Cu (copper sulfate pentahydrate), 1.5 mg I (calcium iodate), 0.3 mg Se (selenium mix), and 0.23 mg Co (cobalt sulfate monohydrate) per kilogram of diet (calculated).

Ingredients used in particular experiments

For all the balance experiments (Experiments 1, 2, 3, 4, and 6) a blue non-toxic food colorant was added to the feed in order to visually mark the feces at the beginning and end of the collection periods as required for the total collection methodology. The dye was Indigo Carmine (certified F. W. 466.36 (860 to 22-0), Aldrich Chemical Company Inc, Milwaukee, WI).

Experiments 1, 2 and 4 assessed digestibility by both total collection and index methods. The external marker added to the diets for the index method was Chromium Sesquioxide, Cr_2O_3 (Fisher Chemicals, Fair Lawn, NJ).

Virginiamycin was supplemented in one or more diets of all the experiments except for the last balance study (Experiment 6). The source of VIR used was Stafac® 20 (Stafac; Phibro Animal Health Co., Fairfield, NJ), which is guaranteed to contain 20 g VIR/lb of product. Phytase was supplemented in Experiments 2, 3, 4, and 6. The source of PHY was Natuphos® 1200G (Natuphos; BASF Corporation, Mount Olive, NJ) guaranteed to contain at least 1200 PU/g of product. Both products (Stafac and Natuphos) were kept under refrigeration (4 to 8°C) at all times.

In Experiment 6, a commercial product rich in phytate P was used. “Ricex-1000™ Stabilized Rice Bran-1000” (Ricex Company, El Dorado Hills, CA) consisted of a mix of stable whole rice bran and germ. The manufacturer claims it provides energy in the form of vegetable fat (5,500 kcal/kg), soluble and insoluble fiber, and high levels of natural vitamin E, and guarantees one year of shelf life.

Monosodium phosphate was used in the short-term growth experiments (Experiments 7 and 8). The monosodium phosphate (MSP) used was Monobasic Sodium Phosphate, $NaH_2PO_4.H_2O$ (Fisher Chemicals, Fair Lawn, NJ) containing 22.44% P.

Dicalcium phosphate (DICAL) was used in the long-term growth experiment (Experiment 5). The DICAL product used was Dynafos® (The Mosaic Co., Plymouth, MN), calculated to contain 24% Ca, and 18.5% total P (18.5% available P).

Summary of the Experiments

In the series of experiments presented in this dissertation a total of 166 crossbred pigs were used in eight experiments: five balance experiments (Experiments 1, 2, 3, 4, and 6) were conducted using a total of 94 growing pigs, while two short-term growth experiments (Experiments 7 and 8) used 40 nursery pigs, and one full-term growing-finishing experiment (Experiment 5) used 32 pigs. Table 3.2 presents a general description of the experiments.

The first four balance experiments (Experiments 1, 2, 3, and 4) shared similar objectives. They are presented jointly in Chapter 4. The long-term growing-finishing experiment is presented in Chapter 5. Experiment 6 was the only experiment not related to VIR, and is presented separately in Chapter 6. The nursery model tested did not work as expected, according to the objectives, so a summary of both short-term growth experiments is presented separately from the body of the dissertation, as Appendix 1.

Experiment 1 assessed nutrient digestibility and balance by total collection in two collection periods, followed by an assessment of digestibility using an index (Cr_2O_3) method (also two collections). A total of 10 pigs were used in this experiment. Experiments 2 and 4 assessed nutrient digestibility and balance by total collection in two collections. In Experiment 2 each total collection was followed by an index methodology (Cr_2O_3) with a different fecal grab sampling

strategy than the one used in Experiment 1. A total of 24 pigs were used in each of the experiments. Experiment 3 assessed nutrient digestibility and balance by the total collection method using the same group of pigs (12 in total) for the two collections conducted.

Experiment 5 measured growth performance characteristics such as feed intake, weight gain and feed conversion ratio over the entire growing-finishing period (3.8 months). Other characteristics assessed in this experiment were: metacarpal and metatarsal breaking strength, ash, ileal microflora populations, and also carcass and meat traits such as dressing percentage, carcass shrink, back fat depth, meat drip losses, loin eye area, and meat color scores.

Experiment 6 assessed nutrient digestibility and balance by the total collection method using a different group of pigs for each of the two collections conducted. In this experiment, nutrient digestibility was assessed on a phytate-rich diet corresponding to a basal corn-SBM diet with graded levels of the rice bran product (0, 7.5, 15 and 30% Ricex-1000™). The effects of PHY (750 U/kg) in the 0 and 30% Ricex-1000™ amended diets were also tested.

Experiments 7 and 8 measured growth performance (feed intake, weight gain and feed conversion ratio) over six weeks. Bone mineral deposition was also assessed by measuring bone breaking strength (femur, metacarpal and metatarsal) and bone ash (metacarpal and metatarsal).

Statistical Analysis

The General Linear Model (GLM) procedure of SAS was used for the analysis of variance in all the experiments in order to test for differences among treatment means. Designed comparisons between means (planned single-degree-of-freedom F tests) were also performed according to the treatment structure of each experiment. Detailed information on experimental design is given in each chapter.

Table 3.1. List of abbreviations

AA---------- Atomic Absorption Spectrophotometry
ADF---------- Acid detergent fiber
ADL---------- Acid detergent lignin
AOAC ---------- Association of Official Analytical Chemists
App ---------- Appendix
aP ---------- Available phosphorus
aCa ---------- Available calcium
ADFI---------- Average daily feed intake
ADG ---------- Average daily gain
BBS---------- Bone breaking strength
BW(i) ---------- Body weight (initial)
BW(f)---------- Body weight (final)
CFU/g ---------- Colony forming units per gram
CF---------- Crude fiber
CP ---------- Crude protein
CCP ---------- Cumulative collection period
EDTA---------- Ethylenediaminetetraacetic acid
EE---------- Ether extract
FDA---------- Federal Drug Administration
F/G---------- Feed conversion ratio (feed/gain)
DD ---------- Deionized water
DE ---------- Digestible energy
DICAL---------- Dicalcium phosphate
DM ---------- Dry matter
EE---------- Ether extract
Exp ---------- Experiment
GLM ---------- General linear model
LSM---------- Least square means
Lig---------- Lignin

Table 3.1. (Continued)

LEA ---------- Loin eye area
PHY---------- Phytase
PU ---------- Phytase units
NA ---------- Not analyzed
NDF---------- Neutral detergent fiber
NFE---------- Nitrogen-free extract
ND ---------- Not detected
No---------- Number
ME ---------- Metabolizable energy
MC ---------- Metacarpals
MT ---------- Metatarsals
Mt ---------- Metric ton
MSP ---------- Monosodium phosphate
RA ---------- Retention as a percent of absorption
Rep ---------- Replication
RFV---------- Relative feed value
RI---------- Retention as a percent of intake
RMSE ---------- Root mean square error
RX ---------- Ricex-1000™
SAS---------- Statistical Analysis System
SEM ---------- Standard error of the mean
SBM ---------- Soybean meal
tCa ---------- Total calcium
tP---------- Total phosphorus
Trt ---------- Treatment
UK ---------- University of Kentucky
W---------- Weight
VIR---------- Virginiamycin

Table 3.2. General description of the experiments

UK code[a]	Exp. No.[b]	Chapter[c]	Exp. type[d]	Digest. method[e]	Additive tested[f]	Trt. No.[g]	Rep. No.[h]	Pig No.	Sex[i]	Groups[j]	Length days[k]	Initial Wt, kg[l]	Final Wt, kg[m]
0201	1	4	Bal	T/I	VIR	2	10	10	M	1	32	68.1	77.3
0301	2	4	Bal	T/I	VIR/PHY	4	6	24	M	2	43	66.2	73.4
0309	3	4	Bal	T	VIR/PHY	4	6	12	M	1	37	53.3	58.2
0402	4	4	Bal	T	VIR/PHY	4	6	24	M	2	51	54.4	60.6
0312	5	5	Growth (long)	-	VIR	4	4	32	M/F	1	116	29.1	113.2
0407	6	6	Bal	T/R	PHY	6	4	24	M	2	31	87.5	95.5
0210	7	App. 1	Growth (short)	-	VIR	5	4	20	M	1	44	15.8	50.0
0311	8	App. 1	Growth (short)	-	VIR	5	4	20	M	1	42	16.5	47.2

[a] Experiment number assigned by the University of Kentucky.
[b] Experiment number assigned for this dissertation.
[c] Chapter number in the dissertation (App: Appendix).
[d] Experiment type: Bal: Balance experiment; Growth: Growth experiment.
[e] T: digestibility by the total collection method; T/I: digestibility by total collection and index method; T/R: digestibility by total collection using regression.
[f] VIR: Virginiamycin (11ppm); PHY: Phytase (750 or 300 Phytase Units).
[g] Number of dietary treatments.
[h] Number of replications.
[i] Sex: M: castrated male; F: female.
[j] Groups: number of groups of pigs used.
[k] Duration of the experiments for the balance experiments includes adaptation and collection periods. Duration for the growth experiments is counted as the first to the last day of feeding experimental diets.
[l] Average body weight (BW): initial BW for the growth experiments; Ave initial BW of all pigs at starting total collection for the balance experiments.
[m] Average body weight: final BW for the growth experiments; Ave BW of all pigs at finishing total collection for the balance experiments.

CHAPTER 4

PHOSPHORUS UTILIZATION BY GROWING-FINISHING PIGS FED A P-DEFICIENT CORN-SOYBEAN MEAL DIET AMENDED WITH VIRGINIAMYCIN AND/OR PHYTASE – Experiments 1, 2, 3, and 4

Introduction

Farm animals have been fed low doses of antibiotics as growth promoters for half a century. Although the mode of action of antibiotics on growth is not well understood, their positive effect on energy and nitrogen utilization has been observed in several animal species, including swine (Vervaeke et al., 1979; Dierick et al., 1986b). Some studies have addressed the effects of penicillin, aureomycin and virginiamycin on mineral utilization in poultry (Migicovsky et al., 1951; Ross and Yacowitz, 1954; Lindblad et al., 1954; Brown, 1957; Buresh et al., 1985), but this type of research is scarcer in swine (Ravindran et al., 1984). Rising concerns regarding pollution of aquatic ecosystems with phosphorus from animal excreta, along with the possible phasing out of antibiotics used at growth promoting levels, justify studying the effects of commonly used antibiotics such as virginiamycin (VIR) on P utilization by swine.

Positive effects of phytase (PHY) amendments on P and Ca utilization by pigs have been profusely described since Natuphos G®, the first recombinant PHY, was approved for commercial use in the U.S. ten years ago. Nevertheless, studies on P utilization by pigs fed diets amended with both PHY and VIR have not been found in the literature.

Nutrient digestibility has been traditionally assessed by either total collection, which is the accepted standard, or by the index method. Digestibility assessment by the latter method is intended to save labor, considering that it does not require quantitative collection of feces - it only requires measuring the concentration of an indigestible indicator (e.g., Cr_2O_3) in feed and feces.

Digestibility results for several nutrients assayed by this method have been regarded as comparable to results obtained by total collection (Schurch et al., 1952). The index method involves a 'grab sampling' procedure for fecal collection. In order to obtain a representative composited sample of feces from individually-penned pigs, researchers have tried collection periods of different lengths, from as short as one day, up to seven consecutive days (Barnicoat, 1945; Clawson et al., 1955; McCarthy et al., 1976; Aherne et al., 1997; Mougham et al., 1991). Clawson et al. (1955) reported that a 1-day collection period consisting of two grab samples was comparable with a 7-day total collection for several characteristics (DM, CP, and EE). As digestibility results are affected by the variation in nutrient concentration in the sample, and as variation is expected to be higher for shorter collection periods, it is possible that a 1-day period may only be valid for those nutrients that are present in high concentrations. No research was found on how samples composited from cumulative periods of different length compare with the total collection method. From a practical point of view, it would be useful to determine how reliable a cumulative collection strategy is in comparison to the total collection method, and which particular nutrient digestibility results, if any, could be trusted.

Objectives

The primary objective of these experiments was to evaluate VIR and PHY amendment effects on digestibility, retention and excretion of nutrients, particularly P, by growing pigs fed a P-deficient diet. This was accomplished in four balance experiments (Experiments 1, 2, 3, and 4) by means of the total collection method. For this purpose, VIR was supplemented alone or along with PHY to corn-soybean meal diets lacking any inorganic source of P.

A secondary objective was to compare the digestibility coefficients obtained by total collection (5 days), as the standard methodology, with the results by the index method (Cr_2O_3) in the first two experiments. The specific

purpose was to establish the reliability of two grab sampling strategies in assessing digestibility of specific nutrients. The strategies were a single-day grab fecal collection (Experiment 1), and a cumulative composite grab collection extending from 1 to 5 days (Experiment 2).

Experimental Procedures

Animals and housing conditions

A total of 70 growing-finishing barrows, crossbreds of (Yorkshire x Landrace) x Hampshire, were used in the four experiments. The average weight to start total collection was 68.1, 66.2, 53.3 and 55.4 kg, respectively, for Experiments 1, 2, 3, and 4 (Appendix 2 presents average starting and finishing weights of treatment groups during adaptation and collection). In each experiment, sibling pigs of similar weight within a replicate were allocated to treatments and randomly assigned to crates. Pigs were individually confined in metabolism crates, as described in Chapter 3. Half-siblings (i.e., a common sire) were used when enough full-siblings were not available. Experiment 1 used ten pigs, Experiment 3 used 12 pigs, and 24 pigs were used in each of the other two experiments. With the 70 animals used 92 observations were obtained for the total collection method, while 68 observations for the index method were obtained with 34 of the pigs, for a total of 162 observations.

Dietary treatments

A basal (B) corn-soybean meal diet not supplemented with any inorganic source of P was prepared separately for each experiment at the feed mill facilities of the University of Kentucky. Conventional corn and dehulled soybean meal (SBM) were used. The limestone was Franklin High Calcium Limestone (Franklin Industrial Minerals, Nashville, TN) which contained 38.5% Ca. Vitamin and trace mineral premixes were provided by the University of Kentucky Feed

Mill. Table 4.1 presents the composition of the basal diet used in all the experiments.

In Experiment 1 two diets were tested: the basal P–deficient diet (B) versus the same diet supplemented with VIR from Stafac® 20 (Phibro Animal Health Co., Fairfield, NJ). Virginiamycin was fed at 11 ppm, corresponding with the level indicated by the FDA-Center for Veterinary Medicine (2004) for growth promotion purposes in swine. For Experiment 2, besides VIR, phytase (PHY) from Natuphos® 1200G (BASF Corp., Mount Olive, NJ) was also used. Four diets were tested: the basal diet, the basal supplemented with 11 ppm VIR, the basal supplemented with 750 phytase units per kg of diet (PU/kg), and the basal plus both additives at the same levels (10 g VIR/ton, and 750 PU/kg diet). The only difference between Experiments 2 and 3 was that the PHY level was reduced from 750 PU/kg to 300 PU/kg for Experiment 3. Both VIR and PHY premixes used in all the experiments came from the same bag of commercial product. Experiment 4 was a repetition of Experiment 3 (supplementing 10 g VIR/ton, and 300 PU/kg diet). The Natuphos® 1200G premix was analyzed before Experiment 4, and the result was 1326 PU/g, which was the value used to calculate PHY concentration in this last experiment, instead of 1200 PU/g. Table 4.2 presents a summary of the dietary treatments tested in the four experiments.

The basal diets were mixed in a 1000-kg capacity horizontal paddle mixer at the University of Kentucky Feed Mill. The experimental diets were prepared in horizontal paddle mixers (either 150-kg or 1000-kg capacity) by blending VIR and/or PHY with the basal diet.

In Experiment 1, two batches of basal diet were prepared. Half of the basal diet was saved for the control treatment (Diet 1), while Stafac® 20 was blended with the other half to make Diet 2.

Two batches of basal diet were prepared for Experiment 2, and one batch was prepared for Experiment 3. Each additive, VIR and/or PHY, was blended with separated fractions of the basal diet to make the experimental diets used in these two experiments.

In Experiment 4, one single batch of basal diet was prepared and then divided into four fractions. One of the quarter fractions was blended with VIR and another one was blended with PHY. The blending of both additives (VIR or PHY) was done in a proportion equivalent to twice the concentration desired for the final experimental diets. To make experimental diets 2 and 3, a part of each concentrated portion was blended with an equal amount of the unblended basal. Diet 4 was prepared by blending together the same amounts of both concentrated fractions. Once the pigs were allotted to start each experiment, diets were weighed as individual meals into labeled plastic bags, and were kept separated by treatment.

Adaptation and collection procedures

As described in detail in Chapter 3, pigs were fed at 3% of body weight during the trials, in a gruel (feed plus water) form, and divided in two daily meals.

In Experiment 1, each pig was used for both sets of collections. When the first set of total and index collections finished, pigs were switched to the alternate diet, provided with a 3-d respite from the crates in 1.22 x 2.44 m pens, and then the adaptation and collection procedures were repeated. Similarly to Experiment 1, a single group of pigs was used in Experiment 3. Two separate groups of pigs were used in each of the other two experiments (Experiments 2 and 4).

In Experiments 1, 2, and 4, fecal collections for the index method ('grab' collections) were conducted after each total collection was completed. Chromic oxide (Fisher Chemicals, Fair Lawn, NJ) was used as the indicator, added to the experimental diets at a rate of 0.25%. The fecal collection procedure for the index method differed among experiments according to their particular objectives. Figures 4.1, 4.2 and 4.3 depict the general flow of events in the experiments, including the number of days between weighings of the pig groups. In Experiment 1 pigs were fed chromic oxide for two consecutive days, starting two days after the second feed marked with Indigo was offered. Collection of fecal samples for the index method was done on one single day for each pig by grabbing a stool when its color appeared bright green, assuming that at this point the indicator

concentration had already stabilized between feed and feces, which occurred most usually on the third day after the initial offering of marked feed.

On the other hand, in Experiment 2 pigs were fed chromic oxide for seven consecutive days. Collection for the index method was conducted for five consecutive days. Collection started when bright green feces were first detected, which generally occurred on the third day after the indicator was initially fed. During the collection period, similar amounts of feces (by approximate size) were grabbed and composited into five cumulative samples labeled: 'CCP1' (corresponding to the first day only), 'CCP2' (composited from days 1 and 2), 'CCP3' (from days 1, 2, and 3), 'CCP4' (from days 1, 2, 3, and 4), and 'CCP5' (from days 1, 2, 3, 4, and 5). Similar to Experiment 1, the Cr indicator was fed during the entire index collection period.

Sample preparation

To obtain a representative sample of urine for nutrient analysis, the collected samples were thawed at room temperature and proportionally composited by weight for each pig according to the recorded daily excretion. Composited samples were kept frozen at all times until analysis.

All frozen feces were dried in a forced-air oven (Tru-Temp, Hotpack Corp., Philadelphia, PA) at 55^{o}C for one week, then air equilibrated, weighed, and ground using a Wiley Laboratory Mill (Model 3, Arthur H. Thomas Co., Philadelphia, PA) through a 1 mm screen. After grinding, feces were composited according to digestibility method. For the total collection method, all ground feces from each collection period were thoroughly mixed in a single bag for each pig. From this bag, a sample for chemical analysis was obtained and re-ground using a smaller, high speed grinder (Type 4041, Model KSM 2-4, Braun Inc., Woburn, MA). The same procedure was used with the fecal samples for the index method, but this time collected feces were mixed according to the objectives of each experiment. In Experiment 1 fecal material collected for the index method (one single stool per pig) was analyzed separately. In Experiment 2, fecal samples taken from each day were composited as previously explained. After being composited, materials

collected for both total collection and index methods were kept in a cold room at 4 to 8°C until chemical analysis.

Laboratory analysis

Feces and feed were analyzed for DM contents. Feces, feed and urine were analyzed for energy, N, P, and Ca concentration. Concentrations of Mg, K, Mn, Zn, Fe, Cu, and Na were assessed in all experiments, except in Experiment 3. Chromium, for the index method, was assessed in feces and feed. Total contents of nutrients in feces, urine, and feed were calculated as the product of nutrient concentration by the total amount of material. Samples were analyzed at least in duplicate, and analysis was repeated when abnormal variation was observed.

Dry matter in feed and feces was assessed according to an adaptation of the AOAC (1995) method, involving overnight drying (105ºC) of the samples in a convection oven (Precision Scientific Co., Chicago, IL) and then calculating moisture contents as the difference between weighings.

Gross energy content was assessed by bomb calorimetry, consisting of the ignition of samples in a pressurized-oxygen environment, and measuring the heat of combustion as the amount of energy transferred to a known mass of water contained in the calorimeter (Model 1261 Isoperibol Bomb Calorimeter, Parr Instruments Company, Moline, IL). Benzoic acid pellets with known combustion heat were ignited at the beginning and end of each set of samples to verify calorimeter measures. Feed and feces samples were assessed in duplicate by a procedure adapted from AOAC (1995). To measure urine energy, samples were oven dried for two days at 55ºC into polyethylene flat bags (Jeb Plastics Inc., Wilmington, DE) prior to combustion. The known heat of combustion per gram of bag material was subtracted from the total heat observed to obtain the sample energy contents (Appendix 3 describes the procedures used to determine gross energy).

Nitrogen was measured using Dumas methodology in an automatic N analyzer (Model FP-2000, LECO Corp., Saint Joseph, MI). Ignition of blanks and

EDTA samples with known N contents was done daily in order to calibrate the equipment and to check for drift in the readings.

Phosphorus in feed and feces was assessed by a gravimetric method (modification of method 968.08 from AOAC, 1990) in which samples were weighed, ashed, acid digested, diluted to 250 mL, and then 50 mL of the liquid was reacted with Quimociac solution, filtered, and the precipitate obtained was weighed to calculate P concentration (Appendix 4 describes the P determination method and the Quimociac preparation procedure).

Phosphorus concentration in urine was assessed as inorganic P by a colorimetric procedure (Procedure No. 360-UVP. Sigma Diagnostics, St. Louis, MO) using a spectrophotometer (Model Ultrospec IIE, 4057 UV/visible, LKB Biochrom Ltd., Cambridge, England). Concentration was measured under ultraviolet light at 340 nm. A commercial reagent was used (Ammonium molybdate, 0.40 mmol/L in sulfuric acid with surfactant) (Catalog No. 360-3, Sigma Diagnostics, St. Louis, MO) along with a set of 3 standards containing 1, 5, and 15 mg/dL P (Calcium /Phosphorus Standard, catalog No. 360-5, Sigma Diagnostics, St. Louis, MO). A blank (DD water plus reagent) and the three standards were used to create calibration curves for the spectrophotometer to test for linearity before urine samples were read in the equipment.

Except for P, all other mineral elements were assessed by Flame Atomic Absorption Spectrophotometry (AA) (Thermoelemental, SOLAAR M5, Thermo Electron Corp., Verona, WI), according to a modification of the procedure from AOAC (1995b) (method 927.02), as described in Appendix 5.

Chromium concentration in feed and feces for the index method was assessed by a modification of the method reported by Williams et al. (1962), involving weighing the samples, ashing and digesting them with potassium bromate and acid manganese sulfate, heating at low temperature, then adding calcium chloride, and finally aspirating them into the AA equipment (Thermoelemental, SOLAAR M5, Thermo Electron Corp., Verona, WI). The Cr stock standard used was from Fisher Scientific (No. SC192). Appendix 6 describes the method and the preparation of the solutions used.

Apparent digestibility coefficients were calculated on a DM basis for both total collection and index methods. Additionally, nutrient retention as well as excretion via feces and urine was calculated for the total collection method. The formulae used are provided in Chapter 3.

Experimental design and statistical analysis

Experiment 1 challenged the null hypothesis of no effect of VIR on mineral digestibility, using 10 replications. It had a crossover design structure in which a single group of ten pigs was used in two collections - each pig receiving a different treatment in each collection. Pigs were matched in five pairs by ancestry. To prevent possible carry-over effects of the first treatment on the second collection, pigs had nine consecutive days between collections to adapt the gastrointestinal tract from the previous diet. The treatment structure was a 1-way treatment classification, and the experimental unit was each pig. The analysis of variance (ANOVA) was done using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS, 1998). The model for analysis included the effects of collection, pair (collection), diet, and diet by collection interaction.

Experiments 2, 3, and 4 utilized a randomized complete block design with pigs blocked by weight. In order to decrease weight variation between the two collection groups, in Experiments 2 and 4 the heaviest group of pigs selected was tested first, leaving the lighter group for the second collection. The treatment structure was a 2-way treatment classification (2 x 2 factorial) with six replicates per treatment, and the experimental unit was the pig. The ANOVA was also obtained using the GLM procedure of SAS. The model included the effects of collection, diet, the diet by collection interaction, and replicate (collection). Several one-degree-of-freedom pre-planned comparisons were performed. Comparisons evaluated the basal diet vs. each additive (VIR or PHY). The error term reported is the standard error of the mean (SEM), except for Experiments 1 (index method analysis) and 2, where unequal numbers of observations were used. In those cases, the root mean square error (RMSE) is reported. It can be converted into

SEM by dividing its value by the square root of the number of observations associated with each specific mean.

Results and Discussion

Total collection

All the animals in Experiments 1, 3, and 4 successfully completed the scheduled time in the metabolism crates for the total collections. All pigs in Experiment 2 finished the trial but one pig did not gain weight. In general, pigs were in good health and condition during the experiments. No intestinal disorders such as diarrhea or constipation were observed either during the adaptation or collection periods. In some experiments a few pigs developed minor bruises in rear feet during confinement, but no abnormal behavior, feed consumption or defecation patterns were observed.

An important condition in any digestion or balance experiment is that the animals be in a positive balance of nutrients, otherwise, results could be biased by the greater tissue catabolism expected for animals losing weight. All but one pig gained weight during collection in Experiment 2, so that one animal was not included in the analysis. Growth performance during collections is not reported as the reliability of differences in growth resulting from such short periods is questionable due to the possible effect of differences in gut fill at weighing times.

Samples of the experimental diets were analyzed for VIR and PHY concentration by Phibro and BASF laboratories, respectively. The assays for the concentration of the additives in the diets found that VIR was close to the target in all experiments. As expected, the diets not amended with the antibiotic were low in VIR (< 2.0 g/ton), while the others, with the exception of Diet 4 in Experiment 3, were close to 10 g/ton (Table 4.3). On the other hand, the assays for PHY concentration were different than expected, particularly for Diets 3 and 4 in Experiment 2. It is not clear whether the differences reflect an issue with the blending of the diets (the blending procedure in Experiment 4 was thought to be

better than the others, which is probably the reason PHY values were closer to expected levels in Diets 3 and 4), or with the lab analysis. In Experiment 2, although the PHY levels in Diets 3 and 4 were different, both were higher than their target levels (Table 4.3). In Experiment 3 the assay for Diets 3 and 4 were considered problematic because differences in results at these relatively low levels of PHY could cause important differences in digestibility due to the enzyme (Cromwell et al., 1995a), possibly interfering with an action of VIR on the remaining substrate. For this reason, the results for Diets 3 and 4 were disregarded in this experiment, the data from Diets 1 and 2 were re-analyzed and only those diet results are presented. In Experiment 4 the assays results were closer to those planned.

Experiment 1

In Experiment 1, the addition of VIR improved the apparent digestibility of several nutrients including DM, Energy, P, Ca, Mg and Zn. Dry matter digestibility increased 0.94% ($P = 0.05$). A similar improvement (0.85%, $P = 0.06$) was observed for energy digestibility. Nitrogen digestibility was numerically but not statistically improved. In this experiment, the greatest improvement in digestibility was observed for P (8.4%, $P < 0.01$), followed by Ca (5.8%, $P < 0.01$), Zn (3.9%), and Mg (3.1%, $P = 0.02$). The apparent digestibility of other minerals tested (K, Fe, Cu, Mn and Na) was not affected by VIR addition ($P > 0.10$). Table 4.4 presents the digestibility results obtained. The observed improvement of 8.4 percent points in P digestibility in Experiment 1 is equivalent to a 0.031% increase in P in the diet calculated to contain 0.37% total P.

