2006

NUTRITIONAL APPROACH TO MINERAL OVER-SUPPLEMENTATION IN GROW-FINISH PIGS: ORGANIC TRACE MINERALS AND PHOSPHORUS BODY ACCRETION

Aitor Balfagón-Romeo
University of Kentucky, tiotowi@hotmail.com

ABSTRACT OF THESIS

NUTRITIONAL APPROACH TO MINERAL OVER-SUPPLEMENTATION IN GROW-FINISH PIGS: ORGANIC TRACE MINERALS AND PHOSPHORUS BODY ACCRETION

The initial study herein assessed mineral digestibility in situations when reduced amounts of inorganic and organic (proteinates) trace minerals (TM) were fed in finishing pigs, and their long-term effects on body mineral status. The second study was a slaughter-investigation that tested the impact of lean growth potential on phosphorus body accretion from 30 to 110 kg.

Organic TM exhibited neither improvement in digestibility nor in total retention; fecal excretion responded quantitatively to mineral intake independently of the source. Contents of copper in kidney and zinc in liver were higher for pigs fed the organic form.

Phosphorus content was linearly related to live weight, empty body weight, and nitrogen content. Phosphorus accretion was very similar for both genetic backgrounds, with gilts retaining more mineral ($P<0.05$) than barrows. A N/P deposition ratio of 5.14 was determined for pigs of both genders and genetic backgrounds to further predict phosphorus requirements based on protein accretion.

Reduction of TM waste from growing-finishing pigs may be best addressed by limiting their dietary inclusion rather than by using organic forms. Data from the second study may be useful for an accurate estimation of phosphorus requirements, which accounts for variations in lean accretion rate.

KEYWORDS: Pigs, Organic Trace Minerals, Phosphorus, Digestibility, Body Accretion

Aitor Balfagón-Romeo

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By

Aitor Balfagón-Romeo

Merlin D. Lindemann

Director of Thesis

David L. Harmon

Director of Graduate Studies

26 July 2006

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THESIS

Aitor Balfagón-Romeo

The Graduate School

University of Kentucky

2006

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THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture
at the University of Kentucky

By

Aitor Balfagón-Romeo

Lexington, Kentucky

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

Lexington, Kentucky

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2006

ACKNOWLEDGEMENTS

My sincere gratitude to my major professor, Dr. Merlin Lindemann, for the quality time he has spent with me, for his generosity and guidance during these two unforgettable years, and to his wife, Susan, for making me feel at home during their lovely family reunions. This appreciation is extended to the other professor of the swine nutrition group, Dr. Gary Cromwell, whose life is an example of service to the academic community. Special thanks also to the third member of my committee, Dr. Richard Coffey, whose recommendations are gratefully acknowledged.

Thanks to my fellow students Beob Gyun Kim and Jorge Agudelo for their valuable help in the animal and the meat labs and for sharing with me their previous experience and knowledge. I also wish to thank Dr. Noel Inocencio for his help in the tedious but necessary process of grinding samples and his assistance in the lab work.

My appreciation to Dr. L. A. Pettey, whose dissertation has served as a model for a generous part of my work in the second experiment.

Special thanks to Alltech, Inc. and the Pig Improvement Company for their economical support, and to Jeremy Burkett and Dr. Kenneth Stalder at Iowa State University for their collaboration with the first experiment.

Thank you to Mr. Jim Monegue and Mr. William “Billy” Patton, who managed and transported the animals required for my experiments. Sincere appreciation is extended to Jim May, for his unquestioned assistance in the processing of all pigs in the meat lab and to David Higginbotham for his assistance in the mixing of experimental diets.

Thank you to my parents, Bienvenido and Josefina, who provided me with their example of life, and not only to them but also to my entire family, for their constant support and love. Thanks also to all my friends from here and there for giving me so many good memories.

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CHAPTER I
Literature Review

Introduction

All forms of living matter require inorganic elements, or minerals, for their normal life processes. The functions of these inorganic elements are extremely diverse. They form the greater portion of the bones and are crucial for a wide variety of regulatory functions in other tissues. They are also essential in maintaining proper osmotic pressure equilibrium as well as acid-base balance.

For many years, a common practice within the swine industry has been to formulate diets with mineral concentrations that exceed the National Research Council (NRC, 1998) requirements estimates, with these extra amounts corresponding to "safety margins". There often was little or no concern about the over-supplementation whenever the goal of maximizing performance was accomplished, and as long as the diets were not excessively expensive (Cromwell, 2005; Kornegay and Verstegen, 2001). These margins are generally established to account for variability in nutrient composition and in nutrient bioavailability of feedstuffs, presence of inhibitors or toxins in ingredients, inadequate processing or mixing of diets, partial loss of nutrients from storage, and other factors (NRC, 1998). In regards to minerals, the discovery of some beneficial properties of copper (Cu) and zinc (Zn) during the nursery period, when used at high doses, has encouraged their use as growth promotants, becoming an additional issue of concern about supplementation beyond that listed as the requirement estimate.

In addition, livestock production is becoming more concentrated in many parts of the world and pork production is no exception (Stalder et al., 2004). High pig density in certain areas of the planet, especially central and northern Europe, can be perceived when examining a recent Canadian report (Saskatchewan Agriculture Food and Rural Revitalization, Statistics Canada, 2003; Table 1.1). More and more people residing in rural areas are not accustomed to practices associated with crop and livestock production. Certainly, producers want to remain profitable in order to continue in the pork business in the future. However, most if not all pork producers also maintain the goal of producing pork in a socially acceptable and environmentally sound manner (Coffey, 1999).

From the mid-1980s onward, environmental concerns have become issues in animal

agriculture (Kornegay and Verstegen, 2001), especially with respect to nitrogen (N) and phosphorus (P), but also with regards to some other minerals. Environmental issues related to water quality have forced livestock and poultry producers to pay much closer attention to their feeding programs so as to limit, primarily, the amount of N and P in the manure produced by animals. Farm animals produce nearly 160 million tonnes of manure annually (Sweeten, 1991). Most of the swine manure is produced in confinement units where the nearby land base may be insufficient to accommodate the waste in an environment-friendly manner, and this excess of N and P in animal manure can contribute to surface and ground water pollution (Cromwell, 2003).

In order to prevent this potential pollution and also to reduce undesirable odors, many governments, especially within the European Union, have regulated the amount of certain minerals that can be applied onto the fields, with special attention to N. We can look at the case of The Netherlands, one of the most intensive farming areas in the world, as an example. The Dutch government, according to the European Directive (91/676/CEE), has established a limit of either 170 kg or 250 kg N/Hectare, depending on the type of crop (grassland or arable land). This amount of total N application concerns the sum of chemical N fertilizer and N in animal manure and other fertilizers. With the new regulation, farms are no longer assessed on the amount of N discharged (lost) into the environment (output), but on the amount of N they use for growing crops (input).

Additionally, the extra and sometimes unnecessary minerals represent an additional cost for the producers. This has forced nutritionists to formulate diets that closer meet the real requirements of the animals in each stage of production. There are several solutions within the swine sector that have been developed during the last two decades; from a better design of facilities that allows for an accurate phase feeding program to the development of low phytic acid corn (Raboy et al., 1994) or genetically-modified pigs (Enviropig®; Golovan et al., 2001) that improve the digestibility of P. From a nutritional standpoint, it has been of particular importance the implementation of commercial production and use of enzymes, like phytase, widely reviewed by Coelho and Kornegay (1996), Cromwell (2003), and Liao et al. (2002), and which will be further discussed. Special interest has also been applied to the utilization of more available forms of minerals.

This review will begin with an overview of organic (sometimes referred to as “chelates” or “proteinates”) trace minerals and their potential improvement of mineral availability, as espoused by some proponents of their use. The section that follows will then focus on the

available literature on the digestibility and accretion of P in body tissues.

Organic Iron, Zinc, Copper and Manganese in Swine Nutrition

Introduction

Much of the research related to trace mineral requirements was carried out more than 30 years ago and may not apply to modern pigs. There is a paucity of information on mineral requirements for current pig genotypes (Close, 2002). Van Lunen and Cole (1998) have suggested that the mineral needs for growth in the modern fast-growing pig hybrids are about twice the level required by the slower growing pigs of some 20-30 years ago because feed intake has been considerably diminished and rate of protein deposition has been greatly increased.

Iron, copper, zinc, manganese, selenium, and iodine are routinely included in trace mineral premixes for swine diets. Many premixes also include cobalt and occasionally chromium and molybdenum are also added. Mateos et al. (2004) analyzed 32 premixes from Spanish manufacturers, pig integrators and private feed mills and stated that commercial trace mineral premixes have higher mineral contents than recommended by research institutions, with the biggest differences generally observed for Cu and Mn. Table1.2 shows their results for grow-finish pigs.

Traditionally, trace mineral supplementation was achieved by the addition of simple inorganic salts such as chlorides, sulfates, carbonates, and oxides. In general, chlorides and sulfates are considered to be more available than carbonates, with oxides having the lowest bioavailability (NRC, 1998). In spite of the lower bioavailability, oxides and carbonates are many times preferred to sulfates as a source of trace minerals because of the lower reactivity in the trace mineral premix and because they contain a higher percentage of mineral, which allows a lower inclusion rate. Also, ZnO is generally preferred to $ZnSO_4$ when used at pharmacological levels to reduce the incidence of diarrhea and to improve piglet performance (Kansas State University, 2003).

Special care has to be taken in regards to cross-contamination of mineral sources. Ca and P are routinely added to pig diets as calcium carbonate and dicalcium phosphate or monocalcium phosphate. The Fe content of these products ranges from 600 to 800 ppm for the Ca source, and from 1,500 to 8,000 ppm for the P sources (NRC, 1998; FEDNA, 2003; Mateos et al., 2004). It is

also of interest to analyze Mn and Zn sources for other trace mineral content. Li et al. (2004) analyzed 12 samples of organic Mn sources and found a Cu content that ranged from 20 to 11,300 ppm, and Zn content varied from 82 to 53,200 ppm. Samples of ZnO analyzed in Spain have shown a Cu content in the range of 200 to 700 ppm (Mateos et al., 2004).

The initial concern about potential P and N delivery excesses is now expanding into other micronutrients and trace elements such as Zn and Cu. Unlike excess land application of N and P, Zn and Cu remain bound to soil particles and do not migrate extensively to water supplies except during soil erosion (Ferket et al., 2002). Therefore, unless they are removed via plant growth, accumulation will occur and eventually result in toxicity of crops. An excess of Zn is toxic to plants, and when the Zn concentration in the soil is over 200 to 300 ppm, the activity of the soil microflora is reduced (Mateos et al., 2006). Revy et al. (2003) indicated that a reduction in the Zn content of piglet diets from 3,000 to 100 ppm, and of fattener diets from 100 to 60 ppm will reduce the concentration of Zn in the slurry from 1,860 to 450 mg/kg DM. Similarly, a study by Jondreville et al. (2002) demonstrated that a reduction of the Cu content from 175 ppm to 6 ppm in piglet feeds, and from 100 ppm to 4 ppm in fattener feeds will reduce the Cu content in the slurry from 911 to 31 mg/kg DM. These and some other works show the efficiency of reducing the trace mineral content of current diets in order to decrease waste onto the environment. On the other hand, a series of studies by Martens et al. (1993) 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. 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 Cu-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 not affected by the mineral additions and no toxicity was reported from the Cu or Zn additions.