No reports of increased P digestibility in pigs due to VIR or any other antibiotic were found in the literature, except for the experiment by Ravindran et al. (1984). They used lighter pigs (35 kg BW) in three balance trials to test the effects of the same level of VIR (11ppm) on nutrient digestibility, mineral absorption, retention, and rate of passage in diets supplemented with P. In a 2 x 2 factorial arrangement, they supplemented VIR to a low fiber corn-SBM meal diet (NDF:

13.5%) and to a high fiber corn-SBM–oats diet (NDF: 20.2%). They tested if VIR would decrease the greater rate of passage expected for the high fiber diet, thus improving nutrient utilization. In agreement with previous reports on the effects of fiber, they found that the high fiber diet decreased DM, energy, CP, and ash digestibility. Interestingly, they also found that VIR increased P digestibility in the high fiber diet (57.7 vs. 63.0%), although it did not affect P digestibility in the low fiber diet (55.2 vs. 55.4%). Similarly, VIR increased Ca digestibility in the high fiber diet (56.5 vs. 66.1%), but not in the low fiber diet (63.5 vs. 62.0). Digestibility of Mg, Cu, Zn, and Mn was increased by VIR in both high and low fiber diets ($P <$ 0.09). Phosphorus and Ca retention as a percent of absorption was also increased by VIR in the high fiber diet (53.7 vs. 58.3%; and 54.4 vs. 64.5%, respectively), but not in the low fiber diet. Interestingly, although VIR slowed the rate of passage ($P <$ 0.01) in both diets (from 20.6 to 26.7 h), the improvement in P digestibility and retention was only observed in the high fiber diet.

Ravindran et al. (1984) did not observe any effect of VIR on P digestibility in their corn-SBM diet, while Experiment 1 did show an important improvement. The reason for the difference could be that their diet had more available P (it was supplemented with 0.90% defluorinated phosphate) while Experiment 1 was P-deficient, so both may not be comparable. This comment is further substantiated later in the discussion of digestibility results of the other experiments.

In Experiment 1, besides the improvement in P digestibility, VIR increased P retained as a % of absorption. Among all the nutrients measured, P was the only one that showed an improvement in retention (as a percent of absorbed) with VIR supplementation (Table 4.5).

In agreement with the improvements in P digestibility and retention observed, total excretion of the mineral (fecal plus urinary) decreased ($P < 0.01$) from 6.11 g/d to 5.49 g/d, a 10.2% reduction (Table 4.6). As expected, of the total P excreted, fecal P accounted for most of the excretion in both control and VIR diets (5.9 vs. 5.3 g, respectively), as compared to urinary P (0.21 vs. 0.19 g,

respectively). Virginiamycin decreased P excretion in both feces and urine by a similar proportion (10.2% in feces and 9.5% in urine).

According to the apparent retention observed in the Experiment 1 (calculated as intake minus fecal and urinary losses), VIR-treated pigs retained 0.78 more grams of P per day (3.9 g total for the 5 d) as compared with the controls.

In Experiment 1, numerical differences in N absorption, retention, and excretion were observed with the addition of VIR (Table 4.6).

Experiments 2, 3, and 4

Virginiamycin effects. In these experiments VIR did not have a main effect on P digestibility. Nevertheless, a VIR x PHY interaction was observed in Experiment 2 (P = 0.06). Considering that differences due to VIR were observed previously in Experiment 1, single-degree-of-freedom comparisons were made between Diets 1 and 2 in Experiments 2, 3, and 4. The comparisons found significant differences in P digestibility favoring VIR in Experiment 2 (34.61 vs. 39.25, $P < 0.03$). Similar improvement was found in Experiment 3 (32.45 vs. 37.66, $P = 0.08$), but no difference was observed in Experiment 4 (33.40 vs. 35.18, $P > 0.10$) (Tables 4.7, 4.8, and 4.9).

The positive effect of VIR on P digestibility observed in Experiments 2 and 3, along with the stronger results initially observed in Experiment 1, agree with Ravindran et al. (1984). It is interesting to note that they reported an effect of VIR only on their high fiber diet, while Experiments 1, 2, and 3 showed a VIR effect in a corn-SBM diet, where most P is unavailable. This observation could imply that the effect of the antibiotic depends on the digestibility level of the diet. This is further substantiated when results of the four experiments are put together showing that the magnitude of increase in P digestibility due to VIR tended to be inversely related to the P digestibility level of the control diet (Figure 4.4).

Lindblad et al. (1954) arrived at a similar conclusion for chicks and poults fed Ca and P deficient diets supplemented with aureomycin. They found that in the absence of the antibiotic, maximum weight gain was obtained with a diet containing 1.0% Ca and 0.6% inorganic P. On the other hand, in the presence of aureomycin maximum gain and feed efficiency resulted when P was decreased to 0.4%. Increasing P above 0.4% in the diet containing antibiotic did not increase the gain. Researchers concluded that the more inadequate the Ca and P in the ration, the greater the percentage increase in weight due to the antibiotic.

The inverse relationship observed between the level of improvement in P digestibility and the digestibility level of the basal diet is in agreement with Braude et al. (1953). These researchers summarized a number of experiments with different antibiotics to conclude that the relative improvement in growth resulting from antibiotic amendments was inversely related to the growth rate of the control pigs.

In contrast to Experiment 1, where the digestibilities of DM, Energy, P, Ca, Mg and Zn were significantly increased by the inclusion of VIR, significant differences were less prominent for these nutrients in the three following experiments. Tendencies for main effect improvements were observed only for N digestibility in Experiment 2 ($P = 0.10$) and also for Zn and Cu digestibility in Experiment 4 ($P < 0.10$) (Tables 4.7, 4.8, and 4.9).

With regard to P retention expressed as a percent of the absorption, there was a main effect of VIR in Experiment 2 (P = 0.03), and numerical differences in Experiments 3 and 4 (Tables 4.10, 4.11, and 4.12). Virginiamycin also tended (P = 0.08) to increase P retention, expressed as a percent of the intake, in Experiment 3 ($P = 0.08$), and numerical differences were observed in Experiments 2 and 4 ($P = 0.14$). The relevance of this last response in P-deficient diets is addressed in the discussion of the effects of PHY.

Phosphorus digestibility and retention data (as a % of absorption) for Diets 1 and 2 in the 4 experiments are summarized in Figure 4.5. Across experiments, the average improvement in P digestibility by amending the basal diet with VIR was 5.0% (range: 1.78 to 8.44%), while the average improvement in P retention (as a % of absorption) was 1.0% (range: 0.07 to 2.36%).

When Diets 1 vs. 2 were compared, numerical improvements by VIR were observed in total excretion of P (fecal plus urinary, g/d), equivalent to 2.3% in Experiment 2, 4.2% in Experiment 3, and 2.8% in Experiment 4 (Tables 4.13, 4.14, and 4.15). Averaging the four experiments, the decrease in total P excreted due to VIR amendment was 4.9%.

Phytase effects. The addition of 750 PU/kg diet had a strong positive effect on the digestibility of several minerals, particularly P and Ca (Tables 4.7 and 4.9). Results from Experiment 2 indicate that this level of PHY inclusion increased P digestibility 78.9% (from 34.6 to 61.9%), for the basal diet, and 55.4% (from 39.3 to 61.0%) for the VIR-added diet ($P < 0.01$). As expected, a smaller effect on P digestibility was observed when PHY level was reduced to 300 PU in subsequent experiments, which agrees with earlier reports of linear responses to different levels of PHY (Lei et al., 1993; Veum et al., 1994; Cromwell et al., 1995; Liu et al., 1995). In Experiment 4, digestibility increased 41.3% (from 33.4 to 47.2%) for the non-VIR diet, and 43.0% (from 35.2 to 50.3%) for the VIR-added diet ($P < 0.01$).

In Experiment 2, no effect of PHY on DM, energy or N digestibility was observed ($P > 0.31$), but it improved Ca digestibility in Experiments 2, 3, and 4 ($P < 0.01$), which is in agreement with other reports (Lei et al.,1993; Pallauf et al., 1992; Pallauf et al., 1994; Jongbloed et al., 1999c; Pallauf and Rimbach, 1999). In regard to micro-minerals, it was interesting to note that PHY decreased Fe and Cu apparent digestibility ($P < 0.05$) in Experiment 2, while Mn digestibility was decreased in both Experiments 2 and 4 ($P < 0.01$). Adeola (1999) did not observe any effect of PHY on Mn absorption.

In Experiment 2, the tendency for an interaction between PHY and VIR for P and Ca digestibility ($P < 0.10$), along with the lack of significant difference between Diets 3 and 4 suggested that the level of inclusion of PHY was probably high enough to release all the phytate-P present in the diet, not leaving any room for further VIR effect. This observation motivated reduction of the PHY level in Experiments 3 and 4 which followed, in order to be able to detect any possible additive effects of VIR.

As expected, a smaller response in digestibility was observed when the level of PHY was lowered in these following experiments. It was known that there is a dose-response relationship between the level of PHY and the apparent total tract P digestibility in pigs fed corn-SBM diets (Cromwell et al., 1995a; Harper et al., 1997; Jongbloed et al., 1999b). In Experiment 2, comparisons between Diets 1 and 3 showed that PHY increased P digestibility 79% (from 34.6 to 61.9%, $P < 0.01$), but it was only 41% (from 33.4 to 47.2%, $P < 0.01$) in Experiment 4.

Phosphorus retention was also increased by PHY additions. The percentages increases resemble the changes observed in P digestibility. In Experiment 2, the high level of PHY used increased P retention (RI) of the basal diet by 83.7% (from 32.9 to 60.5%), and 57.6% (from 37.9 to 59.7%) for the VIR-added diet ($P < 0.01$). Smaller increases in P retention due to PHY were observed when the PHY level was reduced to 300 PU. In Experiment 4, P retention (RI) increased 42.7% (from 32.3 to 46.0%) for the non-VIR diet, and 44.5% (from 34.0 to 49.2%) for the VIR-added diet ($P < 0.01$).

The change in nutrient retention, expressed as a percent of intake, was closely related in magnitude to the change in digestibility (Figure 4.6). In all experiments, P retention (RI) varies according to either positive or negative changes in P digestibility due to VIR and PHY amendment. These changes tend to be very similar in magnitude to the changes seen for P digestibility, which was probably reflecting dietary P availability. As available P in these diets was deficient, urine P should represent a minimum fraction of the total P (fecal plus urinary) excreted. Low urinary P would reflect the increased net retention

expected in a P-deprived animal trying to satisfy P demand during this fast growing stage. It is known that a deficient dietary intake of P stimulates not only active absorption but also renal reabsorption, minimizing urinary losses (Combs, 1998; Crenshaw, 2001; McDowell, 2003). In the study by Rodehutscord et al (1998), it was estimated that the daily inevitable losses of P via urine in growing pigs (50 kg) fed P deficient diets accounted for only about 6% of the total inevitable losses, corresponding to 0.35 mg/kg BW. They concluded that under P deficiency conditions, pigs can completely utilize digestible P without further losses, which also agrees with the findings of Nasi and Helander (1994).

Under these conditions, it would be expected that the coefficient of P digestibility approaches P retention (% intake). Moreover, in the hypothetical case of zero urinary excretion, digestibility and retention (% intake) would be expected to be close or even the same in value. According to this, PHY and VIR are probably not directly causing an increase in P retention (% intake). This retention could be just a reflection of the dietary deficiency and the response of the animal to retain phosphate. As expressed by several researchers (Cromwell, 1999; Underwood and Suttle, 1999; McDowell, 2003) bone assessments, as well as growth performance over longer periods, would probably better help to verify suspected benefits in bioavailability.

In Experiment 4, retention of micronutrients (Fe, Cu, Mn, and Zn) expressed as a percent of absorption was greater than 100% for some of the treatment means (Table 4.12). The reason for these apparently odd results is that digestibility was negative and greater than net retention (which in turn was also negative). In other words, always when digestibility is negative, net retention will also be negative (because some urinary excretion is always expected, regardless of the amount already excreted via feces), and the ratio will be greater than 100%. The urinary excretion level will determine how much greater than 100% retention as a percent of absorption would be in those cases.

Reflecting the increase in digestibility and retention observed with the PHY amendments, total P excretion was notably reduced by PHY. In Experiment 2,

PHY decreased total P excretion of the basal diet by 36.6 % (from 4.8 to 3.1 g/d), and by 34.9% (from 4.7 to 3.1 g/d) in the VIR-added diet ($P < 0.01$). Smaller decreases in P excretion were observed when the PHY level was reduced to 300 PU in Experiment 4. In Experiment 4, P excretion decreased 20.1% (from 4.3 to 3.4 g/d) for the non-VIR diet, and 21.2% (from 4.2 to 3.3 g/d) for the VIR-added diet ($P < 0.01$). (Tables 4.13, 4.14, and 4.15).

From the discussion of the total collection method for the four experiments, it can be concluded that VIR amendment improved apparent P digestibility and total P excretion by 5.0%. In addition, the PHY amendment results confirmed the positive effects of the enzyme on digestibility, retention, and excretion.

Index method

Probably because of the long time in the crates, not all the pigs completed the scheduled collections for the index method in Experiment 1 in good condition. Two pigs in that experiment had low appetite and one of them showed signs of rear legs paralysis and fever during index collection. Because of this, data from those pigs were not included. Although index collections in Experiment 2 took longer no problems were detected, probably because pigs were given a respite from the crates between collections for the total and index methods.

In Experiments 1 and 2 feces usually appeared bright green on the third day after chromic oxide was initially fed. A single fecal sample per pig was collected at that time in Experiment 1. In Experiment 2, grab samples of similar size were collected for 5 consecutive days, starting when bright green feces were first observed.

Experiment 1

In Experiment 1, nutrient digestibility results were generally lower by the index than by the total collection method, which agrees with several reports

(Barnicoat, 1945; Clawson et al., 1955; Mroz et al., 1996). The only exception was Na, which exhibited higher digestibility by the index method than by the total collection method (Table 4.16). No reports of higher digestibility by the index method in comparison to the total collection method, for any nutrient, were found in the literature.

As shown in Figure 4.7, the difference between digestibility methods observed in Experiment 1, was smaller for highly digestible, highly concentrated nutrients. Nutrients having low digestibility (below 60%) and low concentrations exhibited a bigger difference between methods. Micronutrients with very low digestibility (below 25%) by the total collection, such as Zn, Fe, and Cu, had negative digestibility coefficients by the index method. Three out of four of the micro-minerals tested were poorly digestible according to the total collection, and showed nearly zero to negative digestibility by the index method. As pointed out by Schneider and Flatt (1975), it can be expected that the normal variation about the mean may result in negative values for poorly digestible nutrients or for nutrients having a very low concentration in the feed. The negative results might be obtained when the variation in the nutrient concentrations among samples is in excess of the mean concentration of the nutrient. Apgar and Kornegay (1996) did not find such big differences between total collection and index method when they compared mineral balance by both methods in finishing pigs. In their results, the Index method was lower than the total collection method by about one percentage unit. A possible reason for the lower variation observed by these researchers is that they fed elevated levels of Cu in the presence of excess amounts of otherwise 'trace' minerals (Fe, Cu, Mn, and I). They concluded that Cr_2O_3 did not seem to be a reliable marker for estimating trace mineral absorption.

Although digestibility coefficients for highly digestible macronutrients such as N, K, and Na, as well as DM and energy for the two methods appeared quite similar (Figure 4.7), treatment comparisons gave different results. The index method was not able to detect the statistical differences between treatments that were found by the total collection method (Table 4.16). The index method was

not able to detect even strong treatment differences in digestibility for macronutrients such as P and Ca found by the total collection method ($P < 0.01$).

In conclusion, the color of the feces marked with Cr_2O_3 may not be a reliable indicator of Cr concentration. Relying solely on feces color to determine when to collect a single grab sample may lead to wrong conclusions in digestibility assessments.

Experiment 2

In Experiment 2, a separate statistical analysis was conducted using each of the five cumulative composited sample collection periods. This was done in order to observe any trends in digestibility coefficients among the cumulative collection periods (CCP). Separate analysis of each CCP, regardless of diet, showed that digestibility coefficients for all the nutrients were increasing from CCP 1 to CCP 5 (Figure 4.8).

At CCP 4, all micro-minerals but Zn had apparently reached their digestibility plateaus. At CCP 5, the rest of the nutrient digestibility curves apparently reached their plateaus. The increasing trend in digestibility values can be explained by the observed gradual increase in Cr excretion with increasing CCP (Figure 4.9). Chromium fecal excretion also seems to reach its plateau at the last CCP.

Digestibility coefficient values below the plateau (for CCP 1, 2, 3, and 4) do not reflect the real values because Cr concentration was not yet stabilized between feed and feces until CCP 5. Accordingly, CCP 5 was the only period from the index method that could be compared with the total collection method.

In general, the total collection and the index method at CCP5 gave similar results for macronutrients and very dissimilar results for micronutrients (Figure 4.10). As already observed in Experiment 1, some micronutrients (Mn and Cu) had negative digestibility coefficients by the index method.

As all P-values for the main effect of VIR amendment on digestibility were greater than 0.10 by both methods, the conclusions regarding VIR would be the same, regardless of method. Nevertheless, there are big differences in P-values

with the two methods for several nutrients (DM, energy, N, P, and Na), and in all those cases the P-value was greater with the index method. This implies that this method could be less able to detect significant differences among treatments with low expected impact on digestibility values (Table 4.17).

Some of the comparisons regarding the main effect of PHY amendment on macronutrient digestibility (e.g., DM and Mg) found different P-values with the different methods, which would lead to different conclusions according to which method was used. Nevertheless, for nutrients highly impacted by the PHY amendment, such as P and Ca, P-values were both statistically significant ($P < 0.01$) and numerically similar by both methods. With micronutrients, the index method failed to detect significant differences found by the total collection method for the effect of PHY on Fe ($P = 0.02$), Cu ($P = 0.01$), and Mn ($P < 0.01$) digestibility (Table 4.17).

In conclusion, a 5-day CCP for the index method was not appropriate for measuring micronutrients digestibility. The 5-day CCP method would be appropriate to measure digestibilities of macronutrients only when important differences between treatments are expected. This conclusion does not extend to a single grab collection at day 5, because the Cr concentration of the single grab sample would be different than for CCP5 due to the effect of the low Cr concentration in the first days of cumulative collection.

Implications

According to these results, the amendment of a P-deficient corn-SBM diet with VIR or PHY for growing pigs can be expected to increase P digestibility and retention, decreasing excretion, with the expected benefits to the environment. These experiments confirm the well known advantages of PHY in P nutrition for pigs. The experiments also provide new evidence on the positive effects of VIR on P utilization, showing that the antibiotic increases P digestibility by about 5%, on

average. The improvements in P utilization observed in these experiments could represent not only an economic advantage to the producer in terms of inorganic dietary P savings, but also a potential benefit to the environment due to lower P excretion. Further studies are required in order to observe to what extend these improvements in P digestibility and retention transfer into bone mineralization, and to understand the mechanism by which the antibiotic impacts P nutrition.

As regards the issue of methodology, the evaluation of nutrient digestibility by the index (Cr_2O_3) method, the collection of a single fecal grab sample according to its color, although simple, cheap and easy, does not seem to be a reliable alternative to the total collection method. Using a cumulative sample composited over five days was much more accurate than a 1-day collection. This version of the index method might provide acceptable approximative results in cases where it is desirable to have some idea of macronutrient digestibility. Nevertheless, the index method was not suitable for detecting statistical differences in micro-mineral digestibility.

Table 4.1. Basal diet used in Experiments 1, 2, 3, and 4

Ingredient[a]	%
Corn, ground	74.800
Soybean meal (48% CP)	23.300
UK vitamin mix[b]	0.075
UK trace mineral mix[c]	0.075
Limestone	1.400
Phosphate source	0
Salt	0.350
Total:	100.000

Calculated composition		NRC (1998) requirement estimates	
		20-50 kg	50-80 kg
Crude protein (%)	17.28	18.00	15.50
Lysine (%)	0.90	0.95	0.75
ME (kcal/kg)[d]	3346	3265	3265
Calcium (%)	0.60	0.60	0.50
Phosphorus, total (%)	0.37	0.50	0.45
Phosphorus, available (%)	0.07	0.23	0.19

[a] Vitamin premix and trace mineral premix supply nutrients to meet or exceed NRC (1998) requirement estimates.
[b] Diet was calculated to provide (per kg): 4,950 IU vitamin A, 660 IU vitamin D_3, 33 IU vitamin E, 4.8 mg vitamin K (as menadione sodium bisulfite complex), 6.6 mg riboflavin, 16.5 mg pantothenic acid, 33.0 mg niacin, 0.99 mg folic acid, 0.165 mg d-biotin, 24.5 µg vitamin B_{12}, and 3.3 mg vitamin B_6.
[c] The mineral premix in the diet supplies, per kilogram of diet: 135 mg Fe (iron sulfate monohydrate), 135 mg Zn (zinc oxide), 45 mg Mn (manganous oxide), 13 mg Cu (copper sulfate pentahydrate), 1.5 mg I (calcium iodate), 0.3 mg Se (sodium selenite), and 0.23 mg Co (cobalt sulfate monohydrate).
[d] ME: Metabolizable energy.

Table 4.2. Dietary treatments in Experiments 1, 2, 3, and 4

Experiment number	Treatments			
	1	2	3	4
1	B[a]	VIR[b]	-	-
2	B	VIR	PHY(1)[c]	PHY(1)+VIR
3	B	VIR	PHY(2)[d]	PHY(2)+VIR
4	B	VIR	PHY(2)	PHY(2)+VIR

[a] B: Basal diet.
[b] VIR: 10 g virginiamycin/ton of diet (calculated level of inclusion).
[c] PHY(1): 750 phytase units/kg diet (calculated level of inclusion).
[d] PHY(2): 300 phytase units/kg diet (calculated level of inclusion).

Table 4.3. Expected and analyzed phytase (PHY) and virginiamycin (VIR) levels in the diets

		Phytase (PHY) U/kg		Virginiamycin (VIR) g/ton	
Exp.[a]	Trt.[b]	Expected	Analyzed[c]	Expected	Analyzed
1	1	-	-	0	<2.0
	2	-	-	10	9.7
2	1	0	ND[d]	0	<2.0
	2	0	ND	10	12.45
	3	750	962	NA[e]	NA
	4	750	1410	10	8.95
3	1	0	ND	0	<2.0
	2	0	ND	10	8.1
	3	300	420	0	<2.0
	4	300	286	10	6.7
4	1	0	ND	0	<2.2
	2	0	ND	10	8.5
	3	300	448	0	<2.2
	4	300	407	10	8.8
All	Natuphos 1200 G Premix, PU/g	1,200	1,326[f]	-	-

[a] Experiment number.
[b] Treatment number.
[c] Average values (units/kg) of samples analyzed for both collections into each experiment.
[d] ND: Not detected.
[e] NA: Not analyzed.
[f] Value used to calculate PHY addition in Experiment 4. Phytase contents in premix was considered 1200 PU for Experiments 2 and 3.

Table 4.4. Total tract apparent digestibility coefficients (%) by the total collection method. Experiment 1

	Treatment			
Response	Control	VIR	SEM[b]	P-value
DM	88.99	89.93	0.29	0.05
Energy	88.28	89.12	0.27	0.06
N	88.69	89.35	0.35	0.21
P	30.37	38.81	1.11	0.001
Ca	51.51	57.32	0.96	0.003
Mg	55.09	58.15	0.75	0.02
K	85.48	85.38	1.18	0.96
Na	71.98	73.89	2.48	0.60
Fe	23.22	23.38	1.26	0.93
Cu	16.27	17.87	1.01	0.30
Mn	47.79	46.24	2.14	0.62
Zn	21.87	25.77	0.70	0.004

[a]Each mean represents 10 individually penned pigs. Control: Control diet; VIR: 10 g virginiamycin/ton diet.
[b]SEM: Standard error of the mean.

Table 4.5. Nutrient retention as a percent of absorption. Experiment 1

Response	Treatment[a]		SEM[b]	P-value
	Control	VIR		
Energy	96.98	97.04	0.22	0.86
N	57.25	59.86	1.86	0.35
P	91.90	94.26	0.51	0.01
Ca	66.07	70.24	3.07	0.37
Mg	68.47	71.60	1.77	0.25
K	24.29	20.65	7.17	0.73
Na	38.33	43.00	2.96	0.30
Fe	98.61	98.64	0.10	0.84
Cu	94.64	95.44	0.76	0.48
Mn	99.53	99.48	0.05	0.57
Zn	97.37	97.83	0.23	0.19

[a] Each mean represents 10 individually penned pigs. Control: Control diet. VIR: 10 g virginiamycin/ton diet.
[b] SEM: Standard error of the mean.

Table 4.6. Phosphorus and nitrogen balance. Experiment 1

Response	Treatment[a]		SEM[b]	P-value
	Control	VIR		
P				
Intake, g/d	8.49	8.65	0.09	0.23
Excreted (feces), g/d	5.90	5.30	0.09	0.001
Excreted (urine), g/d	0.21	0.19	0.01	0.25
Total excreted, g/d	6.11	5.49	0.09	0.001
Absorption, g/d	2.59	3.35	0.12	0.002
Retention, g/d	2.38	3.16	0.12	0.001
Digestibility (apparent), %	30.37	38.81	1.11	< 0.001
Retention (as a % of intake)	27.95	36.61	1.11	0.001
Retention (as a % of absorption)	91.90	94.26	0.51	0.01
N				
Intake, g/d	57.5	58.7	0.61	0.22
Excreted (feces), g/d	6.5	6.2	0.20	0.37
Excreted (urine), g/d	22.0	21.5	0.91	0.72
Total excreted, g/d	28.5	27.7	0.80	0.53
Absorption, g/d	51.0	52.5	0.66	0.16
Retention, g/d	29.1	31.0	1.08	0.24
Digestibility (apparent), %	88.69	89.35	0.35	0.21
Retention (as a % of intake)	50.75	53.45	1.57	0.26
Retention (as a % of absorption)	57.25	59.86	1.86	0.35

[a] Each mean represents 10 individually penned pigs. Control: Control diet. VIR: 10 g virginiamycin/ton diet.
[b] SEM: Standard error of the mean.

Table 4.7. Total tract apparent digestibility coefficients (LS Means, %) by the total collection method. Experiment 2

	Treatment[a]							
	B	VIR	PHY	VIR+ PHY		P-values[b]		
Response	1	2	3	4	RMSE[c]	VIR	PHY	VIRxPHY
DM	91.61	92.14	91.82	92.28	0.74	0.15	0.59	0.91
Energy	90.85	91.38	90.52	91.04	0.74	0.13	0.32	0.99
N	89.83	90.81	89.43	90.22	1.17	0.10	0.35	0.85
P	34.61	39.25	61.91	60.98	3.15	0.19	<.0001	0.06
Ca	59.49	61.69	71.27	68.74	3.09	0.90	<.0001	0.10
Mg	39.79	41.41	41.24	39.30	5.03	0.94	0.88	0.42
K	90.62	90.88	91.34	90.33	2.28	0.70	0.93	0.53
Na	80.95	83.69	81.41	81.23	2.93	0.32	0.44	0.26
Fe	18.76	17.71	12.26	11.37	5.74	0.70	0.02	0.97
Cu	25.17	25.68	22.08	20.44	3.14	0.68	0.01	0.44
Mn	49.01	41.88	11.01	9.95	10.03	0.36	<.0001	0.49
Zn	15.11	14.74	14.19	12.38	3.82	0.51	0.33	0.67

[a] B: Basal diet; VIR: Basal + 10 g virginiamycin/ton; PHY: Basal + 750 PU/kg diet; VIR+PHY: Basal + 10 g virginiamycin/ton + 750 PU/kg diet.
[b] VIR: Virginiamycin effect; PHY: Phytase effect; VIRxPHY: Virginiamycin by phytase interaction.
[c] RMSE: Root mean square error (number of pigs: 6, 6, 5, and 6 pigs for treatments 1, 2, 3, and 4, respectively).

Table 4.8. Total tract apparent digestibility coefficients (%) by the total collection method. Experiment 3

	Treatment[a]			
	B	VIR		
Response	1	2	SEM[b]	P-values
DM	90.54	90.32	0.31	0.64
Energy	90.41	89.99	0.34	0.43
N	89.63	89.43	0.62	0.83
P	32.45	37.66	1.60	0.08
Ca	57.93	61.22	1.79	0.26

[a] Each mean represents 6 individually penned pigs; B: Basal diet; VIR: Basal + 10 g virginiamycin/ton.
[b] SEM: Standard error of the mean.