The trend towards reducing the trace mineral content of pig diets is expected to continue in the European Union, a decision that might favor the use of phytases and organic sources of trace minerals (Mateos et al., 2004). Moreover, there is evidence of a clear antagonism for

absorption between Fe and Cu. Therefore, current European Union regulations that have reduced the level of Cu in feeds for finishing pigs will also decrease the need for additional iron (Mateos et al., 2006). Furthermore, body iron, as a proportion of body weight, decreases from 20 to 145 kg BW (Mahan and Shields, 1998), indicating that Fe requirements decrease with age. Therefore, current inclusion levels of iron for grow-finish pigs might not be justified and may represent another mineral which could be included to limit Fe load in the environment.

Whether organic trace minerals are ultimately adopted as a means to limit environmental load or not will depend on how well they compare to inorganic minerals in important areas. These areas are absorption and bioavailability, pig performance, and body (tissue) mineral status.

Absorption and Bioavailability of Organic Trace Minerals

According to available information (partially from human nutrition) reviewed by McAnena (2006), iron absorption occurs mainly in the duodenum and healthy adults absorb about 15% of the total Fe contained in a mixed diet. Intestinal mucosal cells carry a specific receptor, which recognizes heme molecules, which are endocytozed into the cell, where Fe is released from the heme molecule by the enzyme heme oxygenase. Non-heme Fe absorption by mucosal cells follows a different mechanism also involving receptors on the cell surface. The rate of absorption is inversely related to mucosal cells' non-heme iron content. The Fe uptake is apparently regulated by one, or several, cell surface proteins but their identity is not yet certain. Dietary Zn and Cu are absorbed in the intestine by both passive diffusion and an active transport pump. Absorption is regulated by metallothionein, which is secreted by mucosal cells in response to high concentrations of divalent metal ions. Metallothionein, however, has a higher affinity for Cu than for Zn, and so high concentrations of Cu can impair Zn absorption both indirectly and directly. Absorption of Mn, which occurs along the whole intestine, is very low and is inversely related to the level of Fe and Ca of the diet.

Organic trace mineral complexes were developed based on the theory that they are more bioavailable, or similar to more naturally-occurring forms in the body than inorganic trace minerals and exhibit improved metabolic utilization resulting in enhanced performance responses and less nutrient excretion (Wedekind et al., 1994). If it can be accepted that uptake of metals from the intestine is the predominant factor influencing their bioavailability, then differences in uptake mechanisms or in the general presentation of the metal in organic versus inorganic form

in the intestinal lumen are likely explanations for the differences noted (Power and Horgan, 2000). An initial theory, widely discussed by Ashmead (1993) proposes that metal amino acid chelates and proteinates utilize peptide and amino acid uptake mechanisms rather than normal metal ion uptake mechanisms in the intestine. The basic concept of this theory is that the metal in question is 'protected' within the complex in a chemically inert form due to the co-ordinate covalent and ionic bonding by the amino acid ligands. Furthermore, transmucosal passage of intact peptides and the existence of peptide carriers in brush-border membranes which utilize a proton-gradient transport mechanism is now firmly established (Gardner, 1998). Indeed, there is good evidence that amino acid absorption in the form of peptides is as important, or perhaps even more important, than absorption of free amino acids in both ruminants and monogastric animals (Webb et al., 1992). Metals using either amino acid or peptide uptake mechanisms would therefore be expected to be absorbed and circulated to target tissues very efficiently. Nevertheless, while considerable circumstantial evidence exists to support such a metal uptake mechanism, direct experimental evidence has failed to identify it (Power and Horgan, 2000). Therefore, there is no conclusive evidence to support the uptake of trace metal chelates or proteinates in intact form through the utilization of amino acid or peptide uptake mechanisms. Indeed, a number of publications suggest that complexes such as zinc methionine are not, in fact, absorbed as intact entities (Hill et al., 1987; Hempe and Cousins, 1989; House, 1999).

Any consideration of uptake mechanisms for metal complexes cannot ignore the possible effects of gastrointestinal pH on the stability or dissociation of such complexes (Power and Horgan, 2000). This topic has been reviewed by Hynes and Kelly (1995), who demonstrated that the distribution of metal species present at given concentrations of metal and amino acids depends on the pH of the solution, and that complexed forms (chelates) of metal ions are not necessarily neutral. Taking these factors into account, it cannot be assumed that metal amino acid chelates and proteinates owe their superior metal bioavailability to uptake mechanisms which allow them to be absorbed as amino acids or peptides in disguise. In the event that such mechanisms do not exist, it is of obvious interest to investigate alternative explanations for the improvements in bioavailability noted for these complexes (Power and Horgan, 2000); for example, their constituent ligands may slow the rate of hydroxyl-polymerization of the metal and allow its effective donation to higher molecular weight binding ligands such as mucin, thereby maintaining them soluble and available to the mucosa for effective absorption.