Table 4.9. Total tract apparent digestibility coefficients (%) by the total collection method. Experiment 4

	Treatment[a]					P-values[b]		
	B	VIR	PHY	VIR+ PHY				
Response	1	2	3	4	SEM[c]	VIR	PHY	VIRxPHY
DM	90.08	89.69	90.01	90.33	0.27	0.91	0.31	0.21
Energy	89.52	88.96	88.95	89.23	0.30	0.65	0.64	0.19
N	89.74	89.84	89.52	89.92	0.49	0.61	0.89	0.76
P	33.40	35.18	47.18	50.31	1.56	0.14	< 0.0001	0.67
Ca	51.02	52.97	60.91	63.20	1.54	0.19	<0.0001	0.91
Mg	37.91	38.66	36.66	40.45	1.59	0.18	0.87	0.36
K	83.66	82.33	83.19	84.78	1.17	0.92	0.42	0.24
Na	67.00	69.27	68.84	70.67	2.20	0.37	0.48	0.92
Fe	-8.16	-8.18	-10.95	-7.61	2.19	0.46	0.62	0.46
Cu	0.92	1.15	-3.42	1.66	1.42	0.09	0.20	0.12
Mn	2.46	0.15	-10.14	-4.89	2.43	0.56	0.004	0.15
Zn	-2.02	-1.77	-10.96	-1.23	2.59	0.08	0.13	0.09

[a] Each mean represents 6 individually penned pigs. B: Basal diet; VIR: Basal + 10 g virginiamycin/ton; PHY: Basal + 300 PU/kg diet; VIR+PHY: Basal + 10 g virginiamycin/ton + 300 PU/kg diet.
[b] VIR: Virginiamycin effect; PHY: Phytase effect; VIRxPHY: Virginiamycin by phytase interaction.
[c] SEM: Standard error of the mean.

Table 4.10. Nutrient retention as a percent of absorption (LSMeans, %). Experiment 2

	Treatment[a]					P-values[b]		
	B	VIR	PHY	VIR+ PHY				
Response	1	2	3	4	RMSE[c]	VIR	PHY	VIRxPHY
Energy	95.81	95.95	95.85	96.34	0.61	0.25	0.44	0.52
N	51.43	49.65	52.77	54.44	4.92	0.98	0.17	0.43
P	95.17	96.53	97.66	97.96	0.76	0.03	<0.0001	0.13
Ca	77.82	72.47	81.48	84.17	5.62	0.59	0.01	0.12
Mg	32.65	30.72	13.78	25.73	14.07	0.42	0.07	0.27
K	38.68	33.28	32.95	36.32	6.82	0.73	0.65	0.16
Na	40.10	41.57	41.59	45.91	8.28	0.43	0.42	0.69
Fe	97.99	98.50	97.31	98.16	1.38	0.27	0.41	0.77
Cu	97.39	97.45	97.39	97.51	0.44	0.63	0.88	0.85
Mn	99.72	99.66	98.50	98.18	0.85	0.62	0.003	0.72
Zn	96.60	96.53	95.04	95.59	1.70	0.75	0.11	0.67

[a] B: Basal diet; VIR: Basal + 10 g virginiamycin/ton; PHY: Basal + 750 PU/kg diet; VIR+PHY: Basal + 10 g virginiamycin/ton + 750 PU/kg diet.
[b] VIR: Virginiamycin effect; PHY: Phytase effect; VIRxPHY: Virginiamycin by phytase interaction.
[c] RMSE: Root mean square error (number of pigs: 6, 6, 5, and 6 pigs for treatments 1, 2, 3, and 4, respectively).

Table 4.11. Nutrient retention as a percent of absorption. Experiment 3

	Treatment[a]			
	B	VIR		
Response	1	2	SEM[b]	P-values
Energy	77.74	79.11	3.58	0.80
N	47.29	50.83	1.62	0.20
P	98.81	99.1	0.18	0.32
Ca	50.33	61.48	2.64	0.04

[a] Each mean represents 6 individually penned pigs. B: Basal diet; VIR: Basal + 10 g virginiamycin/ton.
[b] Standard error of the mean.

Table 4.12. Nutrient retention as a percent of absorption. Experiment 4

	Treatment[a]					P-values[b]		
	B	VIR	PHY	VIR+ PHY				
Response	1	2	3	4	SEM[c]	VIR	PHY	VIRxPHY
Energy	97.93	97.95	97.91	97.93	0.14	0.92	0.87	0.99
N	61.25	61.83	63.63	64.27	2.10	0.78	0.27	0.99
P	96.54	96.61	97.59	97.71	0.23	0.68	0.0005	0.90
Ca	55.26	53.13	67.39	68.33	2.60	0.82	0.0002	0.57
Mg	60.47	59.95	57.70	60.91	2.53	0.60	0.72	0.48
K	35.57	31.26	33.46	31.53	2.83	0.29	0.75	0.68
Na	28.48	33.93	31.76	46.43	2.45	0.002	0.007	0.08
Fe	98.22	98.68	101.10	102.11	2.09	0.73	0.16	0.90
Cu	138.14	170.11	27.59	61.42	50.91	0.53	0.05	0.99
Mn	96.43	130.57	97.19	94.53	17.24	0.38	0.33	0.31
Zn	105.34	95.10	95.97	112.49	9.49	0.75	0.68	0.18

[a] Each mean represents 6 individually penned pigs. B: Basal diet; VIR: Basal + 10 g virginiamycin/ton; PHY: Basal + 300 PU/kg diet; VIR+PHY: Basal + 10 g virginiamycin/ton + 300 PU/kg diet.
[b] VIR: Virginiamycin effect; PHY: Phytase effect; VIRxPHY: Virginiamycin by phytase interaction.
[c] SEM: Standard error of the mean.

Table 4.13. Phosphorus and nitrogen balance (LS Means). Experiment 2

	Treatment[a]					P-values[b]		
	B	VIR	PHY	VIR+ PHY				
Response	1	2	3	4	RMSE[c]	VIR	PHY	VIRx PHY
P								
Intake, g/d	7.24	7.57	7.72	7.61	0.35	0.46	0.11	0.17
Excreted (feces), g/d	4.72	4.63	2.96	2.98	0.29	0.80	<.0001	0.65
Excreted (urine), g/d	0.12	0.10	0.11	0.10	0.03	0.17	0.36	0.75
Total excreted, g/d	4.84	4.73	3.07	3.08	0.31	0.71	<.0001	0.65
Absorption, g/d	2.52	2.94	4.76	4.63	0.31	0.28	<.0001	0.06
Retention, g/d	2.39	2.84	4.65	4.53	0.30	0.23	<.0001	0.05
Digestibility (ap.), %	34.61	39.25	61.91	60.98	3.15	0.19	<.0001	0.06
Retention (as a % of intake)	32.94	37.91	60.52	59.74	3.10	0.14	<.0001	0.05
Retention (as a % of absorption)	95.17	96.53	97.66	97.96	0.76	0.03	<.0001	0.13
N								
Intake, g/d	55.02	57.56	58.66	57.89	2.70	0.46	0.11	0.18
Excreted (feces), g/d	5.57	5.28	6.14	5.62	0.76	0.24	0.19	0.74
Excreted (urine), g/d	23.79	26.62	24.63	24.01	3.15	0.43	0.52	0.22
Total excreted, g/d	29.36	31.89	30.77	29.64	3.27	0.62	0.77	0.21
Absorption, g/d	49.45	52.29	52.52	52.26	2.37	0.23	0.16	0.15
Retention, g/d	25.66	25.67	27.88	28.25	2.59	0.87	0.05	0.88
Digestibility (apparent), %	89.83	90.81	89.43	90.22	1.17	0.10	0.35	0.85
Retention (as a % of intake)	46.20	45.09	47.17	49.14	4.38	0.82	0.21	0.43
Retention (as a % of absorption)	51.43	49.65	52.77	54.44	4.92	0.98	0.17	0.43

[a] B: Basal diet; VIR: Basal + 10 g virginiamycin/ton; PHY: Basal + 750 PU/kg diet; VIR+PHY: Basal + 10 g virginiamycin/ton + 750 PU/kg diet.
[b] VIR: Virginiamycin effect; PHY: Phytase effect; VIRxPHY: Virginiamycin by phytase interaction.
[c] RMSE: Root mean square error (# pigs: 6, 6, 5, and 6 pigs for treat. 1, 2, 3, and 4, respectively).

Table 4.14. Phosphorus and nitrogen balance. Experiment 3

Response	Treatment[a]		SEM[b]	P-value
	Control	VIR		
P				
Intake, g/d	6.03	6.28	0.10	0.15
Excreted (feces), g/d	4.06	3.89	0.08	0.22
Excreted (urine), g/d	0.020	0.019	0.002	0.57
Total excreted, g/d	4.08	3.91	0.08	0.21
Absorption, g/d	1.98	2.39	0.13	0.09
Retention, g/d	1.96	2.37	0.13	0.09
Digestibility (apparent), %	32.45	37.66	1.60	0.08
Retention (as a % of intake)	32.08	37.33	1.61	0.08
Retention (as a % of absorption)	98.81	99.1	0.18	0.32
N				
Intake, g/d	46.04	47.95	0.75	0.15
Excreted (feces), g/d	4.70	4.99	0.32	0.54
Excreted (urine), g/d	22.00	21.22	0.45	0.29
Total excreted, g/d	26.69	26.21	0.41	0.45
Absorption, g/d	41.35	42.95	0.74	0.20
Retention, g/d	19.34	21.73	0.89	0.13
Digestibility (apparent), %	89.63	89.43	0.62	0.83
Retention (as a % of intake)	42.30	45.44	1.47	0.21
Retention (as a % of absorption)	47.29	50.83	1.62	0.20

[a] Each mean represents 6 individually penned pigs. B: Basal diet; VIR: Basal + 10 g virginiamycin/ton.
[b] SEM: Standard error of the mean.

Table 4.15. Phosphorus and nitrogen balance. Experiment 4

Response	Treatment[a]				SEM[c]	P-values[b]		
	B	VIR	PHY	VIR+ PHY		VIR	PHY	VIRx PHY
	1	2	3	4				
P								
Intake, g/d	6.28	6.28	6.34	6.47	0.21	0.76	0.56	0.75
Excreted (feces), g/d	4.21	4.09	3.35	3.21	0.20	0.54	0.001	0.97
Excreted (urine), g/d	0.07	0.07	0.07	0.07	0.005	0.76	0.87	0.94
Total excreted, g/d	4.28	4.16	3.42	3.28	0.20	0.55	0.001	0.97
Absorption, g/d	2.07	2.19	2.99	3.26	0.11	0.100	<0.0001	0.49
Retention, g/d	2.00	2.12	2.92	3.19	0.11	0.10	<0.0001	0.50
Digestibility (apparent), %	33.40	35.18	47.18	50.31	1.56	0.14	<0.0001	0.67
Retention (as a % of intake)	32.26	34.02	46.05	49.17	1.55	0.14	<0.0001	0.67
Retention (as a % of absorption)	96.54	96.61	97.59	97.71	0.23	0.68	0.0005	0.90
N								
Intake, g/d	45.75	45.74	46.17	47.14	1.53	0.76	0.56	0.75
Excreted (feces), g/d	4.67	4.73	4.80	4.76	0.32	0.98	0.81	0.88
Excreted (urine), g/d	15.79	15.85	15.16	15.06	1.17	0.99	0.55	0.95
Total excreted, g/d	20.46	20.58	19.96	19.82	1.28	0.99	0.63	0.92
Absorption, g/d	41.08	41.01	41.38	42.38	1.32	0.73	0.54	0.69
Retention, g/d	25.29	25.15	26.22	27.32	1.16	0.68	0.21	0.60
Digestibility (apparent), %	89.74	89.84	89.52	89.92	0.49	0.61	0.89	0.76
Retention (as a % of intake)	55.00	55.58	59.94	57.81	1.88	0.71	0.29	0.94
Retention (as a % of absorption)	61.25	61.83	63.63	64.27	2.10	0.78	0.27	0.99

[a] Each mean represents 6 individually penned pigs. B: Basal diet; VIR: Basal + 10 g virginiamycin/ton; PHY: Basal + 300 PU/kg diet; VIR+PHY: Basal + 10 g virginiamycin/ton + 300 PU/kg diet.
[b] VIR: Virginiamycin effect; PHY: Phytase effect; VIRxPHY: Virginiamycin by phytase interaction.
[c] SEM: Standard error of the mean.

Table 4.16. Apparent nutrient digestibility LS Means (%) by the total collection method and by the index method. Experiment 1

	Treatment[a]							
	Total collection		Index method		Total	Index	P-values[b]	
Response	B	VIR	B	VIR	SEM[c]	RMSE[d]	Total	Index
DM	88.99	89.93	87.02	87.29	0.29	0.94	0.05	0.59
Energy	88.28	89.12	86.31	86.53	0.27	0.90	0.06	0.33
N	88.69	89.35	87.05	87.39	0.35	1.20	0.21	0.61
P	30.37	38.81	13.82	19.62	1.11	6.83	0.001	0.15
Ca	51.51	57.32	39.19	41.65	0.96	5.30	0.003	0.40
Mg	55.09	58.15	23.05	22.48	0.75	3.10	0.02	0.74
K	85.48	85.38	78.74	80.10	1.18	3.94	0.96	0.53
Na	71.98	73.89	74.3	75.73	2.48	6.78	0.60	0.70
Fe	23.22	23.38	-9.13	-18.22	1.26	3.39	0.93	0.002
Cu	16.27	17.87	-12.93	-15.77	1.01	7.30	0.30	0.48
Mn	47.79	46.24	5.19	-5.01	2.14	15.55	0.62	0.25
Zn	21.87	25.77	-12.58	-13.41	0.70	7.46	0.004	0.84

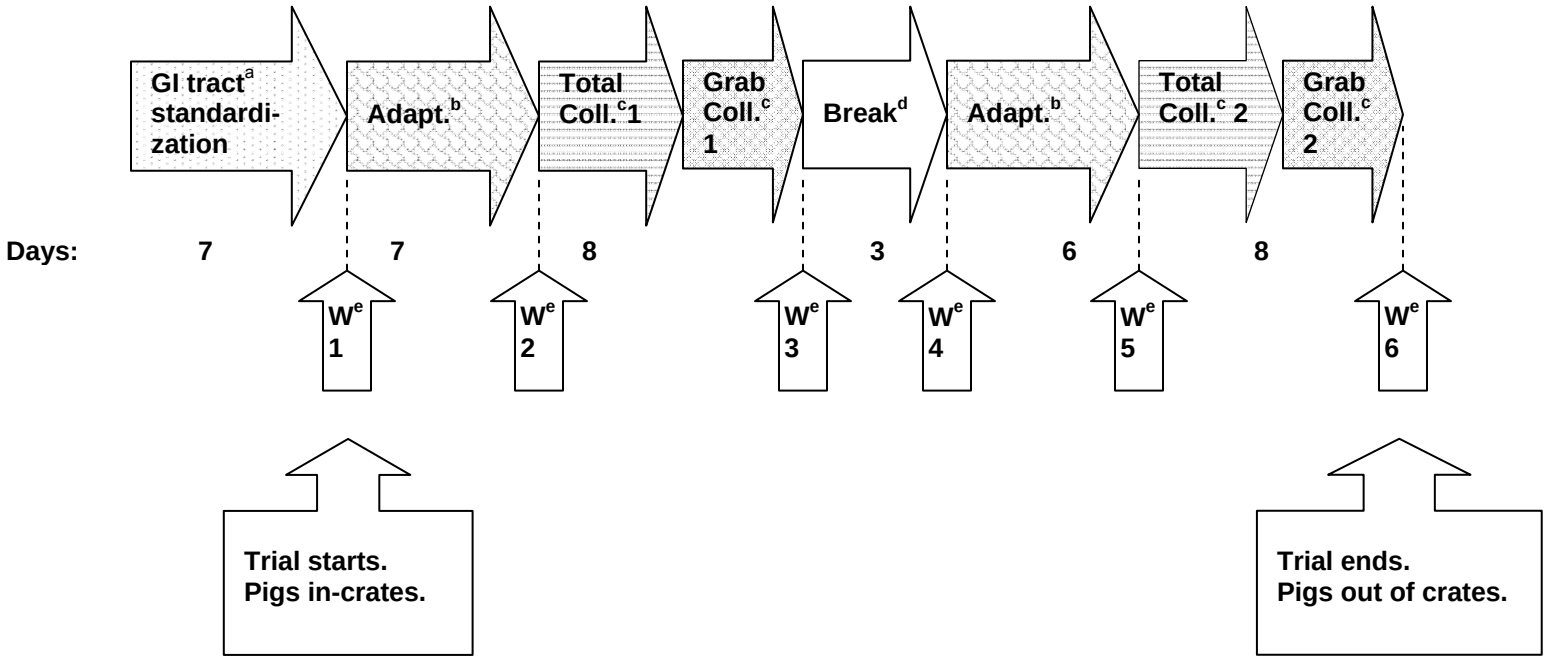
[a] B: Basal diet; VIR: Basal + 10 g virginiamycin/ton.
[b] Represents the P-value for the dietary treatment comparison within each collection method.
[c] SEM: Standard error of the mean.
[d] RMSE: Root mean square error (number of pigs: 10 and 8 pigs for the Control and VIR diets, respectively).

Table 4.17. Apparent nutrient digestibility LS Means (%) by the total collection method and by the index (CCP5) method. Experiment 2

	Treatment[a]								P-values[b]			
	Total collection			Index method (CCP5)[c]			Total	Index	PHY		VIR	
Response	1	2	3	1	2	3	RMSE[d]	SEM[e]	Total	Index	Total	Index
DM	91.61	92.14	91.82	91.13	91.43	91.60	0.74	0.19	0.59	0.08	0.15	0.34
Energy	90.85	91.38	90.52	90.89	90.95	90.62	0.074	0.20	0.32	0.29	0.13	0.62
N	89.83	90.81	89.43	90.16	90.24	89.96	1.17	0.32	0.35	0.29	0.10	0.82
P	34.61	39.25	61.91	25.90	31.02	58.18	3.15	1.98	<0.0001	<0.0001	0.19	0.70
Ca	59.49	61.69	71.27	59.99	63.28	71.66	3.09	1.12	<0.0001	<0.0001	0.90	0.88
Mg	39.79	41.41	41.24	24.59	26.40	33.83	5.03	2.28	0.88	0.01	0.94	0.77
K	90.62	90.88	91.34	87.60	88.68	88.90	2.28	0.91	0.93	0.64	0.70	0.82
Na	80.95	83.69	81.41	84.17	86.59	87.46	2.93	1.28	0.44	0.76	0.32	0.72
Fe	18.76	17.71	12.26	3.93	1.47	1.05	5.74	3.31	0.02	0.29	0.70	0.35
Cu	25.17	25.68	22.08	-5.72	-8.32	-5.08	3.14	3.39	0.01	0.70	0.68	0.58
Mn	49.01	41.88	11.01	-1.70	-12.21	-15.97	10.03	6.35	<0.0001	0.11	0.36	0.29
Zn	15.11	14.74	14.19	76.31	76.44	77.81	3.82	0.74	0.33	0.15	0.51	0.74

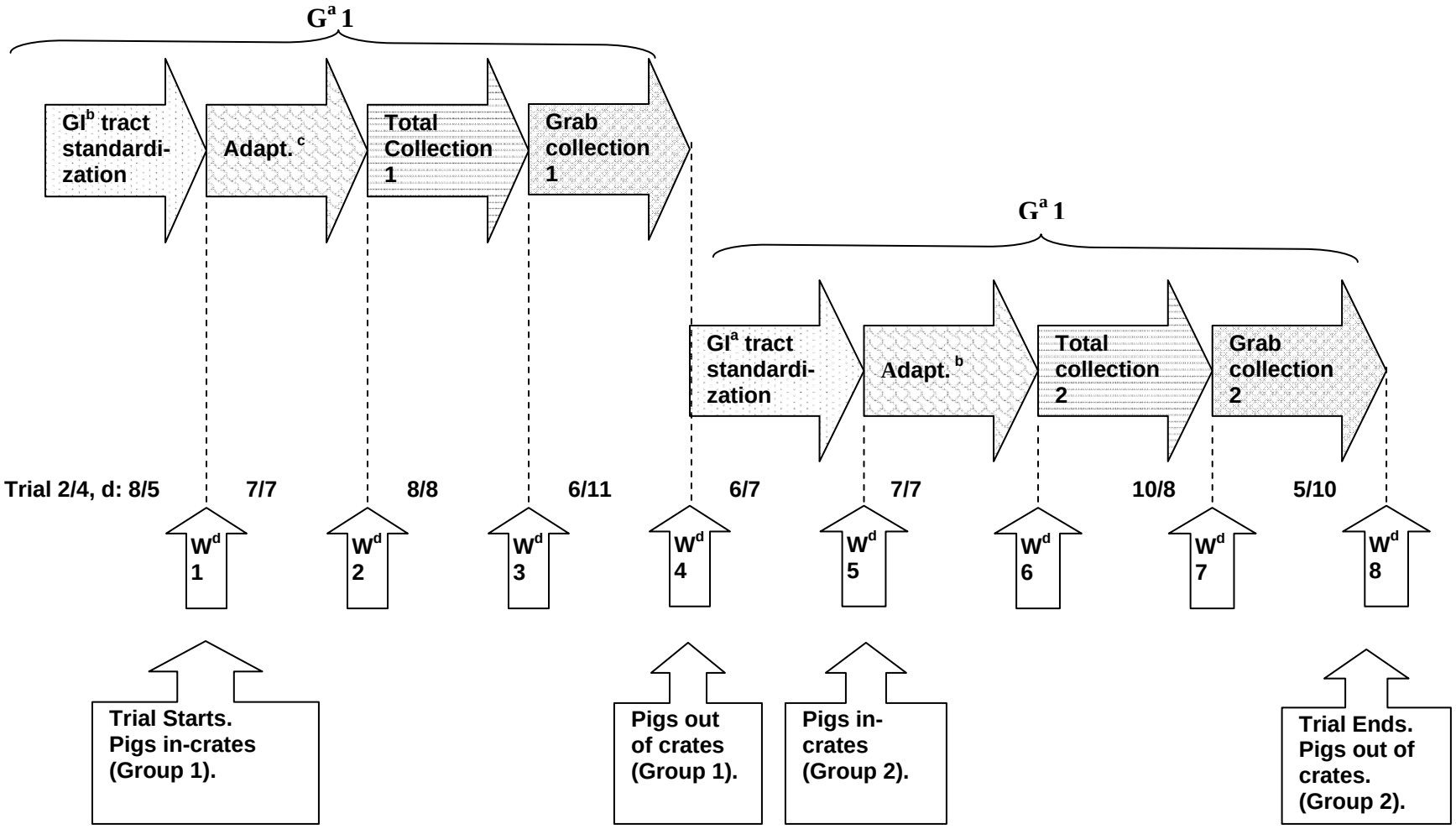
[a] 1: Basal diet; 2: Basal + 10 g virginiamycin/ton; 3: Basal + 300 PU/kg diet.
[b] Represents the P-values for the main effect of PHY and VIR within each collection method.
[c] CCP5: Cumulative collection period 5.
[d] RMSE: Root mean square error (number of pigs: 6, 6, 5, and 6 pigs for treatments 1, 2, 3, and 4, respectively).
[e] SEM: Standard error of the mean.

Figure 4.1. Schedule of events. Experiment 1



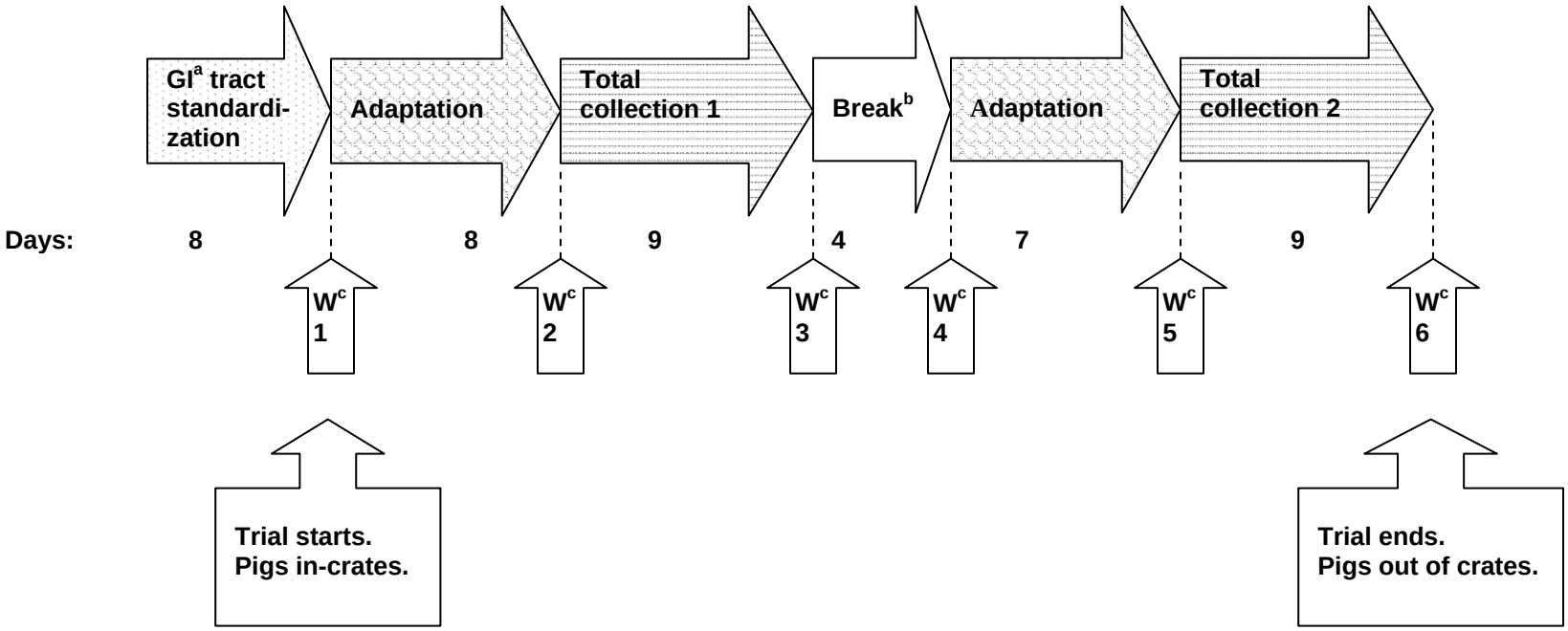
[a]Gastrointestinal tract; [b]Adaptation to amount of diet and to metabolism crate; [c]Collection; [d]Diets switched when break started; [e]Pig weighing.

Figure 4.2. Schedule of events. Experiments 2 and 4



[a]G: Group of pigs; [b]GI: Gastrointestinal tract; [c]Adapt: Adaptation to amount of diet and to metabolism crate; [d]W: Pig weighing.

Figure 4.3. Schedule of events. Experiment 3



[a]Gastrointestinal tract. [b]Pigs fed basal low-P diet during break. [c]Pig weighing.

Figure 4.4. Change in P digestibility to VIR in Experiments 1, 2, 3, and 4 based on the digestibility level of the control diets

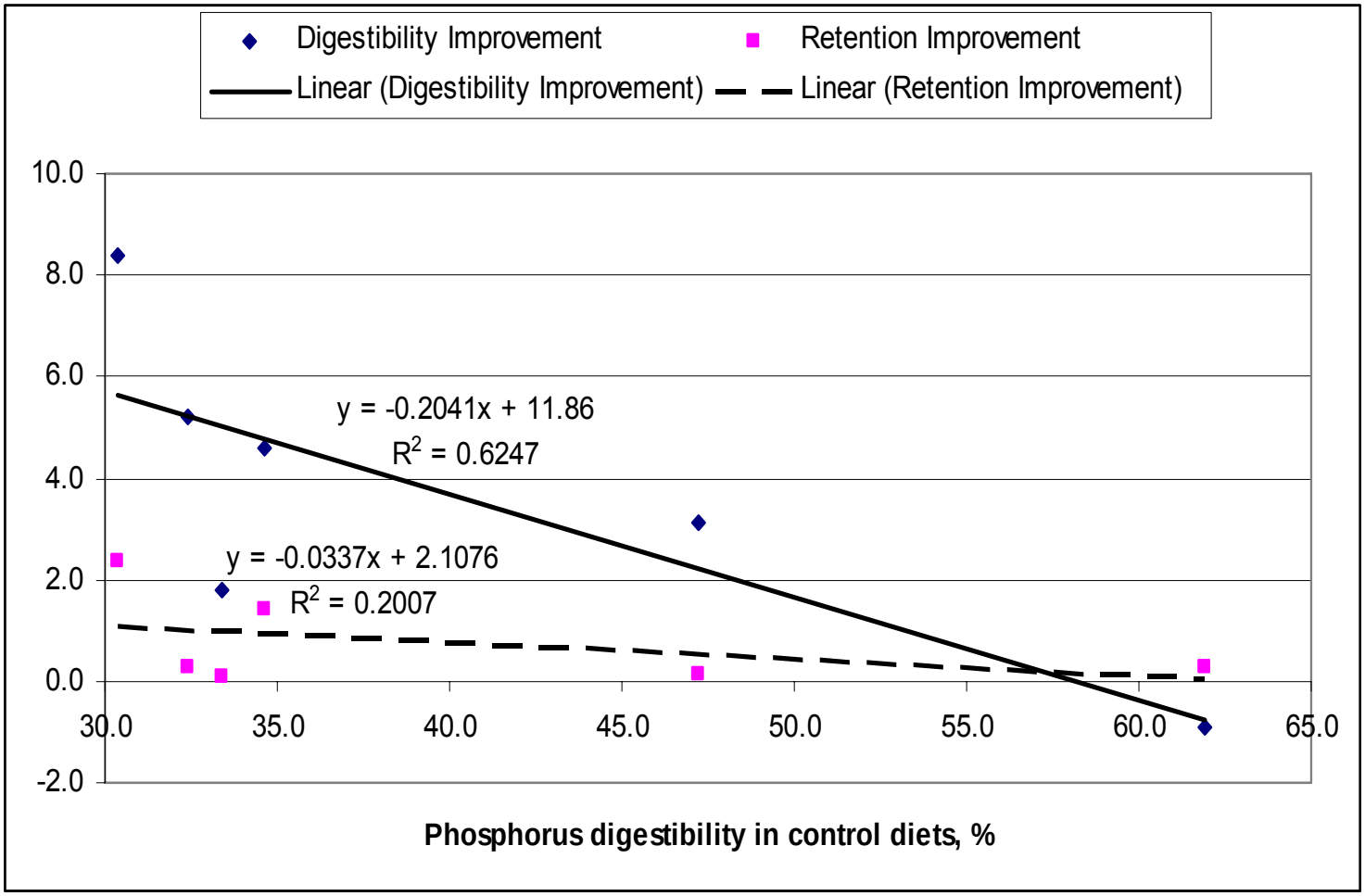


Figure 4.5. Apparent phosphorus digestibility and retention as a % of absorption (Ret. Absorption) by amending Diet 1 with VIR in the experiments

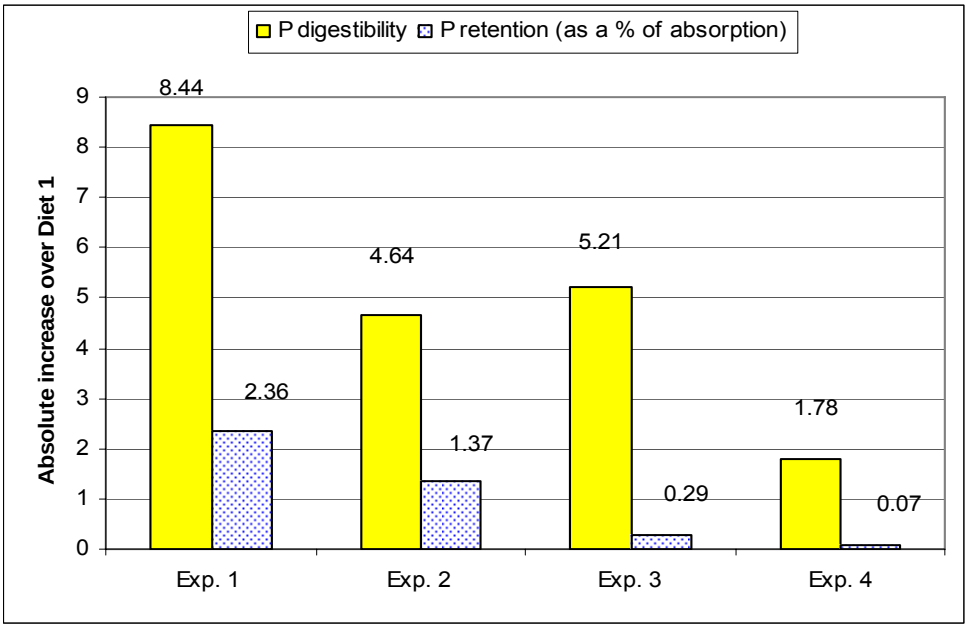


Figure 4.6. Changes in P digestibility and retention by PHY and VIR amendments in all the experiments (the diets compared from the respective experiments are listed below the experiments on the X-axis)

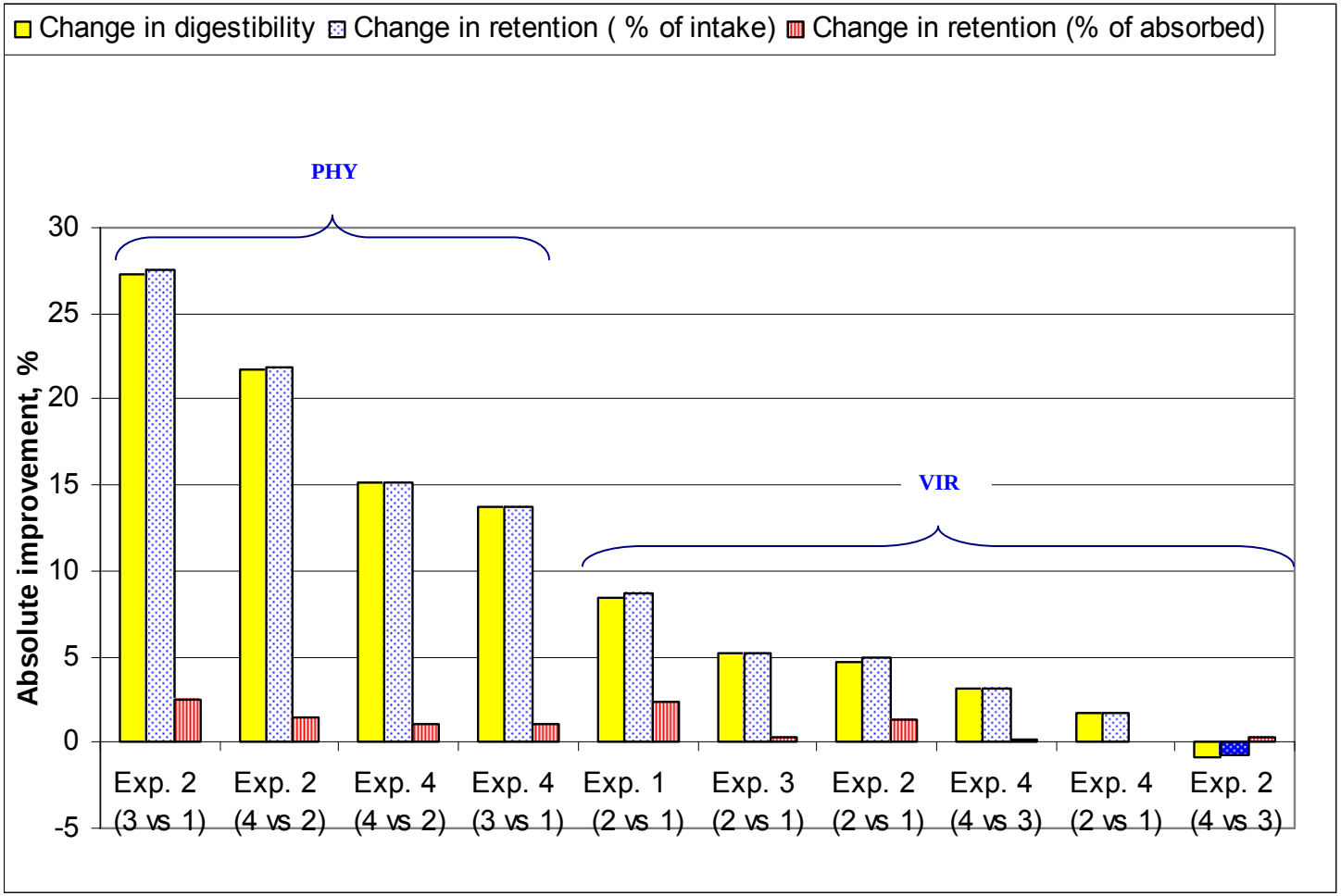


Figure 4.7. Apparent nutrient digestibility by the total collection method and index method, and differences between the methods. Experiment 1

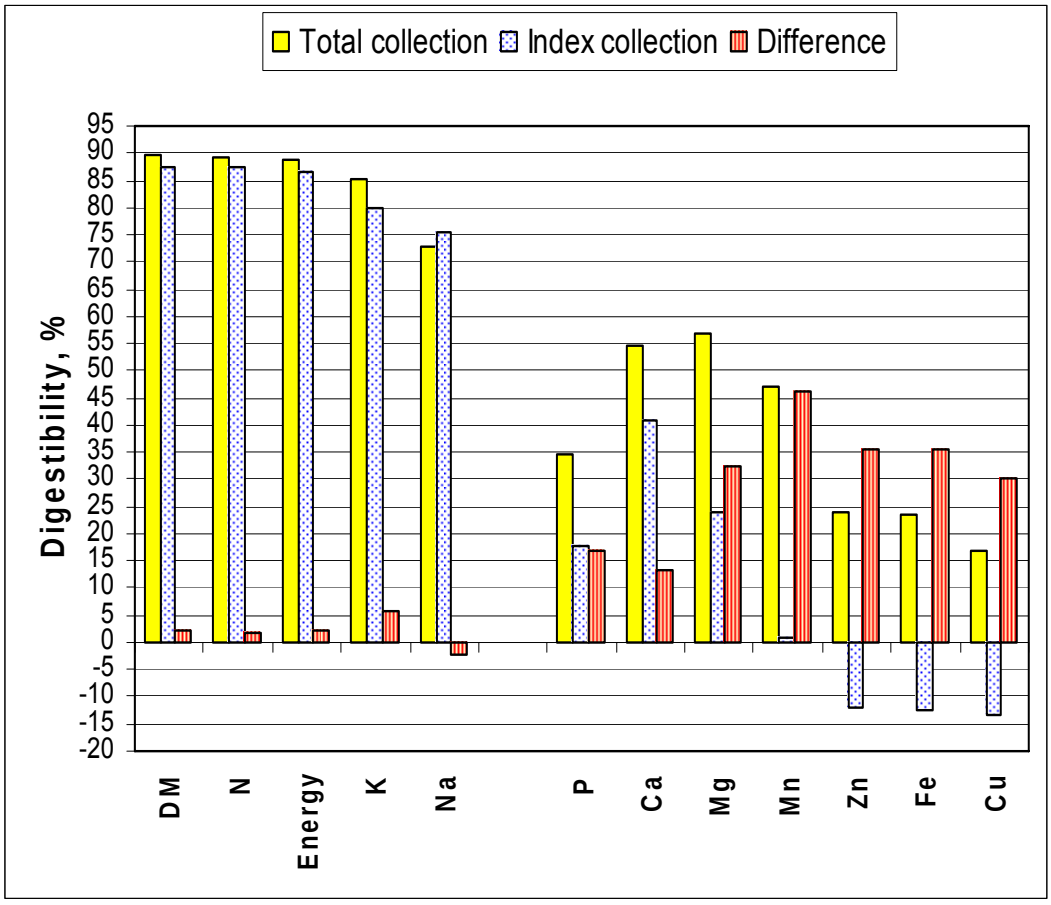


Figure 4.8. Changes in nutrient digestibility patterns with increasing cumulative collection period as measured by the index method. Experiment

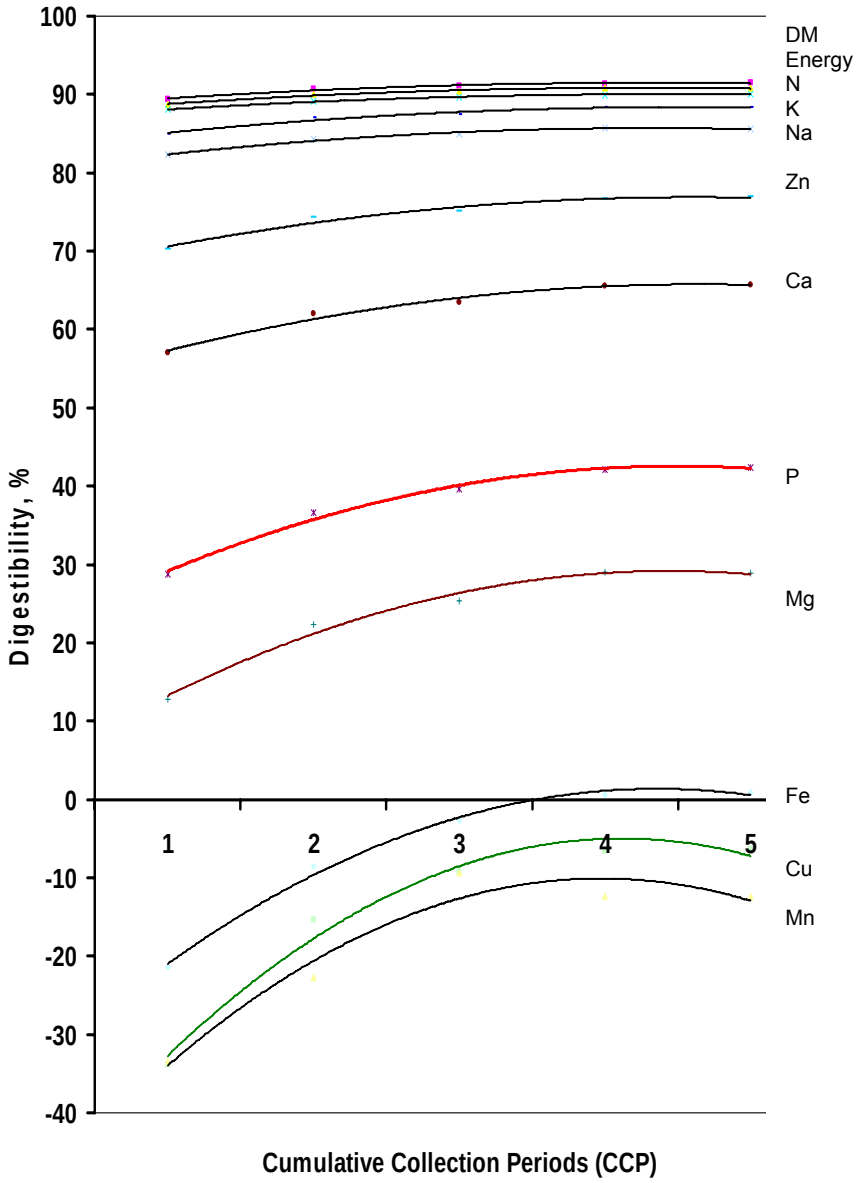


Figure 4.9. Chromium fecal excretion concentration from cumulative period 1 through cumulative period 5. Experiment 2

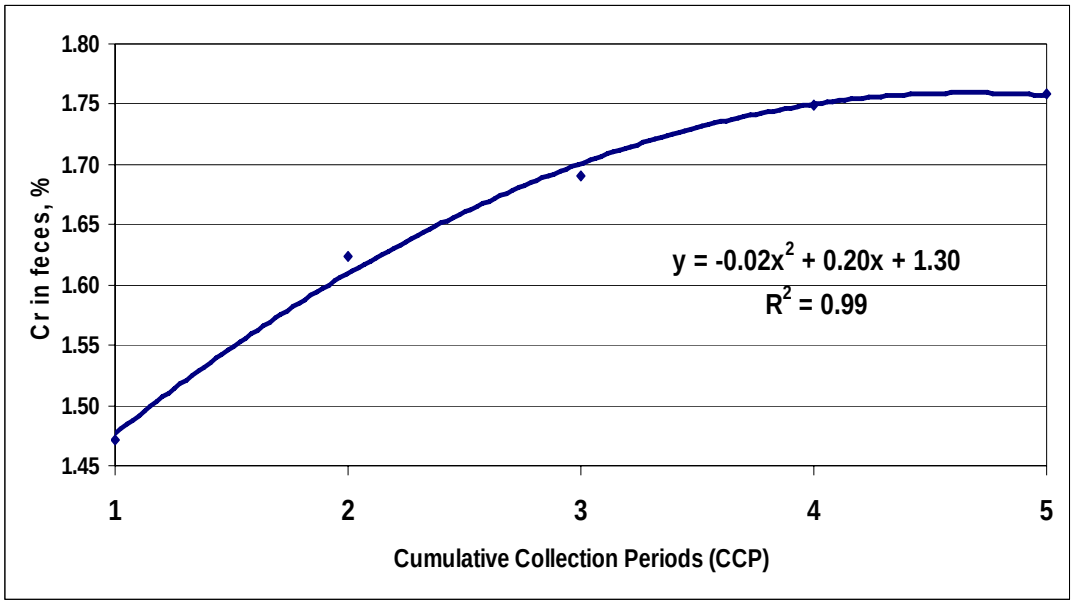
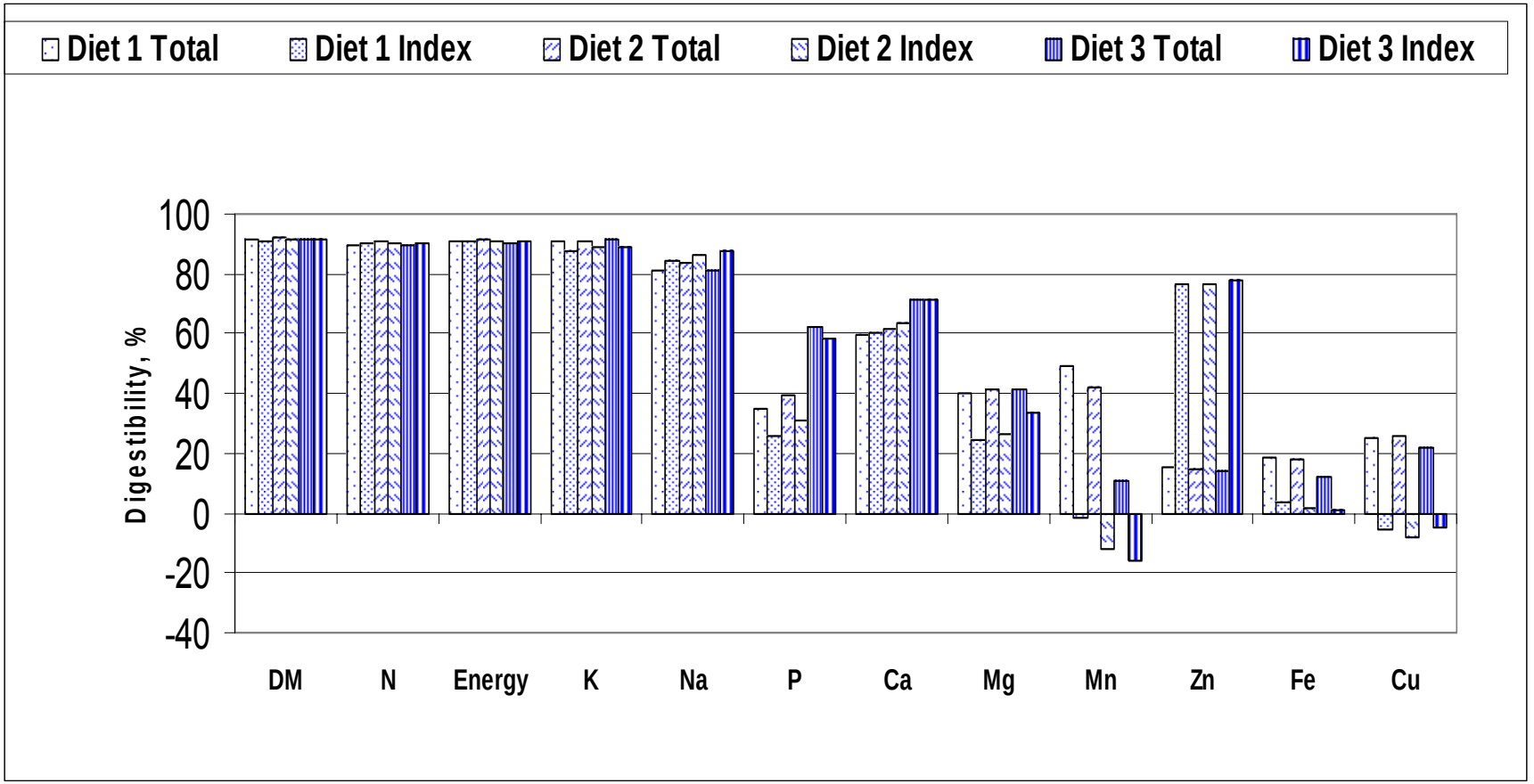


Figure 4.10. Apparent nutrient digestibility by the total collection method and the index method (cumulative collection period 5). Experiment 2



CHAPTER 5

EFFECTS OF PARTIAL DELETION OF DICALCIUM PHOSPHATE IN CONJUNCTION WITH SUPPLEMENTATION OF VIRGINIAMYCIN ON GROWTH, BONE AND CARCASS TRAITS, PORK QUALITY, AND ILEAL BACTERIAL POPULATIONS IN GROWING-FINISHING PIGS – Experiment 5

Introduction

Phosphorus is regarded as the most limiting nutrient in the eutrophication of water resources (Correll, 1998; 1999). Improperly used, swine manure represents a potential environmental risk because of its P content (Sweeten, 1991). The manure P level depends mostly on the fecal P concentration, which is relatively high in pigs fed traditional corn and soybean meal diets. Most of the P in these feedstuffs is present in the form of phytate, which is not digested by the pig, thus most phytate-P is not available and is excreted via feces. The poor digestibility of phytate-P is due to the minimal levels of phytase activity in the mucosa of the small intestine of pigs (Pointillart et al., 1987; Nys et al., 1999), and the negligible phytase activity from resident bacteria in this organ (Kornegay and Yi, 1999).

It is known that antibiotics fed at low levels generally have a growth promoting effect on animals, although their mode of action is not totally understood. It has been suggested that antibiotics such as virginiamycin (VIR) depress the growth of bacteria that compete with the host for nutrients (e.g., lactobacilli and streptococci) while increasing the populations of organisms that synthesize nutrients for the host (e. g. coliforms) (Hays et al., 1973; Cromwell et al., 1976; Vervaeke et al., 1979). It has also been reported that VIR increases absorption and retention of P in young chicks fed corn-soybean meal diets moderately deficient in P (Buresh et al., 1985) and in growing pigs fed high fiber diets (Ravindran et al., 1984).

Experiment 1 results suggested that feeding growing-finishing pigs a P-deficient corn-SBM diet supplemented with growth-promoting levels of VIR improved digestibility and retention of several nutrients, particularly P. The absolute increase in P digestibility with VIR was 8.4% (from 30.4 to 38.8%). This improvement is equivalent to the P contained in 3 lb of dicalcium phosphate (DICAL)/ton of diet (i.e. 0.15% DICAL). It is of interest to determine whether the observed increase in P digestibility is related to an increase in phytate utilizing bacteria populations in the small intestine of pigs fed the antibiotic. It is also of interest to determine if the increased P absorption and retention corresponds with an expected increment in bone mineralization.

Objectives

The main objective of this experiment was to evaluate the effects of removing 0.15% DICAL (0.028% total P) from a common corn-SBM diet supplemented with VIR (11 mg/kg) during the growing-finishing stage of pigs. The response variables evaluated were growth, bone breaking strength (BS), bone ash, carcass traits, and ileal bacterial populations.

Experimental Procedures

Animals and housing conditions

A total of 32 pigs were used (24 gilts and 8 barrows). The pigs were crossbreds of (Yorkshire x Landrace) x Hampshire. The average initial weight was 29.1 ± 0.50 SEM kg. Pigs of similar weight were selected from a larger group and then half-siblings (i.e., a common sire) were allocated to treatments. Same-sex pigs were penned by pairs, resulting in three replications of females and one of males.

The experiment was conducted in a temperature-controlled room (B-6 of the Garrigus Building) at the University of Kentucky campus. A total of 16 pens (1.22 x 2.44 m) were used. Each pen was equipped with a single-hole stainless steel self-feeder and two nipple waterers for ad libitum access to feed and water. The diets were fed in meal form. Temperature in the rooms was kept in the thermo-neutral range at all times. Pens were cleaned on a daily basis using water under pressure. The duration of the experiment was 16 weeks.

Dietary treatments

Four experimental diets were fed. Diets were prepared at the University of Kentucky feed mill. Diets were separately mixed for each stage of growth (growing, developing, and finishing). Diets were based on corn and SBM with or without VIR supplementation (11 ppm), and with or without a partial deletion of DICAL (0.15%). The DICAL product used was Dynafos® (The Mosaic Co., Plymouth, MN), guaranteed to contain 18.5% P and 20 to 24% Ca.

Diet 1 was a common corn-SBM diet with enough DICAL added to meet all NRC (1998) nutrient requirement estimates, including P. Diet 2 consisted of Diet 1 amended with 11 ppm VIR (Stafac® 20, Phibro Animal Health Co., Fairfield, NJ). Diets 3 and 4 met NRC (1998) nutrient requirement estimates, except for P. Both diets were made slightly P-deficient by deleting 0.15% DICAL. Diet 4 was amended with VIR (11 ppm). The treatments were, thus, arranged as a 2 x 2 factorial in level of DICAL and VIR.

A similar ratio of dietary Ca:available P (Ca:aP) among diets was intended for each stage of growth (0.60/0.23, 0.50/0.19, and 0.45/0.15 for the growing, developing, and finishing stages, respectively). Tables 5.1 and 5.2 present the composition of the diets used and the nutrients provided.

Sampling, laboratory analysis, and calculations

Pigs and feed left in the feeders were weighed every two weeks to calculate growth performance (gain, feed intake and feed conversion ratio). At

the end of the experiment, pigs were humanely killed by exsanguination following electrical stunning.

Ileal samples were taken immediately after slaughtering each pig to assess the bacterial profile and digesta pH. Samples consisted of a 20 cm section of the distal ileum and its contents. Sampling involved tying up both ends of the ileal section with a sterilized cotton thread, cutting the ileum portion, and transporting it over ice to the laboratory for pH determination and bacteria culturing. The quantification of phytate-utilizing bacteria populations, as colony forming units per gram of ileal contents (CFU/g), was conducted according to the procedure described by Bae et al. (1999).

The day before slaughtering, pig weight was taken for growth performance and dressing percentage calculations. Hot carcass weight was taken at slaughter. Dressing percentage was calculated using the formula:

Dressing, % = (hot carcass wt / live wt) x 100

At slaughter, front feet were cut at the knee level. The feet were kept frozen in labeled plastic bags for later metacarpal bone breaking strength and bone ash analysis. Cold carcass weight was taken after three days of refrigeration. The carcass weight loss (shrink) during refrigeration was calculated as:

Shrink, % = 100 – (cold carcass wt / hot carcass wt) x 100

After measuring cold carcass weight, back feet were cut at the hock joint for determination of metatarsal bone breaking strength and ash. At this time, a 2.52 cm-thick chop was cut from the right side of each carcass at the 10th rib, and back fat depth was measured on the chops using a ruler. Loin eye area (LEA) was measured on the chops with a plastic grid ruler. Meat color scores were taken on the chops using a Chroma-Meter CR-310 colorimeter (Konica-Minolta, Tokyo, Japan). The colorimeter was calibrated against a white plate

before taking the readings. Chops were then weighed, individually placed into plastic bags, and hung from a cord under refrigeration for drip loss evaluation by weight difference. Six days later, color scores were measured again on the same chops to measure color change. The chops were weighed again to determine drip loss as:

Drip loss, % = 100 – (chop wt at day 6 / chop wt at day 1) x 100

Collecting the third and fourth metacarpal and metatarsal bones for breaking strength assessment required thawing and then autoclaving the feet (AMSCO, American Sterilizer, Erie, Pennsylvania) at 120º C for 3 minutes (using fast-exhaust at the end of the process). Before autoclaving, feet skin was longitudinally cut to allow faster heat penetration, preventing the risk of over-cooking and undesirable bone softening. Once autoclaved, soft tissue was removed and bones were collected and stored frozen in plastic bags. To assess breaking strength, bones were thawed and broken using an Instron machine (Model TM 1123, Instron Corp., Canton, MA). In the machine, each bone was horizontally held over two supports separated 3.2 cm. from each other. Once broken, bones were kept frozen for later ash determination.