The term "bioavailability" is generally used to describe properties of absorption and utilization of nutrients. Trace minerals either free or bound to low molecular weight ligands can be absorbed, and be present in body fluids and tissues, yet they might not be utilized (Mateos et al., 2006). Baker and Ammerman (1995) reported that the bioavailability estimates of organic Cu sources ranged from 88% to 147% of the response to cupric sulfate in domestic species. In general, Cu-amino acids and Cu-proteinates show somewhat greater Cu absorption than Cu sulfate (Hahn and Baker, 1993). Coffey et al. (1994) and Zhou et al. (1994) have found that growth performance was greater in pigs fed a Cu-lysine complex than in pigs fed $CuSO_4$. Nevertheless, many authors have not found any benefit regarding mineral availability or pig performance when replacing the inorganic source of Fe, Zn, Cu, or Mn with different organic sources. The reasons for the inconsistencies found in the literature are not known, but the technological processes used to obtain the commercial products might result in products with similar names (e.g. generic chelate), but containing variable concentrations of bioavailable minerals (Mateos et al., 2006), depending on the amount of mineral that is really bound to the ligand, and the strength of that union.

There is a lack of a simple, standardized methodology for verification of the type and quality of commercial sources of organic minerals. Moreover, no industry methods are available to test the degree of chelation or binding of a mineral element to an organic ligand or to relate the characteristics of the source to an *in vivo* bioavailability. Recently, Li et al. (2004) have proposed a new method based on polarographic analysis that allows prediction of the relative bioavailability of organic Mn sources based on chemical characteristics with more accuracy than methods based on solubility. While this holds some attraction, further work is needed in this respect. Therefore, caution is needed when evaluating sources of organic minerals available on the market. Organic minerals commonly cited in the literature generally belong to one of these categories: 1) metal amino acid chelate that is resultant from the reaction of a soluble metal salt with amino acids; 2) a metal amino acid complex which result from complexing a soluble metal salt with an amino acid; 3) a metal proteinate which is the product resulting from chelation of a soluble salt with partially hydrolyzed protein; and 4) a metal polysaccharide complex which is the product resulting from the complexing a soluble salt with a polysaccharide solution (AAFCO, 1997).

Phytate not only reduces P availability, but also the availability of other mineral cations such as Ca, Co, Cu, Fe, Mg and Zn and, apparently, plant phytates have the highest binding

affinity for Zn and Cu (Mateos et al., 2006). It is well established from research in different species that phytic acid decreases the absorption of Zn, and the inhibitory effect is more pronounced when Ca intake is high. The reason is the formation of insoluble Zn-Ca-phytate complexes in the lumen of the gastrointestinal tract (Lowe et al., 2002). Therefore, phytase supplementation to the diet should improve the utilization of Zn and Cu by the pig. In fact, Revy et al. (2003) estimated that 1,000 units of exogenous phytase per kg of feed is equivalent to the inclusion of 24 ppm of Zn as Zn sulfate in diets for 15 kg BW piglets. On the other hand, Augspurger et al. (2004) demonstrated that pharmacological levels of Zn chelate the phytate complex, thereby decreasing the P releasing efficiency of phytase by 30%. Therefore, the Zn effect on the P-releasing efficacy of phytase could have significant implications in diets for early-post-weaned pigs. However, in this same report it was shown that Cu (200 mg/kg of diet) did not affect phytase efficacy.

Studies by Wu et al. (2001) reported that piglets in the post-weaning period were able to maintain growth performance when 50-100 ppm organic Cu (Bioplex^{TM}) was provided, compared with the customary level of 250 ppm Cu from Cu sulfate. Additionally, they measured the rates of absorption and retention not only of Cu, but also of Zn and Fe. It is well known that Cu can interact negatively with Zn and Fe, and *vice versa*. The authors concluded that increasing the dietary content of Cu increased both the rate of absorption and retention of Cu, but fecal excretion of Cu was lower with the organic Cu diet than with Cu sulfate. However, an accurate comparison between mineral sources cannot be performed because of the differences on their inclusion rates. Piglets fed the 50 or 100 ppm organic Cu also had a higher rate of Zn absorption and retention, with lower fecal Zn excretion ($P < 0.05$). The rate of absorption and retention of Fe was numerically higher on the diets containing organic Cu compared with Cu sulfate (Table 1.3). This indicates that the use of organic sources of Cu may not interfere with Zn or Fe metabolism, yet this may also be caused by the lower amount of mineral used (50 or 100 vs. 250 mg/kg).

Mullan (2002) has recently shown that piglets fed diets containing two levels of Bioplex^{TM} Zn (100 and 250 ppm, respectively) had reduced concentration of Zn in feces ($P < 0.01$) compared with those fed 1500-2250 ppm Zn from ZnO; indeed it was no higher than that in the feces of the control piglets fed no supplemental Zn. Wu et al. (2001), on the other hand, reported that piglets fed 2000 ppm Zn from ZnO retained the most Zn ($P < 0.01$), but the proportion of Zn absorbed and retained in relation to Zn intake was numerically less than that for

the piglets fed 400 ppm Bioplex^{TM} Zn. According to the authors, the piglets fed the ZnO excreted more than four times as much Zn as those receiving organic Zn (Table 1.4), but comparisons between sources are again limited by the study design. The absorption and retention of Cu and Fe was higher for the piglets fed 2000 ppm ZnO, yet the authors do not give an explanation for this fact.