Prior to ash assessment, bones were thawed, cleaned of remaining soft tissues, and defatted. Defatting included cutting bones in half to remove the bone marrow by scooping. Bones were oven-dried overnight at 105ºC to extract additional fat , then wrapped in pairs with cheese cloth, labeled with a metal tag (poultry wing band), and placed in a metal container with petroleum ether to extract the remaining fat. Bones remained submerged in ether for at least three days, changing ether daily until the liquid remained clear of fat for 24 hours. Once defatted, bones were placed overnight in an exhaust hood to evaporate the ether, oven-dried overnight at 105ºC, taken out of the cheese cloth, weighed into pre-weighed porcelain crucibles and ashed overnight at 600ºC in a muffle furnace. Ash content was calculated as a percentage of the dry, defatted bones:

Ash, % = (ash wt / defatted bone wt) x 100

Experimental design and statistical analysis

The treatment structure consisted of a 2 x 2 factorial arrangement of the four treatments with four replicate pens per treatment (pen was the experimental unit). The analysis of variance was done using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS, 1998). The statistical model included the effects of diet and replication.

Results and Discussion

Samples of the experimental diets were analyzed for VIR concentration by Phibro Co. The results are presented in Table 5.3. As expected, the VIR concentration of the diets not amended with the antibiotic (Diets 1 and 3) was below the detection limit of the assay (< 2.2 ppm). Concentrations in Diets 2 and 4 were similar (7.0 and 9.0 ppm, respectively), although somewhat lower than expected (11.0 ppm), particularly for Diet 2.

Growth performance

Although all the animals completed the experiment, one pig lost weight during the finishing period. At slaughter, its lungs revealed pneumonia. The data from this pig were not included in the analysis, and the feed consumption of its pen was adjusted according to the method of Lindemann and Kim (2005). Also, as pigs in one pen exhibited repetitive feed wasting behavior during the experiment, feed consumption and conversion ratio calculations did not include that pen.

Growth was analyzed for the overall experiment and also for the growing, developing and finishing periods separately. No main effects of treatment on growth were found (Table 5.4). The partial deletion of DICAL did not have any

effect on gain, feed intake, or feed conversion (F/G) for the overall growing-finishing period or for any of the separate growth periods.

Similarly, no main effects of VIR on gain, feed intake or F/G were observed. No interactions were observed either between DICAL deletion and VIR supplementation ($P > 0.20$). Nevertheless, the comparison between Diets 1 and 2 showed that VIR had a tendency to improve F/G (2.92 vs. 2.83, respectively, $P < 0.08$). Although nonsignificant, there was also a numerical difference for daily gain between Diets 1 and 2, equivalent to 35 g/d (0.751 vs. 0.786). Cromwell (1976) reported that pigs fed VIR for a similar period (16 weeks) gained 50 g/d more than the control group (784 vs. 734 g/d, $P < 0.01$) and more efficiently (2.86 vs. 3.00, $P < 0.05$). It should be noted that Cromwell (1976) supplemented the pigs with 40 g VIR/ton VIR, while in Diet 2 of this experiment the target concentration was 10 g VIR/ton (11 ppm) and the assayed dietary concentration was 7 ppm.

Although the literature commonly reports experimental results where antibiotics have positively affected growth, some researchers have reported no effects of antibiotic supplementation. Hvidsten and Homb (1961) reported the results of 13 experiments using two levels (12 and 100 mg/kg feed) of aureomycin and terramycin fed to growing-finishing pigs in two different farms. On one farm, only the high level of antibiotic resulted in significant increase in gain, and a nearly significant increase in feed efficiency, while the low level of antibiotic did not. Researchers hypothesized that differences in hygienic conditions could explain the results. A similar result of no growth response was reported by Van Lunen (2003).

It is recognized that antibiotic benefits are more difficult to observe in pigs confined in clean, not overcrowded, facilities, due to the low bacterial load in the environment (Cunha, 1977; Hays, 1978). It is also known that antibiotics have a greater effect on younger pigs (i.e., nursery) than on growing-finishing pigs. The improvement in average daily gain generally observed with the addition of antibiotics to the diet for the whole growing-finishing stage is about 4.2% at research stations (Cromwell, 2001), which are considered cleaner than

commercial facilities. For finishing pigs, the improvement generally observed is reduced to 0 to 3% (Baynes and Varley, 2001).

Regarding the health condition, it has been reported that 'runt' or 'bad doer' pigs respond much better than healthy pigs to antibiotic amendments (Sainsbury, 1975). Also, Speer et al. (1950) reported that healthy, well nourished pigs did not respond to antibiotic supplementation when they were housed in clean and disinfected facilities that had not housed other pigs before. Braude et al. (1953) agreed, reporting that slow-growing pigs fed antibiotics responded better than fast-growing pigs over a series of trials.

In summary, considering that the growth response due to antibiotics appears to be attributed to their action against subclinical disease, which in turn depends heavily on the quality of the environment (Cunha, 1977), the minimal effect of VIR on growth in this experiment could be explained by a combination of good environmental confinement conditions, along with the good health of the pigs used in the trial.

With regard to the P content of the diet, the deletion of 0.15% DICAL/ton of diet was not enough to negatively and significantly affect growth, even though the deletion covered the whole growing-finishing period. These results agree with O'Quinn et al. (1997) who did not find differences in the performance of pigs (from 25 to 118 kg) fed a diet with 25% total P deleted. Other researchers have also reported no differences in growth when more radical P deletions were conducted for shorter periods of time. In some of those studies, either two thirds or 100% of the inorganic P was deleted for the last part of the finishing period without negatively affecting growth (Mavromichalis, 1999; McGlone, 2000; Shaw et al., 2002). A longer deletion of all the inorganic P was reported by Lindemann et al. (1995), who did not find adverse effects on growth after removing 100% of the DICAL for the entire finishing stage (50 to 104 kg BW).

Bone traits

The partial deletion of DICAL negatively affected bone traits (Table 5.4). It decreased both bone ash ($P < 0.01$) and breaking strength ($P < 0.05$) in

metacarpals and metatarsals. On the other hand, VIR addition did not have an effect on bone traits ($P > 0.10$). Although breaking strength was numerically improved by the addition of VIR to both normal and P-deleted diets, the diminished bone strength associated with the partial DICAL removal was not fully restored with VIR supplementation. No DICAL deletion by VIR supplementation interactions on bone characteristics were observed.

Classic studies have demonstrated that bone responses are associated with dietary levels of Ca and P, between certain limits of intake of these minerals Cromwell et al., 1970; Cromwell et al., 1972). In this experiment, bone characteristics showed consistent responses to DICAL deletion. Lindemann et al. (1995) reported reduced bone strength in finishing pigs when 100% of the DICAL was removed from the diet for the entire developing-finishing stage (50 to 104 kg BW). Nevertheless, they did not observe adverse effects on bone strength when the total DICAL deletion started at 63 kg BW. It would appear that the length of the deletion period could have a greater impact on bone demineralization than the amount of P deleted, especially for a moderate deletion such as the one tested in this experiment. On the other hand, VIR amendment did not show any effect on bone traits, either as main effect or for the DICAL-depleted diet ($P > 0.10$).

Carcass and meat traits

Among the carcass and meat characteristics evaluated, neither VIR addition nor DICAL deletion had any independent or combined effects on LEA, back fat depth, dressing percent, drip loss percent or color scores at days 1 and 6 (Table 5.5). These results agree with those of Hvidsten and Homb (1961) who did not find any differences in the quality of pork from pigs fed different antibiotics.

The lack of response to the DICAL deletion agrees with results reported by O'Quinn et al. (1997), who did not find differences in dressing percentage, backfat depth, longissimus muscle area or color scores for pigs fed a 25% total

P-deleted diet for the complete growing-finishing period. These results also agree with those of Lindemann et al. (1995), who reported no differences in back fat depth after removing all the DICAL for the finishing stage, although these researchers also reported a trend for reduced LEA as the length of the DICAL-removal period increased.

A tendency for decreased carcass water holding capacity was observed in the DICAL-deleted diet with added VIR, evidenced by an increase in carcass shrink percent from 3.52 to 4.05% when comparing Diets 3 and 4, respectively (P = 0.08). Nevertheless, the accuracy of the carcass shrink measurement can be questioned because the front limbs, cut and removed at slaughter, were not weighed and added back to the corresponding carcass cold weight three days later. As a result, carcass shrink was overestimated for all the pigs by the amount corresponding to the cut limbs.

Ileal bacterial populations

Phytate-utilizing bacteria were the intestinal organisms of greatest interest. A positive numerical increment in the number of these bacteria was observed in both the normal and P-deleted diets when VIR was added, although the differences were not statistically significant (P = 0.13) (Table 5.6). This numerical difference represents increments of 12.4% and 17.2% over the controls. No literature reports were found on the effects of antibiotics on phytate utilizing bacteria.

The addition of VIR also tended to affect lactobacilli populations (P = 0.11). Virginiamycin strongly decreased lactobacilli in the normal-P diet (P < 0.01), but it did not affect this bacterial population in the P-deleted diet (P = 0.41).

The observed results on changes in lactobacilli numbers in the normal P diet amended with VIR agree with the results of Collier et al. (2003) who reported a decrease in lactobacilli counts in pigs fed a rotating sequence of antibiotics, including VIR. Other researchers have found decreasing numbers of lactobacilli in the feces and incubated ileal contents of pigs fed different antibiotics (Andersen, 1954; Vervaeke et al., 1979). As lactobacilli are abundant populations

of acid-producing bacteria, an increase in ileal pH would also be expected to coincide with a lactobacilli population decrease. Nevertheless, no difference in pH was observed in this experiment. Collier et al. (2003) did not provide data on pH changes. On the other hand, Vervaeke et al. (1979) reported a decrease in lactobacilli numbers, along with a corresponding increase in pH for ileal contents of pigs fed VIR.

Due to known differences in populations of dominant bacterial species in the different segments of the small intestine (Fewins, 1957), and taking into account that the major site of Ca and P absorption is the jejunum (Crenshaw, 2001), it might be important for future research to also assess microbial populations and pH differences at the jejunum level.

Implications

The deletion of 0.15% DICAL had negative effects on bone traits but the magnitude of the deficiency was not enough to affect growth. The VIR amendment was not able to restore the bone de-mineralization caused by the partial DICAL deletion, although numerical improvements were observed. These numerical improvements in bone traits and phytate utilizing organisms merit additional research under the environmental conditions usually seen in commercial farms.

Table 5.1. Composition of experimental diets (%, as fed basis). Experiment 5

	Growing (20 to 50 kg)				Developing (50 to 80 kg)				Finishing (80 to 120 kg)			
Diet:	1	2	3	4	1	2	3	4	1	2	3	4
VIR:[a]	-	+	-	+	-	+	-	+	-	+	-	+
DICAL deletion:	-	-	+	+	-	-	+	+	-	-	+	+
Ingredient[b]												
Corn	72.26	72.26	72.26	72.26	79.06	79.06	79.06	79.06	84.22	84.22	84.22	84.22
SBM (48%CP)[c]	25.50	25.50	25.50	25.50	19.00	19.00	19.00	19.00	14.00	14.00	14.00	14.00
Limestone	0.76	0.76	0.76	0.76	0.66	0.66	0.66	0.66	0.69	0.69	0.69	0.69
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
UK vitamin mix[d]	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
UK trace min. mix[e]	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075
Dicalcium phosphate	0.930	0.930	0.780	0.780	0.735	0.735	0.585	0.585	0.540	0.540	0.390	0.390
Stafac® 20	-	0.025	-	0.025	-	0.025	-	0.025	-	0.025	-	0.025
Corn starch	0.025	-	0.175	0.150	0.025	-	0.175	0.150	0.025	-	0.175	0.150
Total:	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a] VIR: Virginiamycin, 11 mg/kg.
[b] Percent ingredients on as-fed basis.
[c] SBM: Soybean meal.
[d] Supplied per kg of diet: 6,608 IU vitamin A, 881 IU vitamin D_3, 22.03 IU vitamin E, 19.76 mg vitamin K, 22.03 mg pantothenic acid, 44.05 mg niacin, 4.00 mg thiamin, 8.81 mg riboflavin, 6.00 mg vitamin B_6, 22.03 mcg vitamin B_{12}, 1.10 mg folic acid, and 0.22 mg biotin.
[e] Supplied per kg of diet: 135 mg Fe (iron sulfate monohydrate), 135 mg Zn (zinc oxide), 45 mg Mn (manganous oxide), 13 mg Cu (copper sulfate pentahydrate), 1.5 mg I (calcium iodate), 0.3 mg Se (sodium selenite), and 0.23 mg Co (cobalt sulfate monohydrate).

Table 5.2. Calculated chemical composition of the experimental diets. Experiment 5

	Growing (20 to 50 kg)				Developing (50 to 80 kg)				Finishing (80 to 120 kg)			
Diet:	1	2	3	4	1	2	3	4	1	2	3	4
VIR:[a]	-	+	-	+	-	+	-	+	-	+	-	+
DICAL deletion:	-	-	+	+	-	-	+	+	-	-	+	+
Item												
CP, %	18.11	18.11	18.11	18.11	15.59	15.59	15.59	15.59	13.64	13.64	13.64	13.64
Lysine, %	0.96	0.96	0.96	0.96	0.78	0.78	0.78	0.78	0.64	0.64	0.64	0.64
ME, kcal/kg	3334	3333	3340	3339	3347	3346	3353	3352	3355	3354	3360	3360
EE, %	3.58	3.58	3.58	3.58	3.65	3.65	3.65	3.65	3.70	3.70	3.70	3.70
CF, %	3.56	3.56	3.56	3.56	3.31	3.31	3.31	3.31	3.11	3.11	3.11	3.11
tCa, %[b]	0.61	0.61	0.57	0.57	0.50	0.50	0.47	0.47	0.45	0.45	0.41	0.41
aCa, %[c]	0.50	0.50	0.46	0.46	0.41	0.41	0.38	0.38	0.38	0.38	0.34	0.34
tP, %[d]	0.55	0.55	0.52	0.52	0.49	0.49	0.46	0.46	0.43	0.43	0.40	0.40
aP, %[e]	0.23	0.23	0.21	0.21	0.19	0.19	0.16	0.16	0.15	0.15	0.12	0.12
tCa:tP	1.10	1.10	1.09	1.09	1.03	1.03	1.01	1.01	1.04	1.04	1.03	1.03
aCa:aP	2.13	2.13	2.23	2.23	2.17	2.17	2.30	2.30	2.51	2.51	2.75	2.75

[a] VIR: Virginiamycin, 11 mg/kg.
[b] tCa: Total Ca.
[c] aCa: Available Ca, calculated assuming 35.84% total Ca in limestone, assumed 100% available (NRC, 1998).
[d] tP: Total P.
[e] aP: Available P, calculated assuming 18.50% tP, and 17.58% aP in DICAL; 0.69%tP, and 0.159 aP in SBM; 0.28% tP, and 0.039% aP in corn (NRC, 1998).

Table 5.3. Virginiamycin (VIR) levels found by analysis of diets. Experiment 5

Treatment	VIR, ppm	
	Expected	Analyzed
1	0	ND[a]
2	11	7.0
3	0	ND
4	11	9.0

[a] ND: Not detected (the limit of detection was 2.2 ppm).

Table 5.4. Overall growth performance and bone responses. Experiment 5

Diet:[a]	1	2	3	4		P-Values		
VIR:[b]	-	+	-	+	SEM[c]	VIR	DICAL[d]	VIR x DICAL
DICAL deletion:	-	-	+	+				
Response								
Growth performance								
BW (initial), kg[e]	28.86	29.03	28.75	29.43	0.50	0.42	0.78	0.62
BW (final), kg	112.39	116.53	117.50	111.59	4.00	0.83	0.98	0.24
ADG, kg[f]	0.751	0.786	0.797	0.738	0.03	0.75	0.97	0.20
ADFI, kg[g]	2.195	2.227	2.29	2.142	0.04	0.50	0.95	0.30
F/G[h]	2.92	2.83	2.88	2.88	0.02	0.34	0.90	0.37
Bone response								
Metacarpal BS, kg[i]	148.8	151.3	138.8	139.8	4.41	0.71	0.04	0.86
Metatarsal BS, kg	127.7	139.0	118.9	122.7	6.31	0.26	0.08	0.57
Mean BS, kg	138.2	145.1	128.8	131.2	5.07	0.39	0.05	0.67
Metacarpal ash, %	59.5	59.7	59.2	59.1	0.12	0.54	0.002	0.17
Metatarsal ash, %	57.5	57.2	56.5	56.4	0.29	0.54	0.01	0.84
Mean bone ash, %	58.5	58.5	57.8	57.7	0.19	0.78	0.005	0.77

[a] Means for Diets 1, 2, and 3 represent 4 pens - averaging both pigs per pen (except all trait means for Diet 3, which disregarded a pneumonic pig in pen 13). Means for Diet 4 related to ADFI and F/G represent the average of 3 pens (pen 14 wasted feed, so it was disregarded for those two traits).
[b] VIR: Virginiamycin, 11 mg/kg.
[c] SEM: Standard error of the mean.
[d] DICAL: Dicalcium phosphate.
[e] BW: Body weight.
[f] ADG: Average daily gain.
[g] ADFI: Average daily feed intake.
[h] F/G: Feed/gain.
[i] BS: Bone breaking strength.

Table 5.5. Carcass and meat traits. Experiment 5

Diet:[a]	1	2	3	4		P-Values		
VIR:[b]	-	+	-	+	SEM[c]	VIR	DICAL[d]	VIR x DICAL
DICAL deletion:	-	-	+	+				
Response								
Carcass traits								
LEA, cm^{2}[e]	44.03	46.37	44.68	43.55	1.41	0.68	0.46	0.25
Back fat, cm	2.21	2.08	2.16	2.11	0.21	0.68	0.97	0.85
Dressing, %	74.47	74.75	73.67	74.65	0.46	0.20	0.35	0.46
Shrink, %	3.57	3.48	3.52	4.05	0.19	0.28	0.21	0.14
Meat traits								
Drip loss, %	6.08	6.69	6.84	7.86	0.58	0.19	0.13	0.73
Color scores								
Day 1								
L[f]	57.83	58.21	59.57	64.78	2.67	0.32	0.15	0.39
a[g]	16.95	17.31	15.72	15.09	1.18	0.92	0.18	0.69
b[h]	7.87	8.07	7.76	7.9	0.54	0.76	0.79	0.95
Day 6								
L	59.61	59.9	61.03	61.48	0.85	0.67	0.11	0.93
a	14.38	13.73	13.91	13.86	0.88	0.70	0.85	0.74
b	10.38	10.49	10.64	10.54	0.19	0.93	0.40	0.63

[a] Means represent 4 pens - averaging both pigs per pen (except all trait means for Diet 3, which disregarded a pneumonic pig in pen 13).
[b] VIR: Virginiamycin, 11 mg/kg.
[c] SEM: Standard error of the mean.
[d] DICAL: Dicalcium phosphate.
[e] LEA: Loin eye area.
[f] L: Lightness (whiter: higher number; darker: lower number).
[g] a: Red to green color (red: higher number; green: lower number).
[h] b: Yellow to blue color (yellow: higher number; blue: lower number).

Table 5.6. Ileal bacterial counts (Log10 CFU/g[a]) and pH. Experiment 5

Diet:[b]	1	2	3	4		P-Values		
VIR:[c]	-	+	-	+	SEM[e]	VIR	DICAL	VIR x DICAL
DICAL deletion:[d]	-	-	+	+				
Response								
Phytate utilizing	7.35	8.26	7.03	8.24	0.62	0.13	0.79	0.82
Lactobacilli	9.58	7.84	8.25	8.69	0.37	0.11	0.53	0.02
Total coliforms	8.11	8.34	7.93	8.34	0.60	0.60	0.88	0.88
Total anaerobes	10.00	8.61	8.95	9.38	0.68	0.50	0.84	0.21
E. coli	7.63	8.04	7.62	7.78	0.60	0.65	0.83	0.84
Bifido	9.70	8.90	9.02	9.24	0.54	0.61	0.72	0.37
pH	6.9	6.9	6.8	6.8	0.11	0.89	0.32	0.58

[a] CFU/g: Colony forming units per gram of ileal contents.
[b] Means represent 4 pens - averaging both pigs per pen (except all trait means for Diet 3, which disregarded a pneumonic pig).
[c] VIR: Virginiamycin, 11 mg/kg.
[d] DICAL: Dicalcium phosphate.
[e] SEM: Standard error of the mean.

CHAPTER 6

PHOSPHORUS UTILIZATION IN GROWING PIGS FED A PHOSPHORUS DEFICIENT DIET SUPPLEMENTED WITH A RICE BRAN PRODUCT AND AMENDED WITH PHYTASE – Experiment 6

Introduction

Rice bran is a non-traditional, widely available feed ingredient regarded as a source of energy. It has been reported that up to 30% inclusion of rice bran in diets of growing-finishing pigs does not depress growth performance and increases profit margin (Lekule et al., 2001). The energy content of rice bran is equivalent to about 85% of the net energy in corn (2,040 vs. 2,395 kcal/kg, respectively -NRC, 1998). Rice bran also has high levels of P. Among the commonly used feedstuffs for swine listed by the NRC (1998) rice bran has the highest level of total P (1.61%), equivalent to almost six times the amount present in corn (0.28%). Nevertheless, 75% of the P in rice bran is bound as phytic acid, which makes that P unavailable and excreted in pig feces. For this reason, this feedstuff has a greater P polluting potential than does corn.

Phosphorus can potentially become an environmental pollutant where inadequate manure fertilization practices are used (Sweeten, 1991; DeLaune et al. 2000; Hollis and Curtis, 2001; Strak, 2003; Cheeke, 2004). Swine diets can be supplemented with exogenous phytases in order to improve phytate P utilization, thus reducing P excretion. Most studies using phytase have evaluated the effects of the enzyme on traditional feed ingredients such as corn and SBM. However, there is little research on its effects on nutrient utilization in pigs fed alternative feedstuffs. Concerns regarding pollution of water ecosystems with P from animal manure justify a determination of the nutrient digestibilities in rice bran and of studying the effects of phytase on P utilization in pigs fed rice bran.

Objectives

This experiment was intended to establish the digestibility of P and other nutrients in a commercial rice bran product, and to evaluate the effects of increasing levels of inclusion of the product in a P-deficient corn-soybean meal basal diet on nutrient utilization in growing pigs.

Another objective was to evaluate the effects of phytase on nutrient utilization with rice bran at the highest inclusion level in the diet.

Experimental Procedures

Animals and housing conditions

A total of 24 barrows (87.5 ± 2.51kg) were used in the experiment. The pigs were crossbreds of (Yorkshire x Landrace) x Hampshire. Pigs were individually confined in metabolism crates as described in Chapter 3. Two groups of 12 pigs each were used. Half-sibling pigs (i.e., a common sire) of similar weight within a replicate were allocated to treatments and randomly assigned to crates.

Dietary treatments

Six dietary treatments were used. A basal (B) corn-soybean meal diet not supplemented with any inorganic source of P was developed. This diet was similar to the basal used in the previous digestibility studies. To prepare the experimental diets, 0, 7.5, 15, and 30% of the basal was replaced with Ricex-1000™ (RX; Ricex Company, El Dorado Hills, CA) obtaining Diets 1, 2, 3, and 4, respectively. Then, fractions of Diets 1 (0% RX) and 4 (30% RX) were blended with 750 phytase units (PU)/kg diet from Natuphos® 1200G (BASF Corp., Mount Olive, NJ) to obtain Diets 5 and 6 , respectively.

Treatments 1, 2, 3, and 4 were used to test the effects of increasing levels of RX additions on digestibility, retention and excretion of nutrients, and also to

calculate the specific RX nutrient digestibility by regression. Treatments 1, 4, 5, and 6 were used to test the effects of phytase (PHY) on the diets containing 0 and 30% RX, having Diets 1 and 4 as controls.

Ricex-1000™ consists of a mix of stable whole rice bran and germ. The manufacturer claims it includes energy in the form of vegetable fat (5,500 kcal/kg) plus soluble and insoluble fiber. The product is guaranteed to have one year of shelf life, based on its high content of natural vitamin E.

The diets were prepared at the University of Kentucky feed mill. A single batch of basal diet was mixed in a 4000-lb capacity vertical mixer. Each experimental diet was prepared as a single batch in a 2000-lb capacity horizontal paddle mixer by blending RX and/or PHY with different proportions of the basal diet. Once the pigs were weighed to start the experiment, diets were weighed as individual meals into labeled plastic bags, and kept separated by treatment. Samples of the experimental diets were analyzed for phytase concentration by a BASF Corporation laboratory. Tables 6.1, 6.2 and 6.3 present the composition of the ingredients and diets.

Adaptation and collection procedures

Nutrient digestibility was assessed by the total collection method. During the trial pigs were provided feed at 3% of body weight, in a gruel form, divided in two daily meals. The procedures followed are described in detail in Chapter 3.

Sample preparation

To obtain representative samples of urine for nutrient analysis, the daily samples were thawed at room temperature and proportionally composited by weight for each pig according to the daily excretion recorded. Composited samples were kept frozen at all times until analysis.

All frozen feces were dried in a forced-air oven (Tru-Temp, Hotpack Corp., Philadelphia, PA) at 55^{o}C for one week, then air equilibrated, weighed, and ground using a Wiley Laboratory Mill (Model 3, Arthur H. Thomas Co., Philadelphia, PA) to pass a 1 mm screen. Ground feces were then thoroughly

mixed in a single bag per pig. From this bag, a sample was obtained, re-ground using a high speed grinder (Braun, Type 4041. Model KSM 2(4). Braun Inc., Woburn, MA) and kept in a cold room at 4 to 8 °C until chemical analysis.

Laboratory analysis

Feces, experimental diets and feedstuffs (corn, SBM, and RX) were analyzed for DM, energy, fat, N, NDF, ADF, ADL, P, Ca, Mg, K, Mn, Zn, Fe, Cu, and Na. Urine was analyzed for the concentration of energy, N, P, Ca, Mg, K, Mn, Zn, Fe, Cu, and Na. Samples were analyzed at least in duplicate, and analysis was repeated when abnormal variation was observed.

Dry matter in feed and feces was assessed according to an adaptation of the AOAC (1995) method involving overnight drying (105ºC) the samples in a convection oven (Precision Scientific Co., Chicago, IL) and then calculating moisture contents as the difference between weighings.

Gross energy content was assessed by bomb calorimetry (Parr 1261 Isoperibol Bomb Calorimeter, Parr Instruments Company, Moline, IL). Benzoic acid pellets with known combustion heat were ignited at the beginning and end of each set of samples to verify calorimeter measurements. Feed and feces samples were assessed in duplicate by a procedure adapted from AOAC (1995). To measure urine energy, samples were oven dried for two days at 55ºC in polyethylene bags (Jeb Plastics Inc., Wilmington, DE) prior to combustion. The known heat of combustion per gram of bag material was subtracted from the total heat observed to obtain the sample energy content (Appendix 3 describes the procedures used to determine gross energy).

Nitrogen was measured using Dumas methodology in an automatic N analyzer (Model FP-2000, LECO Corp., Saint Joseph, MI). Ignition of blanks and EDTA samples with known N contents was done daily in order to calibrate the equipment and to check for drif in the readings.

Phosphorus in feed and feces was assessed by a gravimetric method (modification of method 968.08 from AOAC, 1990) in which samples were weighed, ashed, acid digested, diluted to 250 mL, and then 50 mL of the liquid

was reacted with Quimociac solution, filtered, and the precipitate obtained was weighed to calculate P concentration (Appendix 4 describes the P determination method used and Quimociac preparation procedure).