Organic Trace Minerals and Pig Performance

Gestation - Lactation

Neonatal pig tissues are Fe-deficient and many studies have been performed in which pregnant sows have been treated with exogenous Fe to improve reproductive performance. The results have been discouraging, especially when inorganic, rather than organic sources of Fe were used (ARC, 1981). The reason is that Fe does not readily cross both the placental and mammary gland barriers, and consequently newborn piglets and sow's milk are very poor in this mineral. However chelated iron proteinates or amino acid-iron chelates given to sows during gestation and lactation at a dose of 60 mg/kg of diet increases the iron content of liver, and promote hemoglobin formation and piglet growth (Ashmead, 1979). A possible explanation for this finding is that the piglets have access to sow feces rich in iron. Hence, not all the authors agree on the extra benefits of supplementing organic iron to sow diets (Fox et al., 1997).

Weanling

The reasons for the positive effect of Cu as Cu-sulfate on pig performance are not fully known. Copper-stimulated growth is largely dependent on a simultaneous increase in feed intake that resembles the mode of action of in-feed growth promoters (Zhou et al., 1994). High Cu levels are very efficacious in weanling pigs reared under poor management conditions, but the beneficial effects are very limited in healthy fattening pigs (Bradley et al., 1983). The response of piglets and other monogastrics to pharmacological levels of Cu seems to be independent of the presence of antibiotics (Cromwell et al., 1998; Nys, 2001; Hill et al., 2001) but it is lower or even absent in the presence of high levels of Zn in the diet (Hill et al., 2000). In piglets, an excess of Cu in the diet reduces Fe reserves in the liver that may lead to anemia and, therefore, when high levels of Cu are used in weanling diets, an excess of Fe (up to 250 ppm) in the feed is recommended (Bradley et al., 1983).

Mullan (2002) has recently shown that piglets fed 100 ppm organic Zn (Bioplex™) had the same growth rate as those fed 1500-2250 ppm Zn from Zn oxide, but those piglets fed 250 ppm organic Zn had superior growth rate ($P<0.01$). Wu et al. (2001), on the other hand, reported that piglets fed 2000 ppm Zn from ZnO had higher growth rates than those fed organic Zn.

Growing - Finishing

By far the largest proportion of feed is used by grower and finisher pigs, and thus there is interest in anything that will improve growth rate, feed conversion and carcass quality without detriment to welfare or food safety (Mullan and D'Souza, 2006). Henman (2001) evaluated the effects of diets containing $CuSO_4$ or organic Cu (Bioplex™) on grower-finisher pig performance in the largest piggery of Australasia (Bunge Meat Industries). Compared with the basal diet, providing 200 ppm of $CuSO_4$ improved growth rate by 5% and feed conversion by 3%. There was no difference in performance between the pigs given 100 ppm organic Cu and those fed 200 ppm $CuSO_4$, indicating that inorganic Cu could be successfully replaced by lower concentrations of organic Cu. Data such as these from a large commercial piggery provide the opportunity to compare the cost effectiveness of the organic alternative with inorganic Cu.

In a more recent experiment, Mullan et al. (2004) compared Cu and Zn provided in either the inorganic or organic (Bioplex™) form to pigs from 25 to 107 kg live weight. Neither the form of trace mineral nor the concentration in the diet had any significant effect on growth rate; the authors concluded that this might suggest that higher concentrations of inorganic minerals can be replaced with lower concentrations of organic Cu and Zn without any detrimental effect on performance. It might also suggest that the need for supplementation of trace minerals in growing-finishing pigs is very small, if any. Additionally, pigs fed Cu and Zn in the organic form were leaner than those fed diets containing inorganic Cu and Zn (11.8 vs. 13.8 mm fat thickness, respectively; $P = 0.016$). The authors were unable to explain the reason for this response, and the improved carcass quality of pigs fed these minerals in the organic forms is the subject of current research.

Apple et al. (2004) observed that the inclusion of 320 to 350 ppm of an amino acid-Mn complex (Availa-Mn™; Zinpro Corp., Eden Prairie, MN) improved feed efficiency and meat quality in pigs. However, 700 ppm of the same complex did not elicit any response. Also, Kats et al. (1994) compared diets containing 24 or 88 ppm of Mn of either an inorganic or chelated source on growth performance and carcass characteristics of pigs. No beneficial effect of the

extra-supplementation on growth performance, backfat thickness or *longissimus* muscle area was reported. Therefore, further research is needed in order to recommend this practice under commercial conditions.

Organic Minerals and Body Mineral Status

In addition to performance, the effects of organic minerals on carcass quality, blood parameters, liver and muscle mineral content have been measured in some experiments. In almost all cases carcass quality and characteristics were not significantly affected (Fremaut, 2003). The effects on blood, liver and muscle mineral content have been variable. Wedekind et al. (1994) depleted Zn stores of pigs during the nursery phase by feeding diets containing no supplemental Zn for 5 weeks. Zinc was then supplemented at 0, 5, 10, 20, 40, and 80 mg/kg as $ZnSO_4$, and at 0, 7.5, and 15 mg/kg as $ZnSO_4$, Zn-Methionine, Zn-Lysine, and ZnO during the growing and finishing phases, respectively. The control growing and finishing diets used in this study contained 32 and 27 mg of Zn/kg, respectively. Supplementation of the control diets with Zn increased plasma and bone Zn, but did not affect pig performance in either phase. In a recent study, Creech et al. (2004) determined the effects of decreasing supplemental concentration of Zn, Cu, Fe, and Mn, and trace mineral source (sulfates vs. a 1:1 sulfate:proteinate combination) on mineral status from weanling through development. Even though performance of pigs was not affected by treatment, plasma Zn and alkaline phosphatase (AP) activity were lower in pigs fed reduced dietary Zn (either organic or inorganic) than in the control group, which provided 15, 100, 100, and 40 mg/kg of supplemental Cu, Zn, Fe, and Mn, respectively, during the growing and gilt-developer phases. AP activity and serum or plasma Zn have been used as indicators of Zn status, however the level of circulating Zn in pigs necessary to maximize Zn dependent functions has not been defined. Plasma Cu concentration and ceruloplasmin activity were generally not affected by treatment either. In agreement with Creech's study, Wedekind et al. (1994) observed that Zn (as $ZnSO_4$) supplementation of diets containing 27 to 32 mg of Zn/ kg increased plasma Zn without affecting pig performance. On the other hand, blood and muscle zinc content were unaffected by diet zinc level or form (ZnO vs. Bioplex™) in a study conducted by Mullan et al. (2002).

Additionally, many studies on the relative bioavailability of Mn have been conducted with poultry for which there is a critical supplemental need for the element. Early studies with poultry, in which growth or leg deformities were measured, were not sufficiently sensitive to

detect differences in bioavailability among supplemental sources (Power and Horgan, 2000). However, over the last two decades, tissue deposition of the element has been used to estimate Mn bioavailability. Such studies have revealed that the most available sources of Mn are manganese-methionine and manganese proteinate (Henry, 1995). Such findings are in agreement with recommendations that liver copper levels, not plasma copper levels, are a better indicator of copper status and relative bioavailability between sources (Lee et al., 1988; Xin et al., 1991).

Whether organic sources of minerals can substitute for greater amounts of inorganic sources with no negative effects on performance and body mineral status in finishing pigs is still unclear. In fact, it is possible that those reduced mineral diets in the organic form had no negative effects compared to the diet with standard levels of inorganic minerals simply because there is not a real need for trace mineral supplementation in that stage of production. Previous research has demonstrated that removing the trace mineral premix (TMP) during the finishing period does not affect growth performance of swine (Kim et al., 1997; Mavromichalis et al., 1999); but, weight ranges for these studies were only 70 to 112 kg and 86 to 116 kg, respectively. Nonetheless, Spears et al. (2001) indicated that removing the Zn and Cu for 22- to 91-kg pigs did not negatively affect growth performance. Other research has shown that removing the vitamin premix and TMP had no effect on growth performance in swine (Edmonds and Arentson, 2001; Shaw et al., 2002). Shelton et al. (2004) used a weight range of 22 to 109 kg and reported that removing the TMP increased liver weight and liver weight as a percentage of final BW. Removing the TMP decreased ($P = 0.08$) Zn concentrations in the bone, muscle, and liver, and decreased Cu and Fe concentrations in the bile but increased ($P = 0.08$) Mn concentrations in the bile and liver of pigs. Data from Shelton et al. (2004) indicate that removing the TMP in diets for growing-finishing pigs may have no negative effects on growth performance or pork quality, but it may have negative effects on carcass traits (hot carcass weight, dressing percent, and kilograms of carcass lean were decreased, $P = 0.10$) and variable effects on tissue mineral content (bone ash percent and bone strength were decreased but liver and kidney weight were increased, $P = 0.10$). Although many researchers showed no adverse effect of removing the TMP on growth performance, on commercial farms there are many stressors that can increase the requirement for TMP including temperature, stocking density, and degree of contamination (Cunha, 1977; Stahly et al., 1997), which suggests caution should be used in considering the total removal of TMP in growing-finishing pigs.

Much of the research related to trace mineral requirements was carried out more than 30 years ago and may not apply to modern pigs. During the last two decades nutritionists developed organic trace mineral complexes, based on the theory that they are more bioavailable than inorganic trace minerals, yet controversy still exists regarding many aspects such as their potential improvement in performance and reduction in mineral waste, as well as in regards to the election of a response to measure bioavailability. Moreover, the largest proportion of feed is used by grower and finisher pigs but only a few studies have focused on this phase of production in comparison with the nursery phase. Consequently, there is a need to verify the digestibility of different sources of trace minerals during the growing-finishing phase, and their retention in different tissues and organs as a long-term measure of bioavailability.

Digestibility and Body Accretion of Phosphorus

Introduction

Phosphorus has more known functions than any other mineral element in the body but less is known about P homeostasis than calcium (Ca) homeostasis. A major role of Ca along with P involves mineralization of bone. Additionally, P is located in every cell in the body with functions ranging from structural components of phospholipids in membranes to energy storage in the form of phosphate diester bonds and even osmotic balance and buffering (Crenshaw, 2001).

Overfeeding of P has led to an increase of P excreted by the pig into the environment. 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). 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).

There is therefore a great need to verify P requirement levels so that diet formulations can more closely match pig requirements at all stages of growth.

Phosphorus Absorption and Digestibility in Growing Pigs

The major sites of P absorption are in the upper parts of the small intestine. P is absorbed as the inorganic phosphate from both dietary inorganic sources and from organic sources after hydrolysis by phosphatases in enterocytes (Jongbloed, 1987). Phosphate can also be absorbed as a structural part of organic compounds such as phospholipids. Phosphate absorption occurs in the small intestine by both active and passive transport systems (Breves and Schroder, 1991), and it is stimulated by vitamin D. The active transport mechanisms may become saturated in high P intake situations and thus passive diffusion may become more prevalent as a route of P absorption (Breves and Schroder, 1991). Intracellular transport of P may occur by carrier proteins mediated by Vitamin D, but the mechanisms are still unclear.