Phosphorus concentration in urine was assessed as inorganic P, initially by a colorimetric procedure using a commercial kit (Procedure No. 360-UVP. Sigma Diagnostics, St. Louis, MO) and then by a modified microscale method for soluble P developed at the University of Kentucky by D'Angelo et al. (2001), and described in Appendix 13.

All other minerals were assessed by Flame Atomic Absorption Spectrophotometry (AA) (Thermoelemental SOLAAR M5, Thermo Electron Corp., Verona, WI) according to a modification of the procedure from AOAC (1995b) (method 927.02), described in Appendix 5.

Fiber fractions (NDF, ADF, and ADL) were sequentially analyzed using gravimetric procedures for detergent fiber described by Harmon (2003) (Appendices 7, 8, 9, and 10). A fiber digester (Ankom 200 Fiber Analyzer, Ankom Techonology Corp., Fairport, NY) was used to separate the NDF and ADF fractions from defatted samples. Defatting of the samples prior to fiber analysis was conducted to avoid clogging of the filtration device (polymer filter bags) during the detergent procedures.

Total fat was assessed by a gravimetric method using a Soxtec Tekator fat extractor (Soxtec System HT 1043 Extraction Unit, Tecator Inc., Herndon, VA). Defatting involved weighing the sample on a filter paper (Catalog # 09-795E, Fisher Scientific, Pittsburgh, PA), placing it in a cotton thimble and immersing the thimble in re-circulating hot petroleum ether contained in the pre-weighed metal cup.

Apparent total tract digestibility and retention were calculated using the formulae in Chapter 3.

Experimental design and statistical analysis

Two sets of treatments were separately analyzed according to the objectives. Each set consisted of four diets.

The first set of treatments consisted of treatments 1, 2, 3, and 4, which had increasing levels of RX (0, 7.5, 15, and 30%, respectively) and was used to indirectly calculate the digestibility of nutrients in the RX product. For each nutrient, the estimation of digestibility in RX was done by regressing the percent of digestibility in these experimental diets on the percent of the nutrient provided by RX to each diet. The formula used to calculate the percent of the nutrient provided by RX was:

Nutrient provided by RX to each experimental diet, % =

$$100 \left[\frac{(\%\text{Nut. BD} \times \%\text{BD})}{(\%\text{Nut. BD} \times \%\text{BD}) + (\%\text{Nut. RX} \times \%\text{RX})} \times 100 \right]$$

%Nut. BD: percent nutrient in the basal diet.

%BD: percent basal diet included in the experimental diet.

%Nut. RX: percent nutrient in RX.

%RX: percent RX included in the experimental diet.

The composition of the basal diet was calculated as the average of Diets 1 and 5. The nutrient contents in the basal diet as well as in RX were analyzed values. The numbers obtained with this formula were plotted against the coefficients of digestibility observed in each diet, in order to obtain regression equations to calculate digestibility in a hypothetical diet consisting of 100% RX product.

The digestibility responses observed in Diets 1, 2, 3, and 4, and the corresponding fraction of nutrients provided by RX in those diets, were also tested for linearity (linear and quadratic trends) using the GLM regression

procedure of SAS. As the fractions of nutrients provided were not in a regular scale across the diets (Table 6.5), procedure IML of SAS was conducted to generate the set of contrast coefficients to be used in the regression of each nutrient.

The second set of treatments was: 1) Basal; 4) Basal + 30% RX; 5) Basal + PHY; and 6) Basal + 30% RX + PHY. This group was analyzed as a 2 x 2 factorial for the main effects of RX (0 or 30%), PHY (0 or 750 PU/kg diet), and the interaction between RX and PHY. The analysis of variance was obtained using the GLM procedure of SAS (SAS, 1998).

Results and Discussion

All the animals successfully completed the experiment. All pigs gained weight during collections, suggesting that all of them were in positive nutrient balance during the experiment (Appendix 11 presents the average starting and finishing weights of treatment groups during the adaptation and collection periods). Pigs were in good health and condition during the experiment. No intestinal disorders such as diarrhea or constipation were observed either during the adaptation or collection periods. Some pigs exhibited minor bruises on rear feet during the confinement, but no abnormal behavior, feed consumption or defecation patterns were observed. Growth performance during collections is not reported as the reliability of differences in growth resulting from such short periods is questionable due to the possible effect of differences in gut fill at weighing times.

According to the lab assay, PHY concentrations in diets not amended with the enzyme (Diets 1, 2, 3, and 4) was below the detection limit of the assay. The PHY concentration of Diet 5 was about 17% lower than that of Diet 6, and both were not far from the 750 PU/kg target (Table 6.4).

Increasing levels of inclusion of Ricex-1000™ in the diet

The increasing levels of several nutrients in Diets 1 through 4 (i.e., gross energy, fat, fiber, and P) were the result of the increasing levels of added RX. As expected, Diets 1 and 5 had a very similar composition. Similarly, Diets 4 and 6 were very close in composition (Table 6.3).

Table 6.5 presents the calculated nutrient contribution of RX to Diets 1, 2, 3, and 4. According to the analysis, RX contains 21.0% fat, 2.46% N (15.4% CP), 19.2% NDF, and 1.75% P. The NRC (1998) estimates that rice bran contains 13.0% fat, 2.13% N (13.3% CP), 23.7% NDF, and 1.61% P. Comparing the RX product with the NRC (1998) estimate, the major difference is that the RX product contains about 82% more fat, while the levels of fiber, N and P are somewhat similar. The primary reason for the difference is that RX contains some of the rice germ. From Table 6.5, it was calculated that at 30% inclusion of RX (Diet 4), this product contributes about 78% of the total fat, 66% of the total P, and about half of the fiber in the diet.

Table 6.6 presents the digestibility coefficients obtained for Diets 1, 2, 3, and 4. The digestibility of several dietary components, including DM, energy, N, fiber, Ca, and P tended to decrease as the proportion of RX in the diet increased from 0 to 30%. The degree of depresion in digestibility was much more marked for DM, energy, N, fiber, and Ca ($P < 0.001$). Phosphorus digestibility also tended to decrease linearly with increasing amounts of RX ($P < 0.05$). The quadratic trend for this nutrient was non sigificant ($P = 0.80$).

The results agreed with those of Campabadal et al. (1976) who reported an almost linear reduction in DM and CP digestibility when increasing levels of rice bran (0, 20, 25, 30, 35, 40, and 45%) were included in a corn-SBM diet for finishing pigs.

These results are in general agreement with most research, which demonstrated an inverse relationship between the level of dietary crude fiber and digestibility coefficients for various nutrients in growing pigs (Schulze et al., 1994;

Lenis et al., 1996; Phuc et al., 2000; Le Goff and Noblet, 2001; Souffrant, 2001; Wenk, 2001). Ranhan et al. (1971) reported that growing pigs fed increasing levels of crude fiber (4.0, 6.8, 8,6, and 11.0%) exhibited an indirect relationship between DM digestibility and the crude fiber content of the diet. The DM digestibility started to be negatively influenced at a dietary level of 6.8% crude fiber. Other researchers have reported similar findings when the dietary level of cellulose was increased. Farrel and Johnson (1970) reported a decrease in DM and energy digestibility in growing pigs fed diets containing 8 and 26% cellulose. Gargallo and Zimmerman (1981) also reported decreased DM, N, and cellulose digestibility with increasing levels of cellulose in the diet. Kornegay (1978) substituted a basal corn-oats-alfalfa meal-SBM diet with 15 and 30% soybean hulls for growing pigs, finding that as the hulls were substituted for the basal diet, digestibility coefficients for DM, energy, CP, and fat were decreased, while ADF digestibility increased. Lindemann et al. (1986) also reported decreased DM, N, energy, ash, and fiber digestibility with graded levels of peanut hulls (0, 7.5, 15, and 30%) included in the diet of finishing pigs.

Several possible modes of action have been proposed to explain the decreased digestibility caused by fiber. Some researchers have explained it as a physical entrapment of the nutrient in the bulk of the bolus, with consequent inaccessibility to enzyme action (Bailey, 1974 ; Bursch et al., 1986). It has also been reported that high fiber diets tend to increase the rate of passage through the alimentary canal, decreasing the opportunity for enzymatic digestion and absorption (Gargallo and Zimmerman, 1981; Wenk, 2001). Nevertheless, the increased rate of passage is not always observed (Lindemann et al., 1986). Apparently, fiber can reduce the digestibility of DM and energy because of its resistance to digestion by the endogenous enzymes secreted into the small intestine (Bach-Knudsen et al., 1991), or probably because of the increased viscosity of the intestinal contents produced by certain fiber components, such as gums (Rainbird et al., 1984). There is conflicting evidence in the literature regarding the modes of action of fiber in the digestive tract. Bach-Knudsen

(2001) explains some of the disagreements between experiments as due to the several different fractions that constitute dietary fiber, the different proportions of these fractions present in the feed ingredients used, and the different physiological effects of these fractions.

In regards to the magnitude of the impact of fiber on energy digestibility; in a series of digestibility studies using a variety of fiber sources, including rice bran substituted at 25% in the diet, Le Goff and Noblet (2001) found that the energy digestibility in growing pigs is reduced by one percentage point for each one percent additional NDF in the diet. In this experiment, although depressed, energy digestibility was not affected by RX addition to the extent estimated by Le Goff and Noblet (2001).

Contrary to the trend in energy digestibility, fat digestibility increased linearly ($P < 0.01$) with increasing levels of RX. These results agree with Campabadal et al. (1976), who reported increased digestibility of EE when increasing levels of rice bran were added to the diet. The effect of increased fat digestibility probably reflects the increasing level of fat intake. In a recent review of experiments with horses, where various feeds were tested, Kronfeld et al. (2004) reported an exponential increase in apparent digestibility of fat as fat content of the diet increased. They also reported a linear ($P < 0.001$) relationship between fat absorbed (g/d), and fat intake (g/d) for 23 different feeds.

In this experiment, the lignin content of Diets 1 through 4 increased (Table 6.3), reflecting the RX substitution levels. The apparent digestibility coefficients of lignin decreased as the lignin contents increased, but the values were relatively high for all the diets, ranging from 68.5 to 37.3%. Although lignin is generally considered an indigestible material (Kotb and Luckey, 1972; Schneider and Flatt, 1975), other researchers have observed relatively high digestibility coefficients for lignin. Kornegay (1978) reported 44.1 to 51.2% digestibility coefficients for lignin in his diets containing 15 and 30% SBM hulls, respectively. Lindemann et al. (1986) reported 42.2, 32.4, 30.7, and 21.3% digestibility coefficients for lignin in a basal corn-SBM diet substituted with 0, 7.5, 15, and 30% peanut hulls for finishing pigs.

Table 6.7 presents retention data (as a % of absorption) for all nutrients assayed. Calcium and Mg were the only nutrients that exhibited a significant trend ($P < 0.01$), with increased retention as the proportion of RX increased in the diet. The increase in Ca retention was likely the response of the pig to the observed decrease in apparent digestibility of the mineral (Table 6.6). The intake of this mineral decreased linearly with increasing levels of RX ($P < 0.01$), due to the fact that the whole basal diet, which included the limestone supplementation, was substituted with RX. As a result, the level of Ca inclusion was reduced from 0.60% in Diet 1 to 0.43% in Diet 4. Total Ca intake per day dropped linearly ($P < 0.01$) from 16.5 g in Diet 1 to 13.0 g in Diet 4. Nevertheless, this 21% difference in Ca intake is less than half the difference observed in the amount of Ca absorbed, which dropped 45%, in a linear manner ($P < 0.01$), from 7.7 to 4.2 g/d for Diets 1 and 4, respectively. It can be assumed that the decrease in absolute absorption of Ca was not only due to a decrease in absolute intake, but also to a concomitant decrease in Ca digestibility, which dropped linearly ($P < 0.01$) from 46.8 to 32.7% for Diets 1 and 4, respectively. The decrease in Ca intake and absorption apparently led to an increase in retention, as evidenced in urinary Ca excretion, which decreased from 2.8 to 0.6 g/d for Diets 1 and 4, respectively.

Complete balance data are provided for N and P, the two nutrient elements of primary interest from an environmental stand point (Table 6.8). As expected, total P intake/day increased as RX increased. Because most of this P was phytate P, and no phytase was supplemented to these diets, a simultaneous linear increase in fecal excretion was also observed. Fecal P excretion was 118% higher for Diet 4 than for Diet 1 ($P < 0.01$), raising questions on its potential environmental impact. Phosphorus retention (% of intake) decreased linearly ($P < 0.5$) with greater RX, reflecting the same trend observed in the digestibility for this nutrient ($P < 0.05$). The retention (% of intake) and digestibility data were closely related across these diets, which is related to the tight control of urinary P excretion by pigs eating P-deficient diets. This is further supported by the observed lack of increase in urinary P ($P = 0.97$) with increasing RX supplementation. It is interesting to note that P digestibility is not always

depressed with fiber supplementation. Kornegay et al. (1995) reported a linear increase in apparent P digestibility by weanling pigs fed increasing levels (0, 8, or 16%) of peanut hulls added to a corn-SBM diet.

The N intake decreased as RX supplementation increased ($P < 0.05$), reflecting the lower CP content of RX, in comparison to SBM. The amount of fecal N linearly increased ($P < 0.01$), but the urinary N did not change ($P = 0.36$). Fecal N was 24% higher for Diet 4 than for Diet 1, which raises concerns regarding greater environmental N problems with high levels of RX in pig diets. The higher fecal N excretion observed agrees with the findings of Lenis et al. (1996), who reported an increase in N excretion in the feces of growing pigs fed semi-purified diets with 15% added NDF. The urinary excretion of N reportedly decreased with the NDF-added diet, which was not observed in this experiment.

Nutrient digestibility in Ricex-1000™

Table 6.6 also presents the estimated digestibility coefficients for nutrients contained in RX. The coefficients used in the regression analysis, as well as the variables used to calculate these coefficients are presented in Appendix 12. Compared to the basal diet, RX was estimated to have lower digestibility values for most nutrients, including P. Digestibility of Ca and Na in RX were estimated to be negative. Several methods of excluding portions of the data were attempted (in the event that these results were dependent on a single treatment or individual pig), but both values were negative for all combinations of data from the four diets used to calculate digestibility in the RX product. A possible reason for the observed negative values for these two nutrients could be the low amounts provided by RX in these experimental diets (Table 6.5), which is in agreement with observations by Schneider and Flatt (1975). Additionally, the Ca:P ratio in the diets may have contributed to the low Ca digestibility observed. It is possible that osmotic imbalances in the gut, derived from the increased fiber intake, may explain the negative digestibility observed for Na (Lindemann et al., 1986).

Digestible and metabolizable energy (DE, and ME, respectively) in the RX product were estimated by regressing the energy contents (DE or ME, in kcal/kg) on the percent of RX substituted in each of the first four diets (Diets 1, 2, 3, and 4). The linear regression estimates were 3967 and 3869 kcal/kg of RX for DE and ME, respectively (on 'as fed' basis).

Phytase amendment of the diet containing 30% Ricex-1000™

Table 6.9 presents the digestibility coefficients for the lowest and highest levels of RX substitution (0 and 30%), amended with 0 and 750 PU, and the corresponding main effects of RX and PHY, and their interaction effect (if any). Phytase amendment increased the digestibility of P, Ca, and fat at both low and high levels of RX substitution. The PHY main effect was strongly significant ($P < 0.01$) for P and fat digestibility, and moderately significant ($P < 0.05$) for Ca digestibility. The relative increase in P digestibility due to the PHY amendment of the 0% RX diet was 87% (from 25.5 to 47.4), and was 81% (from 20.4 to 37.0) in the 30% RX diet. It is possible that the comparatively lower improvement observed in the 30% RX diet was due to an insufficient level of PHY for cleaving all the phytic P present in this diet. Nevertheless, on a grams/day basis, P absorption in the 30% RX diet amended with PHY almost doubled (4.73 to 8.04 g/d) the increase observed in the 0% RX diet amended with the enzyme (2.49 to 4.12 g/d) (Table 6.11).

The magnitude of increase in Ca digestibility due to PHY amendment was 4.1% for the 0% RX diet, and 10.9% for the 30% RX diet. The magnitude of increase in fat digestibility due to PHY amendment was 6.3% for the 0% RX diet, and 3.3% for the 30% RX diet.

Several researchers have reported increased P digestibility in common diets amended with PHY (Jongbloed et al., 1992; Cromwell et al., 1993; Lei et al., 1993; Mroz et al., 1994; Cromwell et al., 1995a; Yi et al., 1996; Han et al., 1997), but the literature is scarce on research using rice bran and the enzyme.

No research reports were found regarding total tract apparent digestibility of fat when PHY was supplemented to pigs. Akyurek et al. (2005) reported that

broiler chicks fed corn-soybean meal diet supplemented with PHY had improved ileal crude fat digestibility. Ravindran et al. (2001) reported that mineral-phytate complexes may contribute to the formation of insoluble metallic soaps in the gastrointestinal tract, which is a constraint on lipid utilization. By preventing the formation of mineral-phytate complexes, PHY may reduce the degree of soap formation in the gut, enhancing fat utilization (Ravindran et al., 2001).

In this experiment, PHY did not have any effect on the apparent total tract digestibility of N (P = 0.44) or on N retention as a percent of absorption (P = 0.40), which agrees several reports (Yi et al., 1996; Han et al., 1997). Ketaren et al. (1993) reported that PHY addition to diets of growing pigs did not have any effect on the apparent digestibility of protein, altough they observed an increase in N retained as a percent of intake. On the other hand, other researchers have reported a positive effect of PHY on N digestibility (Mroz et al., 1994; Kemme et al., 1999; Zhang and Kornegay, 1999) and retention (Ketaren et al., 1993; Mroz et al., 1994; Li et al. 1998) in pigs.

In regard to P retention, PHY did not increase retention as a percent of absorption (P = 0.17), although there was a numerical difference between Diets 1 and 4, favoring PHY. The enzyme amendment improved absolute retention of P, and decreased P excretion in both diets (P < 0.01) (Table 6.11).

Implications

According to these results, the estimated digestibility coefficients for most RX-derived nutrients in growing pigs were lower than those for a basal low-P corn-SBM diet. Compared to the basal diet, digestibility of the RX product was very low for DM, energy, CP, and P (relative differences of: 80, 88, 83, and 59%, respectively). Digestibility was particularly low for Ca (negative coefficient). For this reason, the inclusion of increasing levels of RX in a basal corn-SBM diet for growing pigs would be expected to linearly decrease the digestibility of nutrients. The exception is the digestibility of the fat fraction, which is expected to increase

with increasing levels of the product in the diet. The amendment of a corn-SBM low P diet containing 30% RX with 750 PU/kg will increase P digestibility. Further research is required to define the optimum level of PHY amendment to such diets in order to release the maximum amount of phytic P.

Table 6.1. Basal diet composition. Experiment 6

Ingredient	%		
Corn, ground	76.925		
Soybean meal (48% CP)	21.00		
UK vitamin mix[a]	0.100		
UK trace mineral mix[b]	0.075		
Limestone	1.400		
Phosphate source	0.0		
Salt	0.500		
Total:	100.000		
		NRC (1998) requirement estimates	
Calculated composition		50 to 80 kg	80 to 120 kg
Crude protein (%)	16.36	15.5	13.2
Lysine (%)	0.83	0.75	0.60
ME (kcal/kg)[c]	3341	3265	3265
Calcium (%)	0.60	0.50	0.45
Phosphorus, total (%)	0.36	0.45	0.40
Phosphorus, available (%)	0.06	0.19	0.15

[a] Supplied per kg of diet: 6,608 IU vitamin A, 881 IU vitamin D_3, 22.03 IU vitamin E, 19.76 mg vitamin K, 22.03 mg pantothenic acid, 44.05 mg niacin, 4.00 mg thiamin, 8.81 mg riboflavin, 6.00 mg vitamin B_6, 22.03 mcg vitamin B_{12}, 1.10 mg folic acid, and 0.22 mg biotin.
[b] Supplied per kg of diet: 135 mg Fe (iron sulfate monohydrate), 135 mg Zn (zinc oxide), 45 mg Mn (manganous oxide), 13 mg Cu (copper sulfate pentahydrate), 1.5 mg I (calcium iodate), 0.3 mg Se (sodium selenite), and 0.23 mg Co (cobalt sulfate monohydrate).
[c] Metabolizable energy.

Table 6.2. Calculated composition of the experimental diets[a]. Experiment 6

Trt:	1	2	3	4	5	6
Rice bran, %:	0	7.5	15	30	0	30
PHY, U/kg:[b]	-	-	-	-	750	750
Item						
CP, %[c]	16.36	16.22	16.08	15.80	16.35	15.80
Lysine, %	0.83	0.81	0.79	0.75	0.83	0.75
ME, kcal/kg[d]	3340	3303	3266	3193	3338	3191
EE, %[e]	3.63	4.89	6.16	8.69	3.63	8.69
CF, %[f]	3.39	5.31	7.23	11.07	3.38	11.07
tCa, %[g]	0.60	0.56	0.51	0.43	0.60	0.43
aCa, %[h]	0.50	0.46	0.43	0.35	0.50	0.35
tP, %[i]	0.36	0.45	0.54	0.73	0.36	0.73
aP, %[j]	0.06	0.09	0.11	0.17	0.06	0.17
tCa : tP	1.66	1.23	0.94	0.59	1.66	0.59
aCa : aP	7.90	5.22	3.73	2.13	7.91	2.13
Na, %	0.22	0.20	0.19	0.15	0.22	0.15
Cl, %	0.34	0.32	0.30	0.26	0.34	0.26
K, %	0.70	0.77	0.83	0.96	0.70	0.96
Mg, %	0.18	0.22	0.27	0.35	0.18	0.35
Fe, mg/kg	194	185	177	159	194	159
Cu, mg/kg	19.5	18.3	17.0	14.5	19.5	14.5
Mn, mg/kg	57.9	72.8	87.7	117.4	57.9	117.4
Zn, mg/kg	160	149	137	114	160	114

[a] Based on rice bran calculated composition (NRC, 1998).
[b] PHY: Phytase from Natuphos® 1200G.
[c] CP: Crude protein.
[d] ME: Metabolizable energy.
[e] EE: Ether extract.
[f] CF: Crude fiber.
[g] tCa: Total Ca.
[h] aCa: Available Ca.
[i] tP: Total P.
[j] aP: Available P.

Table 6.3. Analyzed nutrient composition of the experimental diets, the basal diet, and the feedstuffs used. Experiment 6

	Experimental diets						Basal[a]	Feedstuffs		
Trt:	1	2	3	4	5	6	diet	Corn	SBM	RX
RX, %:[b]	0	7.5	15	30	0	30	-	-	-	-
PHY:[c]	-	-	-	-	+	+	-	-	-	-
Item										
DM, %[d]	87.9	88.3	88.9	90.2	87.8	90.3	87.9	86.5	89.2	95.0
Gross energy, kcal/kg	3915	3988	4082	4237	3926	4269	3920	3916	4162	5051
Fat, %	2.5	4.0	5.4	8.3	2.7	8.7	2.6	3.2	0.7	21.0
N, %	2.9	2.8	2.7	2.7	2.9	2.8	2.9	1.5	8.4	2.5
NDF, %[e]	9.2	9.9	10.7	12.6	8.6	13.0	8.9	12.4	15.4	19.2
ADF, %[f]	2.7	3.2	3.8	4.6	2.6	4.7	2.7	3.1	6.1	8.3
ADL, %[g]	0.3	0.4	0.5	0.8	0.3	0.8	0.3	1.3	4.7	5.0
P, %	0.38	0.48	0.56	0.80	0.38	0.83	0.38	0.29	0.69	1.75
Ca, %	0.64	0.62	0.57	0.52	0.64	0.53	0.64	0.01	1.09	0.05
Mg, %	0.14	0.18	0.22	0.28	0.13	0.29	0.14	0.09	1.09	0.51
K, %	0.67	0.73	0.78	0.91	0.68	0.94	0.67	0.32	2.10	1.53
Na, %	0.16	0.16	0.14	0.11	0.15	0.11	0.15	0.001	0.002	0.011
Fe, ppm	200	200	180	200	160	200	181	20	100	220
Cu, ppm	12	14	13	10	14	13	13	0.4	12.6	5.7
Mn, ppm	50	70	70	80	50	90	52	3	37	133
Zn, ppm	130	130	120	120	140	140	100	20	40	60

[a] Calculated as the average between Diets 1 and 5.
[b] RX: Ricex-1000™.
[c] PHY: Calculated phytase level supplemented was 750 PU/kg diet.
[d] DM: Dry matter.
[e] NDF: Neutral detergent fiber.
[f] ADF: Acid detergent fiber.
[g] ADL: Acid detergent lignin.

Table 6.4. Analyzed phytase levels. Experiment 6

Treatment	PU/kg[a]	
	Expected	Analyzed
1	0	ND[b]
2	0	ND
3	0	ND
4	0	ND
5	750	614
6	750	738

[a] PU/kg: Phytase, units/kg of diet.
[b] ND: Not detected.

Table 6.5. Nutrients (%) contributed by RX to each experimental diet[a]. Experiment 6

Trt:[b]	1	2	3	4
RX, %:[c]	0	7.5	15	30
Item				
DM[d]	0	8.1	16.0	31.7
Energy	0	9.5	18.5	35.6
Fat	0	39.5	58.7	77.6
N	0	6.5	13.1	26.8
NDF[e]	0	14.9	27.5	48.0
ADF[f]	0	20.1	35.4	57.1
ADL[g]	0	61.2	77.5	89.3
P	0	27.3	44.9	66.5
Ca	0	0.7	1.4	3.3
Mg	0	23.5	40.1	61.9
K	0	15.6	28.6	49.3
Na	0	0.6	1.2	2.9
Fe	0	9.0	17.8	34.4
Cu	0	3.4	7.2	15.8
Mn	0	17.2	31.1	52.3
Zn	0	3.2	6.6	14.7

[a] Analyzed values used to calculate regression coefficients by PROC IML of SAS.
[b] Each mean represents 4 individually penned pigs.
[c] RX: Ricex-1000™.
[d] DM: Dry matter.
[e] NDF: Neutral detergent fiber.
[f] ADF: Acid detergent fiber.
[g] ADL: Acid detergent lignin.

Table 6.6. Apparent (%) digestibility of nutrients at increasing levels of RX and apparent digestibility in the RX product. Experiment 6

Trt:[a]	1	2	3	4			P-value
RX, %:[b] Response	0	7.5	15	30	RX Dig.[c]	SEM[d]	(Linear)
DM[e]	90.12	89.23	87.28	84.58	72.25	0.29	< 0.0001
Energy	89.81	89.33	87.73	86.01	78.79	0.28	< 0.0001
Fat	78.51	78.83	81.07	83.72	84.12	0.93	0.003
N	89.72	89.28	87.28	85.81	74.38	0.37	< 0.0001
NDF[f]	68.16	67.69	64.00	56.92	45.30	1.90	0.0014
ADF[g]	72.81	70.22	65.24	52.32	39.35	1.62	< 0.0001
ADL[h]	68.45	56.81	38.47	37.29	35.07	3.67	< 0.0001
P	25.49	27.05	16.24	20.43	14.94	2.3	0.028
Ca	46.78	48.17	38.93	32.73	-420	1.80	< 0.0001
Mg	29.26	33.41	29.56	26.80	26.24	1.94	0.26
K	89.47	85.30	85.37	82.90	78.96	0.78	0.0003
Na	78.22	73.61	57.92	51.51	-850	3.54	0.0003
Fe	20.80	35.83	22.17	24.02	21.83	1.73	0.52
Cu	3.98	20.90	11.95	12.50	32.08	1.45	0.12
Mn	4.60	24.74	13.09	17.96	27.99	1.87	0.006
Zn	3.98	9.06	0.27	17.62	86.08	1.36	< 0.0001

[a] Each mean represents 4 individually penned pigs.
[b] Ricex-1000™.
[c] RX Dig: Regressed digestibility of Ricex-1000™.
[d] SEM: Standard error of the mean.
[e] DM: Dry matter.
[f] NDF: Neutral detergent fiber.
[g] ADF: Acid detergent fiber.
[h] ADL: Acid detergent lignin.