Under normal circumstances regulation of P homeostasis occurs by controlling the absorption rate of inorganic phosphate (P_i) in the upper small intestines and by renal Pi excretion. These processes are mainly mediated by parathyroid hormone (PTH) and calcitriol (1,25-dihydroxycholecalciferol, 1,25-$(OH)_2D_3$). The concerted action of increased/decreased circulating calcitriol/PTH on the intestinal tract, bone and kidneys normalizes P_i levels in plasma (Schroder et al., 1996).

Fernandez (1995a) found that the balance of Ca seemed to be regulated solely by the intestine and declined relatively with decreasing intake. Balance of P responded in a similar manner but was also modulated by renal action. In further kinetics studies (Fernandez, 1995b) with radioactive Ca and P, it was shown that bone accretion was constant and independent of the level of intake. Conversely, bone resorption decreased with increasing intake, thus the increased amounts of Ca and P entering the system by increased absorption were counteracted by a reduced amount of mineral leaving bone (Fernandez, 1995c).

There is no argument that the digestibility of P in various diets by pigs is highly dependent on the form of P (phytic acid, phospholipids, etc.) in the individual ingredients in the diet. However, the effect of body weight on P digestibility is quite variable across studies, and is a debated factor. Pettey (2004) carried out an extensive literature review of available data. In that review, Jongbloed (1987), one of the primary researchers on P needs of pigs, concluded that digestibility of P decreased with age. In partial support of that concept, a comparison of P balance data of pigs consuming three levels of available P at three different body weights revealed that apparent digestibility was unchanged to 80 kg, but decreased at heavier weights

(O'Quinn et al., 1997). However, several other studies included in this review have disagreed with these conclusions. Regardless of P or Ca level in the diet, apparent P digestibility increased almost 13% in 100 kg pigs as compared with 65 kg pigs (Eeckout et al., 1995). Similar results were observed in 35 and 65 kg pigs as well (Fernandez, 1995a). Kemme et al. (1997) determined that increasing body weight from 60 to 90 kg increased apparent P digestibility in pigs housed in metabolic crates, but not in pigs reared in pens. Pettey (2004) concluded that level of dry matter intake, P intake, and genetic differences in pigs across experiments may account for discrepancies in digestibility differences with body weight, as previously stated by Jongbloed (1987).

According to Shen et al. (2002), differences in P contents among studies are the largest single factor responsible for the large variability in the apparent P digestibility values in corn reported in the literature. In their experiment, the relative contribution of the endogenous P outputs, as a percentage of total dietary P contents, decreased exponentially as dietary P content increased. Furthermore, they reported that the average apparent P digestibility and availability values are 22% in the literature, whereas true P digestibility is 57% in corn as determined from their study. According to these authors, the current literature data of apparent digestibility and availability underestimate the true digestive utilization of P in corn for growing pigs by approximately 35%, and current diet formulation on the bases of total, apparent P digestibility and availability values in corn inevitably leads to P overfeeding and excessive P excretion in pigs.

The continued overfeeding of P has led to an increase of P excreted by the pig and thus a greater P content of manure put back into the environment. Although pigs contribute approximately 10% to total manure output by domestic farm animals, their output of P is approximately 23% of the total P output (Sweeten, 1991). The major reason for the high concentration of P in swine and poultry manure is that most of the P in cereal grains and oilseed meals is bound in an organic complex called myo-inositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate, commonly referred to as phytic acid or phytate. Unfortunately, pigs and poultry do not have sufficient amounts of phytase in their digestive tract to degrade phytate, so most of the P from the grains and oilseed meals is excreted in the feces and, consequently, formulating diets on an "available P" basis rather than a "total P" basis allows one to more precisely meet the P requirements without having overages of nutrients (Cromwell, 2003).

A recent series of experiments by Hastad et al. (2004) comparing commercial

environment pigs' requirements with NRC (1998) recommendations illustrate the vast range of P requirements estimates and the difference between commercial and experimental conditions. Their results suggest that 33- to 55-kg pigs require approximately 0.22% available P (aP), which corresponds to 0.60 g of aP/Mcal of ME or 3.30 g of aP/d to maximize ADG and G:F compared with NRC (1998) estimates of 0.23%, 0.70 g of aP/Mcal of ME, and 4.27 g of aP/d for 20- to 50-kg pigs. Finishing pigs (88 to 109 kg) require at least 0.19% aP, corresponding to 0.53 g of aP/Mcal of ME or 4.07 g aP/d compared with NRC (1998) estimates of 0.15%, 0.46 g of aP/Mcal of ME and 4.61 g of aP/d for 80- to 120-kg pigs. Even though the percentage of bone ash and bending moment continued to increase with increasing aP, recommendations of Hastad et al. (2004) were based on growth responses, illustrating the need to also clarify response criteria when discussing requirements for pigs.

Estimation of Phosphorus Requirements

Two approaches for estimating P requirements have been utilized, the empirical approach (balance or feeding experiments) and the factorial approach. The advantages and disadvantages of both methods have been extensively discussed by Jongbloed (1987). The empirical approach is the traditional one and has been used for decades to estimate the Ca and P needs of pigs. Scientifically, the factorial method is considered to be advantageous for several reasons, not least that it allows a more accurate approach because it can be applied across various systems of production. However, requirement estimates produced with the factorial approach need to be confirmed in feeding experiments.

The NRC (1998) Swine subcommittee did not use a modeling approach to estimate the mineral requirements probably due to the fact that there was not sufficient data available in the literature to develop an accurate model. As a result, all the estimates were based on empirical data from research studies (Cromwell and Baer, 2005). Estimates were made for six weight classes of growing-finishing pigs.