Table 6.7. Retention of nutrients (as a % of absorption) at increasing levels of RX inclusion. Experiment 6

Trt:[a]	1	2	3	4		P-value
RX, %:[b]	0	7.5	15	30	SEM[c]	(Linear)
Response						
Energy	96.22	96.57	96.67	96.67	0.15	0.07
N	66.46	62.60	66.33	64.70	2.78	0.90
P	96.00	99.29	97.87	97.78	1.21	0.41
Ca	63.94	65.82	67.26	84.95	2.78	< 0.001
Mg	50.53	67.98	72.79	81.42	4.28	< 0.001
K	69.72	57.51	51.69	60.48	8.76	0.47
Na	45.80	48.55	47.48	46.32	3.86	0.94
Fe	98.98	99.49	99.27	99.40	0.12	0.09
Cu	78.12	98.20	96.80	96.73	6.95	0.18
Mn	102.97	99.59	99.42	99.41	3.45	0.51
Zn	89.62	95.23	101.35	98.07	5.28	0.31

[a] Each mean represents 4 individually penned pigs.
[b] RX: Ricex-1000™.
[c] SEM: Standard error of the mean.

Table 6.8. Phosphorus and nitrogen balance at increasing levels of RX inclusion. Experiment 6

Trt:[a]	1	2	3	4		P-value
RX, %:[b]	0	7.5	15	30	SEM[c]	(Linear)
Response						
P						
Intake, g/d	9.84	11.77	14.38	20.18	0.31	< 0.01
Excreted (feces), g/d	7.36	8.57	12.05	16.05	0.19	< 0.01
Excreted (urine), g/d	0.09	0.02	0.05	0.09	0.03	0.97
Absorption, g/d	2.49	3.20	2.33	4.12	0.30	< 0.05
Retention, g/d	2.39	3.18	2.28	4.03	0.30	< 0.05
Digestibility (apparent), %	25.49	27.05	16.24	20.43	2.10	< 0.05
Retention (as a % of intake)	24.53	26.87	15.88	19.97	2.14	< 0.05
Retention (as a % of absorption)	96.00	99.29	97.87	97.78	1.21	0.41
N						
Intake, g/d	74.47	68.72	70.03	67.22	1.68	< 0.05
Excreted (feces), g/d	7.69	7.39	8.91	9.54	0.30	< 0.01
Excreted (urine), g/d	22.53	23.04	20.63	20.36	2.02	0.36
Absorption, g/d	66.78	61.33	61.12	57.68	1.52	< 0.01
Retention, g/d	44.25	38.29	40.49	37.32	1.61	< 0.05
Digestibility (apparent), %	89.72	89.28	87.28	85.81	0.37	< 0.01
Retention (as a % of intake)	59.66	55.90	57.88	55.50	2.35	0.34
Retention (as a % of absorption)	66.46	62.60	66.33	64.70	2.78	0.90

[a] Each mean represents 4 individually penned pigs.
[b] RX: Ricex-1000™.
[c] SEM: Standard error of the mean.

Table 6.9. Apparent (%) digestibility of nutrients when supplementing phytase (PHY) to low and high RX diets. Experiment 6

	Experimental diets							
Trt:[a]	1	4	5	6				
RX, %:[b]	0	30	0	30		P-value		
PHY:[c]	-	-	+	+	SEM[d]	PHY	RX	PHYxRX
Response								
DM[e]	90.12	84.58	90.70	85.00	0.36	0.20	< 0.01	0.82
Energy	89.81	86.01	90.00	85.76	0.33	0.92	< 0.01	0.53
Fat	78.51	83.72	84.83	87.00	1.14	< 0.01	0.01	0.22
N	89.72	85.81	90.12	86.01	0.38	0.44	< 0.01	0.80
NDF[f]	68.16	56.92	70.29	56.74	2.35	0.69	< 0.01	0.64
ADF[g]	72.81	52.32	74.95	51.82	2.27	0.73	< 0.01	0.57
ADL[h]	68.45	37.29	69.26	26.59	4.06	0.25	< 0.01	0.19
P	25.49	20.43	47.44	37.03	2.12	< 0.01	< 0.01	0.24
Ca	46.78	32.73	50.86	43.65	3.07	< 0.05	< 0.01	0.29
Mg	29.26	26.80	33.25	24.47	1.93	0.68	< 0.05	0.14
K	89.47	82.90	91.79	82.48	0.78	0.26	< 0.01	0.11
Na	78.22	51.51	79.96	55.43	2.27	0.24	< 0.01	0.64
Fe	20.80	24.02	24.09	27.58	2.00	0.13	0.13	0.95
Cu	3.98	12.50	5.28	10.35	1.69	0.81	< 0.01	0.32
Mn	4.60	17.96	0.79	12.13	2.16	0.05	< 0.01	0.65
Zn	3.98	17.62	1.87	17.70	3.08	0.75	< 0.01	0.73

[a] Each mean represents 4 individually penned pigs.
[b] RX: Ricex-1000™.
[c] PHY: Calculated phytase (PHY) level supplemented was 750 PU/kg diet.
[d] SEM: Standard error of the mean.
[e] DM: Dry matter.
[f] NDF: Neutral detergent fiber.
[g] ADF: Acid detergent fiber.
[h] ADL: Acid detergent lignin.

Table 6.10. Retention of nutrients (as a % of absorption) when supplementing phytase (PHY) to low and high RX diets. Experiment 6

	Experimental diets							
Trt:[a]	1	4	5	6				
RX, %:[b]	0	30	0	30		P-values		
PHY:[c]	-	-	+	+	SEM[d]	PHY	RX	PHYxRX
Response								
Energy	96.22	96.67	96.57	96.78	0.16	0.18	0.07	0.46
N	66.46	64.70	68.19	69.03	3.45	0.40	0.89	0.71
P	96.00	97.78	98.74	90.64	1.46	0.17	0.06	< 0.01
Ca	63.94	84.95	78.41	89.18	2.78	< 0.01	< 0.01	0.10
Mg	50.53	81.42	57.47	80.11	4.79	0.57	< 0.01	0.41
K	69.72	60.48	64.37	56.61	6.90	0.52	0.25	0.92
Na	45.80	46.32	44.77	52.39	6.89	0.72	0.57	0.62
Fe	98.98	99.40	99.21	99.46	0.13	0.29	0.03	0.56
Cu	78.12	96.73	88.92	96.55	6.71	0.45	0.08	0.43
Mn	102.97	99.41	111.72	99.13	5.38	0.45	0.17	0.42
Zn	89.62	98.07	92.60	98.45	2.49	0.52	0.02	0.62

[a] Each mean represents 4 individually penned pigs.
[b] RX: Ricex-1000™.
[c] PHY: Calculated phytase level supplemented was 750 PU/kg diet.
[d] SEM: Standard error of the mean.

Table 6.11. Phosphorus and nitrogen balance when supplementing phytase (PHY) to low and high RX diets. Experiment 6

Trt:[a]	1	4	5	6				
RX, %:[b]	0	30	0	30		P-values		
PHY:[c]	-	-	+	+	SEM[d]	PHY	Ricex	PHYxRX
Response								
P								
Intake, g/d	9.84	20.18	10.03	21.82	0.34	< 0.05	< 0.01	< 0.10
Excreted (feces), g/d	7.36	16.05	5.30	13.79	0.38	< 0.01	< 0.01	0.78
Excreted (urine), g/d	0.09	0.09	0.06	0.76	0.09	< 0.01	< 0.01	< 0.01
Total excreted, g/d	7.45	16.15	5.36	14.55	0.30	< 0.01	< 0.01	0.44
Absorption, g/d	2.49	4.12	4.73	8.04	0.27	< 0.01	< 0.01	0.01
Retention, g/d	2.39	4.03	4.67	7.28	0.26	< 0.01	< 0.01	< 0.10
Digestibility (apparent), %	25.49	20.43	47.44	37.03	2.12	< 0.01	< 0.01	0.24
Retention (as a % of intake)	24.53	19.97	46.88	33.48	2.08	< 0.01	< 0.01	0.06
Retention (as a % of absorption)	96.00	97.78	98.74	90.64	1.46	0.17	0.06	< 0.01
N								
Intake, g/d	74.47	67.22	75.89	72.70	1.76	< 0.10	< 0.05	0.28
Excreted (feces), g/d	7.69	9.54	7.52	10.15	0.35	0.55	< 0.01	0.29
Excreted (urine), g/d	22.53	20.36	22.06	19.59	2.41	0.80	0.36	0.95
Total excreted, g/d	30.22	29.89	29.58	29.74	2.45	0.88	0.97	0.92
Absorption, g/d	66.78	57.68	68.37	62.55	1.52	< 0.10	< 0.01	0.30
Retention, g/d	44.25	37.32	46.31	42.96	1.98	< 0.10	< 0.05	0.39
Digestibility (apparent), %	89.72	85.81	90.12	86.01	0.38	0.44	< 0.01	0.80
Retention (as a % of intake)	59.66	55.50	61.46	59.34	2.99	0.37	0.32	0.74
Retention (as a % of absorption)	66.46	64.70	68.19	69.03	3.45	0.40	0.89	0.71

[a] Each mean represents 4 individually penned pigs.
[b] RX: Ricex-1000™.
[c] PHY: Calculated phytase level supplemented was 750 PU/kg diet.
[d] SEM: Standard error of the mean.

CHAPTER 7

SUMMARY OF DISSERTATION

Concerns regarding pollution of water ecosystems with phosphorus from animal excreta, along with the possible phasing out of antibiotics used at growth promoting levels, motivated these studies on the effects of the antibiotic virginiamycin (VIR) and an exogenous phytase (PHY) on the utilization of P and other nutrients by growing swine.

Experiments 1 through 4 evaluated VIR and PHY amendments on digestibility, retention and excretion of nutrients, particularly P, by pigs fed a corn-SBM diet without an inorganic source of P. The main objective was to evaluate a possible effect of VIR on P utilization.

Experiment 1 compared VIR with the basal diet, finding that VIR increased P digestibility by 8.4%, and also increased retention (as a % of absorption) by 2.4%.

Experiments 2, 3, and 4 included PHY amendments at two different levels. Phytase was initially included at 750 PU/kg of diet (Experiment 2) to test for possible additive effects between VIR and PHY on P digestibility. As no additive effects were observed, and considering that 750 PU were probably enough to cleave most of the dietary phytic P, not leaving room for observing further possible VIR effects, the level of the enzyme was later reduced to 300 PU/kg (Experiments 3 and 4). In agreement with profuse findings reported over the last two decades, PHY amendments improved P digestibility and retention. Improvements of P digestibility due to PHY amendments were between 14 and 27%. Retention, as a percent of absorption, was improved between 0.7 and 2.5%. As expected, improvements were greater at the higher level of PHY inclusion. On the other hand, VIR effects were not as strong as initially observed in Experiment 1, but differences favoring the antibiotic were still observed when the basal diet was compared with the VIR-amended diet. On average, dietary

addition of VIR improved P digestibility and total excretion by 5.0%, and P retention (as a % of absorption) by 1.0% in the four experiments. Table 7.1 presents a summary of the results.

Parallel to the work on the amendments, a comparison of methodologies was conducted. Interested in testing the reliability of simple grab sampling procedures to assay digestibility by the index method (Cr_2O_3), two different sampling procedures were compared to the standard total collection methodology in the first two experiments. The procedures were a single-day grab fecal collection (Experiment 1), and a cumulative composite grab collection extending from 1 to 5 days (Experiment 2). In Experiment 1, it was found that a single-day fecal sample - grabbed according to the color of the feces - although cheap and simple, is not a reliable alternative compared to the total collection method. In Experiment 2, the index method using a cumulative composited sample during five days proved to be more accurate than the 1-day collection and could provide approximate results when it is desired to have a general idea on the digestibility of macronutrients. Nevertheless, the procedure did not fully match the capabilities of the standard method. It was not able to detect statistical differences between treatments for several of the nutrients assayed.

Interested in further evaluating the impact of VIR on P utilization, a full term growing-finishing trial was conducted. It was designed to test the effects of partial dietary P deletion (0.028% P, or 0.15% DICAL) in VIR-supplemented diets upon growth performance, bone traits, ileal flora populations, carcass, and meat characteristics. The amount of P deleted was calculated from the results observed in the first digestibility experiment. The P deletion did not affect growth, but had negative effects on bone traits. The VIR amendment was not able to restore the bone de-mineralization caused by the partial DICAL deletion, although numerical improvements were observed. The observed improvements in bone traits and phytate utilizing bacteria indicated a possible mode of action that merits further research under different environmental conditions.

A final balance trial assessed nutrient digestibility of a commercial rice bran-containing product (RX) rich in phytate P, by the regression method.

Simultaneously, the effects of phytase were assessed on nutrient digestion, retention and excretion in a basal corn-soy diet supplemented with a high level of RX and these results were compared with those of a non-supplemented diet. The estimated digestibility of most nutrients in the RX product was lower than in the basal diet. Compared to the basal diet, digestibility of the RX product was very low for DM, energy, CP, and P (relative difference: 80, 88, 83, and 59%, respectively). Digestibility was particularly low for Ca. Diets substituted with increasing levels of RX for growing pigs were found generally to have a linear decrease in nutrient digestibility, with the only exception being fat, where digestibility was increased. Additionally, the amendment of the 30%RX diet with 750 PU/kg increased P digestibility. Further research is required to define the optimum level of PHY amendment to this feedstuff in order to release the maximum amount of phytic P.

In summary, the antibiotic VIR improved P digestibility in pigs fed diets not amended with inorganic P. This improvement, around 5.0%, although much lower than the improvements obtained with PHY, is still significant. The mechanism of action appears to be related with an increase in phytate-utilizing bacteria in the small intestin, but more research is required. Additionally, byproduct feeds such as rice bran, represent opportunities as partial substitutes for traditional feedstuffs, but the levels of rice bran inclusion should be carefully considered because of negative effects on digestibility.

Table 7.1. Improvements in apparent digestibility (total collection) and retention resulting from subtracting control diet from amended diet in Experiments 1, 2, 3, and 4

	Improvement by VIR, %		Improvement by PHY, %	
Exp	Digestibility	Retention As a % of absorption	Digestibility	Retention As a % of absorption
1[a]	8.44	2.36	-	-
2[b]	4.64	1.37	27.31	2.49
3[c]	5.21	0.29	16.25	0.68
4[d]	1.78	0.07	13.78	1.05
Ave.[e]	5.02	1.02	19.11	1.41

[a] Virginiamycin included at 11 ppm; Phytase not used in Experiment 1.
[b] [b]Virginiamycin included at 11 ppm and phytase at 750 U/kg.
[c] Virginiamycin included at 11 ppm and phytase at 300 U/kg.
[d] Virginiamycin included at 11 ppm and phytase at 300 U/kg.
[e] Average of the four experiments.

Appendix 1, Effects of Virginiamycin on Growth, Ileal Bacterial Populations And Bone Traits Evaluated in a Nursery Model With Pigs Fed a Phosphorus Deficient Corn-Soybean Meal Diet – Summary of Experiments 7 and 8

Introduction

It is known that subtherapeutic dietary levels of antibiotics generally improve growth and feed utilization in pigs. Although their modes of action are not well understood, it is believed that antibiotics depress growth of bacteria that compete with the host for nutrients and increase organisms that synthesize nutrients for the host. Some antibiotics also increase Ca utiliation in poultry (Buresh et al., 1985). In previous experiments (Experiments 1, 2, 3, and 4) the antibiotic virginiamycin (VIR) improved P digestibility in growing-finishing pigs. A logical step following this finding would be to determine its effects on bone traits associated with mineral deposition, and its possible impact on ileal microbial populations that liberate P from the phytate molecule.

Objectives

To evaluate the effects of VIR on growth performance and bone traits in nursery pigs fed ad libitum a basal, low-P corn-SBM diet supplemented with two levels of the antibiotic. Further, to evaluate VIR effects on ileal microbial populations, particularly phytate utilizing organisms.

Experimental Procedures

Animals and housing conditions

Twenty growing barrows (crossbreds of Hampsire or Duroc x (Yorkshire x Landrace)) were used in each experiment. The average starting weight was 15.8 and 16.6 kg for Experiments 7 and 8, respectively. Each experiment tested five dietary treatments with four replicates per treatment. In each experiment, sibling pigs of similar weight within a replicate were allocated to treatments and randomly assigned to crates. Half-siblings (i.e., a common sire) were used when inadequate full-siblings were available. Pigs were individually confined in raised-deck pens with welded-wire floors in two rooms at the Univerity of Kentucky

campus (Garrigus building). Pens were equipped with a four-hole stainless steel self-feeder. Water was supplied ad libitum. Room temperature was kept in the thermo-neutral range. Rooms and pens were cleaned daily with water under pressure.

Dietary treatments

Diet composition is outlined in Appendix 1, Table 1.1. In both experiments, Diets 1, 2, and 3 had graded levels of added P from MSP (0, 0.1, and 0.2% added P, respectively). In Experiment 7, Diets 4 and 5 consisted in Diet 1 supplemented with VIR (5.5 and 11 ppm, respectively). In Experiment 8, Diets 4 and 5 consisted of Diet 2 supplemented with VIR (5.5 and 11 ppm, respectively). All diets met NRC (1998) nutrient estimates, except for P (Appendix 1, Table 1.2).

Sampling and laboratory analysis

Pigs were weighed and feed intake determined every ten days in both experiments. Each experiment lasted 40 days. Pigs were humanely killed by exsanguination following electrical stunning, and samples were collected. The femurs, front and back feet were removed. Feet and femurs were collected for bone strength and ash assessment, following the procedures outlined in Chapter 5. In Experiment 7, immediately after slaughtering each pig, a sample (approx. 20 cm long) of the distal portion of the ileum with its contents was collected and transported over ice to the lab for pH reading and microbial culture, including phytate utilizing bacteria, according to the procedure by Bae et al. (1999).

Experimental design and statistical analysis

Each experiment was analyzed separately. The effects of incremental dietary aditions of MSP in Diets 1, 2, and 3 were analyzed as linear and quadratic contrasts using procedure GLM (SAS, 1998).

In Experiment 7, contrasts were also made between Diets 1 and 4 (basal vs. basal plus 5.5 ppm VIR), and also between Diets 1 and 5 (basal vs. basal plus 11 ppm VIR) to test for the effects of the low and high levels of VIR supplementation, respectively. The two levels of VIR supplementation were also contrasted with each other. In Experiment 8 similar comparisons were made, but Diet 2 was used instead of Diet 1as control diet. Contrasts were made between Diets 2 and 4 (basal vs. basal plus 5.5 ppm VIR), Diets 2 and 5 (basal vs. basal plus 11 ppm VIR), and Diets 4 and 5. In Experiment 8, LSMeans were calculated to account for a limping pig not included in the analysis.

Results and Discussion

Experiment 7

The diet assay for VIR was close to the target (<2.0 g/ton for Diet 1, 5.9 g/ton for Diet 4, and 9.2 g/ton for Diet 5). All pigs finished the trial. Results are presented in Appendix 1, Table 1.3. As expected, linear effects in Diets 1, 2 and 3 were highly significant ($P < 0.0001$) for P intake, ADG, and both bone traits, indicating an increase in growth and bone response to graded levels of P intake. A linear effect ($P = 0.016$) was found for ADFI, probably reflecting an improvement in appetite with graded levels of dietary P. The linear effect for F/G was not significant ($P = 0.12$), which could be a result of differences in orts recovery. Apparently, addition of MSP did not affect microbial populations ($P > 0.20$). When comparing VIR diets (Diets 4 and 5) with their control (Diet 1), no effect on gain was observed at either level of VIR (P > 0.10). Similarly, no effects of VIR were found on bone breaking strength or ash ($P > 0.20$). Differences observed in bacterial counts (Coliforms, E. coli, and phytate utilizing bacteria), are difficult to explain, because the direction of the changes do not correspond with the increasing levels of VIR fed in Diets 1, 4 and 5.

Experiment 8

The diet assay for VIR was close to the target (< 2.0 g/ton for Diets 1, 2, and 3; 6.1 g/ton for Diet 4; and 9.6 g/ton for Diet 5). A limping pig fed Diet 1 was excluded from the analysis. Similar to Experiment 7, the expected linear effects in Diets 1, 2 and 3 were highly significant ($P < 0.0001$) for P intake, growth and bone traits, indicating an increase in growth and bone response to graded levels of P intake. No significant difference was observed between Diet 4 and the control (Diet 2) for daily gain. Virginiamycin tended to increase ($P = 0.07$) daily gain in the group fed 11 ppm of the antibiotic. Similarly to Experiment 1, no effects of VIR were found on either bone breaking strength or ash ($P > 0.10$), except for a tendency to lower MC strength ($P = 0.09$) in Diet 5, which is difficult to explain considering that pigs on Diet 5 grew faster than the control pigs (Appendix 1, Table 1.4).

Implications

The nursery model did not work as expected to establish the post-absorptive effects of the increased P digestibility observed with growing-finishing pigs in previous experiments. The model also failed to detect a suspected increase of phytate utilizing bacteria in the small intestine of pigs fed VIR. Although it is not clear why the expected treatment effects were not observed, a difference in stress level may explain it. Pigs in the digestibility trials probably endured higher levels of stress because of the restricted feeding, the confinement conditions, and the lack of excercise. The lower level of stress in the nursery trials could have limited the response to the antibiotic. Irrespective of the reasons, the nursery model did not contribute to an understanding of the VIR effect on P digestibility.

Appendix 1. (Continued)

Table 1.1. Composition of Experimental Diets. Experiments 7 and 8

Exp:	7					8				
Trt:	1	2	3	4	5	1	2	3	4	5
Ingredient										
Corn	71.765	71.765	71.765	71.765	71.765	71.765	71.765	71.765	71.765	71.765
Corn starch	1.000	0.555	0.110	0.988	0.975	1.000	0.555	0.110	0.988	0.975
Stafac® 20	-	-	-	0.0125	0.025	-	-	-	0.0125	0.025
Corn oil	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30
SBM (48%CP)	23.5	23.5	23.5	23.5	23.5	23.5	23.5	23.5	23.5	23.5
Calcium carbonate	1.48	1.48	1.48	1.48	1.48	1.48	1.48	1.48	1.48	1.48
Monosodium phosphate	-	0.445	0.89	-	-	-	0.445	0.89	0.445	0.445
Salt (non iodized)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
UK trace min. mix[a]	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075
UK vitamin mix[b]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Santoquin	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Lysine HCl	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
DL-Methionine	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
L-Threonine	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Total:	100	100	100	100	100	100	100	100	100	100

[a] Supplied per kg of diet: 135 mg Fe (iron sulfate monohydrate), 135 mg Zn (zinc oxide), 45 mg Mn (manganous oxide), 13 mg Cu (copper sulfate pentahydrate), 1.5 mg I (calcium iodate), 0.3 mg Se (selenium mix), and 0.23 mg Co (cobalt sulfate monohydrate).
[b] Supplied per kg of diet: 6,608 IU vitamin A, 881 IU vitamin D_3, 22.03 IU vitamin E, 19.76 mg vitamin K, 22.03 mg pantothenic acid, 44.05 mg niacin, 4.00 mg thiamin, 8.81 mg riboflavin, 6.00 mg vitamin B_6, 22.03 mcg vitamin B_{12}, 1.10 mg folic acid, and 0.22 mg biotin.

Appendix 1. (Continued)

Table 1.2. Calculated chemical composition of experimental diets. Experiments 7 and 8

Exp:[a]	7					8				
Trt:[b]	1	2	3	4	5	1	2	3	4	5
Item										
CP, %[c]	17.12	17.12	17.12	17.12	17.12	17.12	17.12	17.12	17.12	17.12
Lysine, %	1.04	1.04	1.04	1.04	1.04	1.04	1.04	1.04	1.04	1.04
ME, kcal/kg[d]	3398	3380	3362	3397	3397	3398	3380	3362	3380	3379
EE, %[e]	3.51	3.51	3.50	3.51	3.51	3.51	3.51	3.50	3.51	3.51
CF, %[f]	3.42	3.42	3.42	3.42	3.42	3.42	3.42	3.42	3.42	3.42
Ca, %	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67
tP, %[g]	0.36	0.47	0.59	0.36	0.36	0.36	0.47	0.59	0.47	0.47
aP, %[h]	0.07	0.18	0.29	0.07	0.07	0.07	0.18	0.29	0.18	0.18
Ca:P	1.85	1.42	1.15	1.85	1.85	1.85	1.42	1.15	1.42	1.42

[a] Exp: Experiment.
[b] Trt: Dietary treatment.
[c] CP: Crude protein.
[d] ME: Metabolizable energy.
[e] EE: Ether extract.
[f] CF: Crude fiber.
[g] tP: Total P.
[h] aP: Available P.

Appendix 1. (Continued). Table 1.3. Summary of performance, bone, and bacteria results (LSMeans). Experiment 7

							P- values			
Trt:	1	2	3	4	5	CV	Linear[a]	1 vs. 4	1 vs. 5	4 vs. 5
Response										
P intake, g										
Total	211	306	395	233	207	7.6	<.0001	0.16	0.80	0.10
Added	0	64.75	134.5	0	0	11.6	<.0001	1.00	1.00	1.00
Performance										
ADFI, kg	1.38	1.54	1.61	1.52	1.36	7.6	0.016	0.10	0.76	0.06
ADG, kg	0.766	0.865	0.979	0.756	0.705	5.9	<.0001	0.77	0.10	0.16
FG	1.81	1.78	1.64	2.02	1.93	7.6	0.12	0.05	0.24	0.38
Bone strength, kg										
MC	39.40	62.00	92.50	45.00	39.60	15.5	<.0001	0.38	0.97	0.40
MT	31.80	64.90	90.00	40.30	36.10	18.1	<.0001	0.23	0.53	0.55
Ave MC + MT	35.59	63.47	91.23	42.63	37.85	15.6	<.0001	0.26	0.71	0.44
Femur	88.13	207.63	274.75	97.88	99.13	19.8	<.0001	0.66	0.62	0.95
Bone ash, %										
MC	50.76	55.86	57.10	51.06	50.76	2.8	<.0001	0.78	1.00	0.78
MT	50.28	55.53	57.39	50.64	50.36	3.0	<.0001	0.76	0.94	0.81
Ave MC + MT	50.52	55.69	57.25	50.85	50.56	2.9	<.0001	0.77	0.97	0.80
Bacteria, CFU/g										
Coliforms	5.88	5.90	5.99	5.16	6.77	10.7	0.80	0.14	0.07	0.004
E. coli	5.40	4.47	5.67	5.03	6.36	12.0	0.56	0.43	0.06	0.01
Lactobac	7.71	7.56	7.92	6.77	6.90	11.0	0.72	0.13	0.18	0.83
T. Anaerobes	8.96	8.80	9.45	8.30	8.31	10.9	0.48	0.35	0.35	0.99
Bifido	8.85	9.02	9.11	8.43	8.20	10.2	0.69	0.51	0.32	0.72
Phytate	6.67	5.53	5.91	4.47	5.87	23.5	0.44	0.04	0.41	0.17
Ileal pH	6.87	6.93	6.49	6.90	6.81	4.9	0.14	0.89	0.80	0.70

[a] Linear contrasts for diets 1, 2, and 3. Each mean represents 4 individually penned pigs.

Appendix 1. (Continued). Table 1.4. Summary of performance and bone results (LSMeans). Experiment 8

							P- values		
Trt:	1	2	3	4	5	CV	Linear[a]	1 vs. 4	1 vs. 5	4 vs. 5
Response										
P intake, g										
Total	176	271	329	265	286	5.5	<.0001	0.61	0.18	0.08
Added	-1.1	58.4	117.0	57.3	61.7	7.8	<.0001	0.74	0.37	0.22
Performance										
ADFI, kg	1.189	1.426	1.427	1.397	1.504	5.0	0.001	0.58	0.14	0.05
ADG, kg	0.552	0.782	0.815	0.754	0.843	5.7	<.0001	0.38	0.07	0.01
FG	2.16	1.83	1.75	1.86	1.79	4.4	<.0001	0.62	0.50	0.25
Bone strength, kg										
MC	26.35	55.63	66.56	44.63	43.69	18.6	<.0001	0.12	0.09	0.89
MT	23.32	52.06	59.50	44.44	47.88	17.8	0.0002	0.22	0.49	0.57
Ave MC + MT	24.84	53.84	63.03	44.53	45.78	16.9	<.0001	0.13	0.18	0.83
Femur	78.91	176.38	201.50	159.50	180.75	12.7	<.0001	0.28	0.77	0.18
Bone ash, %										
MC	49.49	55.15	56.07	54.08	54.21	2.3	<.0001	0.25	0.31	0.89
MT	50.25	55.95	57.05	54.66	55.32	2.0	<.0001	0.13	0.44	0.42
Ave MC + MT	49.87	55.55	56.56	54.37	54.76	2.0	<.0001	0.16	0.34	0.63

[a] Linear contrasts for diets 1, 2, and 3. Each mean represents 4 individually penned pigs.

Appendix 2. Pig Weights by Treatment. Experiments 1, 2, 3, and 4

Exp. No	Digest. method [a]	Trt	Adaptation		Collection	
			BW (i), kg [b]	BW (f), kg [c]	BW (i), kg [d]	BW (f), kg [e]
1	Total 1	Control	58.0	59.2	59.2	68.2
		VIR	56.5	58.5	58.5	69.0
	Total 2	Control	72.2	75.9	75.9	84.4
		VIR	76.6	78.7	78.7	87.9
2	Total 1	B	55.3	56.1	56.1	60.6
		VIR	55.3	57.3	57.3	63.8
		PHY	55.6	59.2	59.2	64.5
		VIR+PHY	55.2	57.6	57.6	63.5
	Index 1	B	-	-	60.6	67.7
		VIR	-	-	63.8	70.3
		PHY	-	-	64.5	74.8
		VIR+PHY	-	-	63.5	71.7
	Total 2	B	71.4	72.4	72.4	79.1
		VIR	72.1	75.2	75.2	83.5
		PHY	72.3	75.6	75.6	83.2
		VIR+PHY	72.4	75.9	75.9	85.3
	Index 2	B	-	-	79.1	87.1
		VIR	-	-	83.5	89.2
		PHY	-	-	83.2	89.5
		VIR+PHY	-	-	85.3	90.5
3	Total 1	B	45.5	45.9	45.9	49.4
		VIR	45.6	48.6	48.6	50.9
		PHY	45.0	46.7	46.7	50.9
		VIR+PHY	44.5	45.2	45.2	50.0
	Total 2	B	57.3	59.1	59.1	65.0
		VIR	58.2	61.1	61.1	67.9
		PHY	57.3	59.8	59.8	65.5
		VIR+PHY	57.3	60.3	60.3	66.1
4	Total 1	B	45.8	46.7	46.7	51.1
		VIR	46.2	48.2	48.2	53.2
		PHY	46.8	49.1	49.1	55.9
		VIR+PHY	46.8	49.1	49.1	55.9
	Index 1	B	-	-	51.1	55.3
		VIR	-	-	53.2	59.4
		PHY	-	-	55.9	60.8
		VIR+PHY	-	-	55.9	60.8
	Total 2	B	60.6	63.0	63.0	69.2
		VIR	58.3	62.0	62.0	66.8
		PHY	58.3	61.7	61.7	69.7
		VIR+PHY	58.3	63.6	63.6	71.7
	Index 2	B	-	-	69.2	79.5
		VIR	-	-	66.8	75.9
		PHY	-	-	69.7	79.4
		VIR+PHY	-	-	71.7	79.2

[a] Digest. method: Total 1: Digestibility assessment by total collection, 1st collection; Total 2: Digestibility assessment by total collection, 2nd collection; Index 1: Digestibility assessment by index method, 1st collection; Index 2: Digestibility assessment by index method, 2nd collection.
[b] Ave weight at the start of the adaptation period.
[c] Ave weight at the end of the adaptation period.
[d] Ave weight at the start of the collection period.
[e] Ave weight at the end of the collection period.

Appendix 3. Gross Energy Determination

Feed, feces, and urine gross energy (cal/g) were assessed by bomb calorimetry, using a Parr 1261 Isoperibol Bomb Calorimeter (Parr Instruments Company, Moline, IL). This is an adaptation of the method by the AOAC (1995). It includes running a couple of benzoic acid pellets (6318 cal/g) at the beginning and at the end of every set of samples to test for precision and accuracy of the equipment. The calorimeter used has two bombs and two metal buckets. Each one has been calibrated to be used with a particular bucket.

Feed and feces samples

Feces and feed samples were ground, weighed (1 g), and pelleted in duplicate prior to combustion in the calorimeter.

Urine samples

Prior to assess urine gross energy, samples were dried into plastic bags. The bags used were: flat 1.5” x 3” clear 2 mil product No. 01-0103-2, 47.60 for 1000 (Jeb Plastics, Inc., Wilmington, DE). The average bag energy content (cal/g) was established by conducting 20 individual bag combustions. Urine samples were centrifuged at 1,500 rpm for 10 minutes prior to gross energy assessment. Each bag was cut and sealed to generate 3 smaller bags able to hold about 3 g of urine each. Bags were weighed, opened and placed in tared metal crucibles over a scale. Then the urine sample was pipetted into the bag, and the bag plus urine weight was recorded. Then bags were placed in a draft oven at 40 ºC until dry - 48 to 72 hours. Once bags were dry they were combusted in the bomb calorimeter. Urine energy was calculated as:

Gross energy, cal/ml =
(Total energy released – (bag wt, g. x plastic energy factor))/wet urine wt

Appendix 4. Phosphorus Determination in Feed, Feedstuffs, and Feces

This gravimetric procedure is a modification of method 968.08 from AOAC, 1990.

Feed (4.0 g) and feces (1.0 g) were weighed in quartz crucibles and dry-ashed overnight in a muffle furnace at 600°C. After cooling down in a desiccator, samples were digested with 40 mL 3N HCl on a hot plate at high temperature, and let boiling (15 min). Digested solutions were then quantitatively transferred into 250 mL volumetric flasks and diluted to volume with DD water. Flasks were shaken, sealed with parafilm paper, and left overnight to settle. Then, 50 mL aliquots of the solutions were transferred into Erlenmeyer flasks, heated to boiling on hot plates, 50 mL of Quimociac reagent added, and left some minutes on the hot plates until the color changed from 'milky' yellow to clear yellow. Then solutions were vacuum-filtered in pre-weighed porcelain gooch crucibles using fiberglass filter paper circles. Crucibles with the filtered precipitate were then oven-dried overnight at 105^{o}C, then cooled down in a desiccator and weighed.

Total phosphorus concentration was calculated as:

$$\text{Total P, \%} = \left[\frac{(\text{Precipitate wt., g} \times V_1)}{V_2} \times \frac{(0.013997 \times 100)}{\text{Sample wt, g}} \right]$$

V_1: Initial volume after quantitative transferring HCl-digested sample (250 cc).
V_2: Aliquot of V_1 to be reacted with Quimociac (50 cc).

Appendix 4. (Continued)

Quimociac reagent preparation procedure

To prepare 1 L of Quimociac reagent:

1. Dissolve 70 g sodium molybdate dehydrate ($Na_2MoO_4 \cdot 2H_2O$) into 150 mL DD water.
2. Dissolve 63.8 g citric acid dehydrate [$HOCCOOH(CH_2COOH)_2 \cdot H_20$] into 150 mL deionized (DD) water, add 85 mL concentrated nitric acid (HNO_3) and allow to cool.
3. Add the molybdate solution to the citric-nitric solution while stirring.
4. Add 5 mL synthetic quinoline (C_6H_4N:CHCH:CH) to a mixture of 100 mL DD water and 35 mL concentrated nitric acid.
5. Slowly add the quinoline mixture to the molybdate-citric-nitric solution, while stirring.
6. Let solution stand overnight.
7. Filter solution through a No. 2 Whatman filter.
8. Add 280 mL of acetone (CH_3COCH_3) and dilute to 1 L with DD water.

Appendix 5. Mineral Determination by Atomic Absorption Spectrophotometry

Except for P, all the minerals in feed, feces and urine were assessed by Flame Atomic Absorption Spectrophotometry (AA) (Thermoelemental SOLAAR M5, Thermo Electron Corp., Verona, WI), according to a modification of the procedure from AOAC (1995b) (method 927.02).

Samples of the volumes (250 mL) obtained during the gravimetric assessment of P were aspirated in the AA equipment. Before aspiration, samples were re-diluted according to the concentration expected for each mineral. The dilution factors used in each case were multiplied by the AA result to calculate concentration in the original sample.

The assessment by AA included the reading of a blank and five working standards at the beginning of each set of samples (typically 40 to 70) to generate a regression curve used to calculate concentration according to the amount of light absorbed by the mineral during atomization.

The flame used was air/C_2H_2 for all the minerals, except for Ca and Cr, which used N_2O/C_2H_2. The wave lengths used were: 769.9, 589.6, 428.9, 422.7, 324.8, 285.2, 279.5, 248.3, and 213.9 nm for K, Na, Cr, Ca, Cu, Mg, Mn, Fe, and Zn, respectively.

The working standards chosen were the optimum sets of concentrations that provided the best linear response for each mineral under test. As part of the quality control, blank and standards were aspirated after each set of samples to check for drifting of the values during the suction process. Samples were re-analyzed when drift was observed. Samples were re-diluted (Hamilton Digital Diluter, Reno, NV) and re-analyzed when sample concentration was above the highest standard used. The re-dilution for the macro-minerals was aimed to obtain readings distributed around the middle of the set of standards. The concentration of all the stock solutions used to prepare the working standards was 1000 ppm (Fisher Scientific, Pittsburg, PA.).

Appendix 6. Chromium Determination

This is a modification of the method reported by Williams et al. (1962). Feed and feces samples were weighed (0.5 g) in porcelain crucibles, dry-ashed overnight at 600°C in a muffle furnace, then digested by adding 4 mL potassium bromate solution and 3 mL acid manganese sulfate solution. After adding both solutions, samples were heated at low temperature on a hot plate until simmering stopped and a golden-brown color developed. Samples were then quantitatively transferred into tared 100 mL plastic containers with 12.5 mL calcium chloride solution added, and the volume was brought to 100 mL with DD water. Then, samples were thoroughly mixed by shaking the containers and allowed to settle overnight. Next day, aliquots of the non-disturbed solutions were carefully transferred to test tubes for subsequent aspiration into the AA equipment.

Reagents Preparation for chromium analysis

Potassium bromate solution:

Dissolve 45 g potassium bromate ($KBrO_3$) in 1 L DD water.

Acid manganese sulfate solution:

Dissolve 2.27 g manganese sulfate ($MnSO_4 \cdot 1\ H_2O$) in 30 mL DD water and carefully add to 970 mL of 85% phosphoric acid (H_3PO_4).

Calcium chloride solution:

Dissolve 14.65 g calcium chloride ($CaCl_2 \cdot 2\ H_2O$) to 1 L DD water.

Appendix 7. Ankom Procedure for NDF Determination

Reagents

Neutral Detergent solution (ND):

Add 30.0 g sodium lauryl sulfate, USP; 18.61 g ethylenediaminetetraacetic disodium salt, dehydrate; 6.81 g sodium tetraborate decahydrate; 4.56 g sodium phosphate dibasic, anhydrous; 10.0 mL triethylene glycol, in 1 L distilled H_2O; Agitate and heat to facilitate solubility. Check pH range to 6.9 to 7.1.

Alpha-amylase:

Heat-stable bacterial alpha-amylase: activity = 340,000 Modified Wohlgemuth Units / mL. One Modified Wohlgemuth Unit is that activity which will dextrinize 1.0 mg of soluble starch to a defined size dextrin in 30 minutes.

Acetone:

Use grade that is free from color and leaves no residue upon evaporation.

Apparatus

Digestion apparatus (ANKOM200/220 Fiber Analyzer), filtration device (ANKOM Technology, F57 Filter Bags), Impulse bag sealer - Requires high enough temperature to melt and seal polymer in filter bags (ANKOM Technology - 1915/1920), desiccator (ANKOM Tech. MoistureStop weigh pouch-F39).

Procedure

Prepare Sample. Label filter bag with black permanent pen and weigh filter bag record weight, and tare balance. Weigh 0.5 g (±0.05 g) of air-dried sample, ground to pass through a 1mm screen, directly into filter bag. Weigh one blank bag and include in digestion to determine blank bag correction. Seal the bags closed within 0.5cm from the open edge using the heat sealer.

Spread sample uniformly inside the filter bag by shaking and lightly flicking the bag to eliminate clumping.

A maximum of 24 bags may be placed in the bag suspender. All nine trays are used regardless of the number of bags being processed. Place three bags per tray and then stack trays on center post with each level rotated 120 degrees. The weight is placed on top of the empty 9th tray to keep the bag suspender submerged.

Samples Containing soy-product or >5% Fat:

Extract fat from samples by placing 24 bags with samples into a 500 mL bottle with a top. Pour enough acetone into bottle to cover bags and secure top. Shake the container 10 times and allow bags to soak for 10 minutes. Repeat with fresh acetone. Pour out acetone and place bags on a wire screen to air-dry (approximately 5 minutes).

Exception: Roasted soy - Due to special properties of roasted soy a modification to the fat extraction is required. Place roasted soy samples into a 500 mL bottle with a top. Pour enough acetone into bottle to cover bags and secure top. Shake the container 10 times and pour off acetone. Add fresh acetone and allow samples to soak for twelve hours. After soak time, drain off acetone as stated above and allow to air-dry before next step.

When processing 24 sample bags add 2000 mL of detergent solution at ambient temperature into ANKOM Fiber Analyzer vessel. If processing less than 20 bags add 100 mL/bag of detergent solution (minimum of 1500 mL (ensure bag suspender is covered)) and 4.0 mL of heat stable alpha-amylase if samples contain starch.

Place bag suspender with samples into the solution in vessel.

Turn agitate and heat on (set to 100ºC) and confirm that bag suspender is agitating properly. Set timer for 75 minutes and push Start. Close and seal lid of vessel.

Appendix 7. (Continued)

After 75 minutes (timer will beep) have elapsed turn agitate and heat off, open the drain valve and exhaust hot solution before opening lid. Warning: The solution in vessel is under pressure. The valve should be opened first to remove pressure before lid can be opened. Ensure exhaust hose is securely positioned for safe disposal of effluent. After the solution has been exhausted close valve and open the lid. Add approximately 2000 mL of hot H_2O and lower lid but do not tighten. With starch containing samples add 4 mL heat stable amylase to each of the first 2 rinses. Set heat at 95ºC, turn agitate and heat on and rinse for 7 minutes. Exhaust water and repeat rinses for a total of three times or until water is at neutral pH. After final rinse remove filter bags from bag suspender and gently press out excess water. Place in beaker and cover with acetone. Allow bags to soak 3 minutes, then remove and lightly press out excess acetone. Spread bags out and allow acetone to evaporate. Complete drying in oven at 105ºC for at least 2 hours. Warning: Do not place bags in the oven until acetone has completely evaporated. Longer drying period may be required depending oven and frequency of sample introduction. Remove bags from oven, place directly into a desiccator and flatten pouch to remove air. Cool to ambient temperature and weigh bags.

Appendix 8. Ankom Procedure for ADF Determination

Reagents

Acid Detergent solution (AD):

Add 20 g cetyl trimethylammonium bromide (CTAB) to 1 L 1.00N H_2SO_4 previously standardized. Agitate and heat to dissolve.

Acetone – same as for NDF procedure.

Apparatus

Same as for NDF procedure.

Procedure

The procedure is conducted with the samples in the bags already processed for NDF. The procedure follows the same steps as performed for NDF, except that the solution to be used is AD instead of ND solution.

Appendix 9. Ankom Procedure for ADL Determination Using Beakers

Reagents

Sulfuric acid (72% by weight) mix manually by standardizing reagent grade H_2SO_4 to specific gravity 1634 g/L at 20 ºC or 24.00N.

Add 1200 g H_2SO_4 to 440 mL H_2O in 1 L volumetric flask with cooling.

Standardize to 1634 g/L at 20ºC by removing solution and adding H_2O or H_2SO_4 as required.

Apparatus

3 L beakers.

Procedure

The procedure is conducted with the samples in the bags already processed for ADF. After performing ADF determinations, place dried bags/samples into 3L beaker IN HOOD and add sufficient quantity (approximately 250 mL) of 72% H_2SO_4 to cover bags.

Important: Bags must completely dry and at ambient temperature before adding concentrated acid. If moisture is present in the bags, heat generated by the H_2SO_4 and H_20 reaction will affect the results (sample inside bag will char).

Place 2L beaker inside 3L beaker to keep bags submerged.

Agitate bags at start and at 30-minute intervals by pushing and lifting 2 L beaker up and down approximately 30 times.

After 3 hours pour off H_2SO_4 and rinse with hot (90 to 100ºC) H_2O to remove all acid.

Repeat rinses until pH is neutral.

Rinse with approximately 250 mL of acetone for 3 minutes to remove water. (Warning: bags should not be placed in the oven until acetone is completely evaporated).

Appendix 9. (Continued)

Complete drying in oven at 105ºC for at least 2 hours. Longer drying period may be required depending on oven and frequency of sample introduction into the oven.

Remove bags from oven and place directly into a desiccator and flatten to remove air.

Cool to ambient temperature and weigh bags.

Ash entire bag in pre-weighed crucible (30 or 50 mL) at 525ºC for 3 hours or until C-free, cool and calculate weight loss.

Calculate blank bag ash correction using weight loss upon ignition of a blank bag sequentially run through ADF and lignin steps.

Appendix 10. Calculations for Sequential NDF/ADF Analyses

Values to record:

Weight	Convention	Notes
Blank bags:		
Initial (tare) weight	B1	
Weight following NDF extraction	B2	
Weight following ADF extraction	B3	
Weight following ADL extraction	B4	
Weight following ASH	B5	= (pan + ash) – (pan tare)
Sample bags:		
Sample weight	WT	
Sample dry matter	DM	
Bag tare weight	S1	
Weight following NDF extraction	S2	Includes bag wt
Weight following ADF extraction	S3	Includes bag wt
Weight following ADL extraction	S4	Includes bag wt
Weight following ASH	S5	Includes bag wt

Calculations:

Correction factors:

C1 =B2/B1
C2 =B3/B1
C3 =B4/B1
C4 =B5/B1

Ash-free NDF,ADF, ADL, on DM basis:

NDF =[S2-(S1*C1)-S5+(S1*C4)]/[WT*DM]
ADF =[S3-(S1*C2)-S5+(S1*C4)]/[WT*DM]
ADL =[S4-(S1*C3)-S5+(S1*C4)]/[WT*DM]

Appendix 11. Pig weights (kg) by Treatment. Experiment 6

Treatment	Adap. (i) [a]	Adap. (f) [b]	Coll. (i) [c]	Coll. (f) [d]
1	86.1	89.1	89.1	97.4
2	81.9	84.7	84.7	93.5
3	81.8	86.3	86.3	93.9
4	84.2	84.8	84.8	93.9
5	83.5	88.9	88.9	95.6
6	85.7	91.5	91.5	98.9

[a] Average weight at the start of the adaptation period.
[b] Average weight at the end of the adaptation period.
[c] Average weight at the start of the collection period.
[d] Average weight at the end of the collection period.

Appendix 12. Variables for Regression Coefficients, and Regression Coefficients Obtained

The variables used to calculate regression coefficients were the analyzed nutrient concentration in the diet, and the percent nutrient contributed by Ricex-1000™ (from analyzed values). Coefficients were calculated using procedure IML (SAS, 1998). Only linear and quadratic coefficients were considered.

RX inclusion level, %:[a]	0	7.5	15	30
DM in diet, %	87.87	88.28	88.87	90.25
DM from RX, %	0	8.06	16.02	31.65
Regression coef. (Linear)	-0.5958	-0.2511	0.0893	0.7576
Regression coef. (Quadratic)	0.5620	-0.3303	-0.6394	0.4078
Energy in diet %	3920	3988	4082	4253
Energy from RX, %	0	9.46	18.52	35.57
Regression coef. (Linear)	-0.6057	-0.2450	0.1004	0.7504
Regression coef. (Quadratic)	0.5567	-0.3493	-0.6264	0.4191
Fat in diet %	2.61	3.96	5.35	8.50
Fat from RX, %	0	39.52	58.71	77.55
Regression coef. (Linear)	-0.7652	-0.0771	0.2571	0.5852
Regression coef. (Quadratic)	0.3953	-0.6525	-0.3101	0.5673
N in diet %	2.87	2.78	2.74	2.72
N from RX, %	0	6.49	13.12	26.83
Regression coef. (Linear)	-0.5842	-0.2577	0.0760	0.7659
Regression coef. (Quadratic)	0.5677	-0.3083	-0.6536	0.3942

[a] Ricex-1000™.

Appendix 12. (Continued)

RX inclusion level, %:[a]	0	7.5	15	30
NDF in diet %	8.91	9.91	10.73	12.78
NDF from RX, %	0	14.85	27.51	47.96
Regression coef. (Linear)	-0.6417	-0.2197	0.1401	0.7213
Regression coef. (Quadratic)	0.5339	-0.4193	-0.5733	0.4587
ADF in diet %	2.69	3.19	3.82	4.66
ADF from RX, %	0	20.10	35.39	57.08
Regression coef. (Linear)	-0.6734	-0.1924	0.1734	0.6924
Regression coef. (Quadratic)	0.5088	-0.4813	-0.5183	0.4908
ADL in diet %	0.26	0.39	0.50	0.83
ADL from RX, %	0	61.22	77.46	89.30
Regression coef. (Linear)	-0.8288	0.0614	0.2976	0.4698
Regression coef. (Quadratic)	0.2495	-0.7491	-0.1051	0.6047

[a] Ricex-1000™.

Appendix 12. (Continued)

RX inclusion level, %:[a]	0	7.5	15	30
P in diet %	0.38	0.48	0.56	0.82
P from RX, %	0	27.26	44.92	66.45
Regression coef. (Linear)	-0.7116	-0.1519	0.2107	0.6528
Regression coef. (Quadratic)	0.4702	-0.5550	-0.4410	0.5258
Ca in diet %	0.64	0.62	0.57	0.53
Ca from RX, %	0	0.65	1.40	3.34
Regression coef. (Linear)	-0.5379	-0.2784	0.0210	0.7954
Regression coef. (Quadratic)	0.5872	-0.2234	-0.7011	0.3374
Mg in diet %	0.14	0.18	0.22	0.28
Mg from RX, %	0	23.49	40.06	61.87
Regression coef. (Linear)	-0.6922	-0.1736	0.1922	0.6736
Regression coef. (Quadratic)	0.4911	-0.5178	-0.4818	0.5085
K in diet %	0.67	0.73	0.78	0.92
K from RX, %	0	15.55	28.61	49.32
Regression coef. (Linear)	-0.6461	-0.2162	0.1449	0.7175
Regression coef. (Quadratic)	0.5307	-0.4280	-0.5661	0.4633
Na in diet %	0.15	0.16	0.14	0.11
Na from RX, %	0	0.57	1.23	2.93
Regression coef. (Linear)	-0.5381	-0.2787	0.0216	0.7952
Regression coef. (Quadratic)	0.5867	-0.2232	-0.7014	0.3378

[a] Ricex-1000™.

Appendix 12. (Continued)

RX inclusion level, %:[a]	0	7.5	15	30
Zn in diet %	0.014	0.013	0.012	0.013
Zn from RX, %	0	3.15	6.62	14.68
Regression coef. (Linear)	-0.5585	-0.2707	0.0464	0.7828
Regression coef. (Quadratic)	0.5787	-0.2598	-0.6823	0.3634
Fe in diet %	0.018	0.020	0.018	0.020
Fe from RX, %	0	9.03	17.77	34.41
Regression coef. (Linear)	-0.6027	-0.2470	0.0972	0.7525
Regression coef. (Quadratic)	0.5582	-0.3435	-0.6305	0.4157
Cu in diet %	0.0013	0.0014	0.0013	0.0011
Cu from RX, %	0	3.43	7.17	15.80
Regression coef. (Linear)	-0.5607	-0.2693	0.0484	0.7815
Regression coef. (Quadratic)	0.5782	-0.2644	-0.6797	0.3658
Mn in diet %	0.005	0.006	0.007	0.008
Mn from RX, %	0	17.18	31.10	52.29
Regression coef. (Linear)	-0.6562	-0.2078	0.1555	0.7085
Regression coef. (Quadratic)	0.5231	-0.4477	-0.5492	0.4737

[a] Ricex-1000™.

Appendix 13. Phosphorus Determination in Urine (Micro scale Method)

This unpublished method is a modification of the method for analysis of soluble P developed at the University of Kentucky by D'Angelo et al. (2001). It is commonly used to assess total P in water and soil samples by reducing all P compounds to orthophosphate (it is also a total Kjeldahl method to assess total N). The method has the advantage of controlling - by dehydration - the acid concentration in the sample, leaving the sample in a homogeneous acidic matrix (1.26 N).

The procedure was done in duplicate. For digestion, 1ml of undiluted urine samples were pipetted into 25x200 mm Pyrex ignition tubes (Fisher Scientific, Pittsburgh, PA). Then, samples were added with 2.44 mL of 13.25 N sulfuric acid (a prediluted specialty product from LabChem Inc, Fisher Scientific, Pittsburgh, PA). After this, 0.3 g of K_2SO_4 was added to increase the boiling temperature. Two Hengar granules (Selenium coated Hengar granules, Troemner Inc, Thorofare, NJ.) were also added to promote smooth boiling (prevent overheating) and to catalyze the decomposition of organic compounds during the high temperature digestion stage. Then tubes were placed in a programmable aluminum block digester for 1 hour at 220 ºC, wrapping them in aluminum foil to speed-up water evaporation. When water was fully evaporated (no droplets observed in the tube walls, about 1.5 hours later), each tube was topped with a Teflon ball (to control the acid evaporation) and temperature was increased to 360 ºC for one hour (second boiling stage). Then tubes were added with 24.2 mL of deionized distilled water, vortexed, and samples were poured into cluster cups.

The entire digestion procedure was also conducted for 2 blanks (1.0 mL deionized water). Solutions were then ready for P determination by the modified molybdate reagent. This last part included the preparation of eight working standards from 0 to 1.0 ppm P in 1.26 N sulfuric acid.

Appendix 13. (Continued)

One mL aliquots of standards, digested blanks and samples were pipetted from the cluster cups into a deep well microplate, then added with 40 µl of the modified molybdate reagent (containing no acid) and shook for 10 minutes. Finally, samples in the microplate were added with 40 µl of the Malachite Green reagent (preparation instructions for molybdate and Malachite Green reagents are at the end). Microplates were then shook for 20 minutes on an orbital titer plate shaker (Lab Line, Model 4625, Melrose park, IL) and absorbance was read in a multi-channel optical system (microplate reader) at 630 nm (Biotech, Model EL 311, Winoosky, VT). The equipment calculates P concentration from the standard curve.

Modified Molybdate reagent preparation: a 1.75% (w/v) molybdate solution is made by dissolving 8.75 g of ammonium molybdate•$4H_2O$ and diluting to 500 mL with deionized water. Reagent should be stored in plastic bottle, and is stable for a long time at room temperature.

Malachite Green solution: a 0.035% (w/v) solution of Malachite Green carbinol hydrochloride (Aldrich Chemical Company Inc, Milwaukee, WI) in 0.35% (w/v) aqueous polyvinyl alcohol (Mol. Wt. 30,000 to 50,000; Aldrich) is prepared by dissolving 1.75 g PVA in 450 mL of deionized water which has been preheated to 80 ºC. When cooled down, 0.175 g Malachite Green is dissolved in the PVA solution and diluted to 500 mL with deionized water. The reagent should be stored in plastic bottle. It is stable for a long time at room temperature.

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VITA

The author was born in Medellin, Colombia on August 11, 1962. He attended the Universidad de Antioquia, in Medellin, where he received a Bachelor of Science degree in Animal Science (Zootecnia) in 1990. From 1990 to 1998 he worked for a Colombian swine producing company (Tecniagro, S.A.) as farm manager. In 1998 he was appointed for a teaching position in Swine Production at the University of Antioquia. In 2000 he was awarded a Fulbright scholarship to pursue a Ph. D. degree in Animal Science with a focus in swine nutrition at the University of Kentucky.

Jorge H. Agudelo-Trujillo

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