An attempt to utilize modeling procedures in the factorial estimation of P requirements was conducted by Jongbloed and Everts (1992). This procedure separated the net P requirement into P required for maintenance and P required for growth. Maintenance P was defined as the P lost from the inevitable excretion via urine and feces. The authors assumed that P required for

growth would be directly reflective of the P retained in body tissues as measured by slaughter investigations.

Using the methodology and assumptions of Jongbloed and Everts (1992), Pettey (2004) calculated the net P requirement for every body weight from 25 to 110 kg (and plotted as in Figure 1.1). According to Pettey (2004),

> "It can be seen that the factorially estimated requirement is lower at lighter body weights, but becomes much closer at heavier weights. Much of this change is due to the proportion that maintenance P estimates contribute to the net requirement in Jongbloed and Evert's (1992) data. At 25 kg body weight, maintenance P represents approximately 6% of the net requirement, while at 110 kg body weight it represents almost 17% of the total requirement. It is also interesting to note that the P requirement for growth in the data of Jongbloed and Everts (1992) is based on the relationship of P retention to live weight gain; yet, the live weight gain estimates cited in their published report are much lower than those estimated by NRC (1998) for a pig consuming a corn-SBM diet at a carcass fat-free lean growth rate of 325 g/d. Line 3 in Figure 1.1 shows the factorially estimated (Jongbloed and Everts, 1992) P requirement using the live weight gain of an average barrow (325 g/d lean gain) as estimated by NRC (1998)".

More recent series of experiments were conducted to estimate the maintenance requirements for P (Pettey et al., 2006) and to assess the accretion rates of whole-body P (Pettey et al., 2003; 2004a, b). Their estimates of P requirements (Figure 1.2) were similar to the bioavailable P requirements of NRC (1998), especially in the mid-weight range, but differed some in the lighter- and heavier-weight pigs, in which case their estimates were less than those of NRC (1998). While these studies seem to confirm the NRC (1998) estimates, they are limited to a degree. They were done with a single genotype, and additional work of this type needs to be done with other genotypes of pigs to determine the effects, if any, that the lean growth rate or perhaps other factors related to genotype may have on P requirements (Cromwell and Baer, 2005).

Endogenous Phosphorus Loss

Estimation of dietary influences on P excretion in growing pigs is often confounded by the inability to partition total P excretion into the indigestible dietary P portion and the endogenous P portion. In addition, modeling P requirements for growing pigs includes a factor referred to as maintenance P, which is a reflection of endogenous P excretion via the feces and/or urine. Jongbloed and Everts (1992) estimated from available data that 3 mg/kg body weight

could be considered a reasonable estimate of the maintenance P requirement for pigs consuming a low P diet; yet pigs consuming a diet in excess of P would have a maintenance requirement of 9 mg/kg BW. According to these authors, endogenous or inevitable P losses via urine are considered to be 1 mg/kg BW/d.

By using a regression technique, fecal endogenous P outputs in soybean meal were estimated to be 0.25 g/kg dry matter intake on average across four graded treatments (1.1, 2.1, 3.2 and 4.3 g P/kg diet on a dry matter basis) for 5 to 20 kg BW pigs (Fan et al., 2001), and 0.45 g/kg dry matter intake (accounting for 17.6% of the NRC (1998) recommended available P requirements in growing pigs) for 20 to 50 kg pigs (Ajakaiye et al., 2003). For corn, endogenous P excretion was determined to be 0.67 g/kg dry matter intake for 20 to 45 kg pigs (Shen et al., 2002). According to Pettey (2004), these estimates are unique in that they are the first attempts at associating endogenous P with specific feed ingredients, and they provide evidence that endogenous P excretion may not fluctuate with increasing P intake when fed at levels below the requirement. Moreover, the true ileal and fecal P digestibility values in soybean meal were determined, and there was no difference between true ileal and fecal P digestibility values (50.7 ± 7.1 vs. 48.5 ± 5.4%, $P > 0.05$). This indicates that the large intestine does not play a major role in the absorption of exogenous P (Fan et al., 2001). A more recent study conducted by Pettey et al. (2006) to estimate the endogenous P loss at zero P intake in pigs fed semi-purified diets resulted in 110, 156, and 226 mg P loss/d for 27-, 59-, and 98-kg pigs, respectively. That represents an increase of approximately 1.632 mg for each 1-kg increase in BW from 25 to 100 kg.

Body Distribution of Phosphorus and Tissue Accretion

Reference values for the major distribution of P in the body indicate that approximately 75% of whole carcass P is complexed in the bone matrix, while the remaining 25% is found in soft tissue stores (Crenshaw, 2001). The latter portion is predominately in the lean muscle mass, mostly due to the greater overall mass of muscle tissue compared with other tissues in the body. In the whole body of the pig, P can also be found in visceral tissue and blood (Just Nielsen, 1972; Mahan and Shields, 1998). In bone, Ca is incorporated with P at a ratio of 2.2:1 in the form of hydroxyapatite ($Ca_{10}(PO_4)_6$) crystals, and this ratio appears to be constant even when pigs are fed diets deficient in Ca (Nicodemo et al., 1998).

Just Nielsen (1972) determined the distribution of P to be 77% in bone, 17% in muscle, 3.5% in viscera, and 0.9% in fat tissue. In bone ash, Ca represents 37 to 39% while P represents 17 to 19% (Crenshaw, 2001). However, in the young pig, the relationship among the dissectible components (adipose tissue, lean, and bone) and the chemical components (lipid, protein, water, and ash) is not constant. During the first few weeks following birth, the protein content of the fat-free body of the pig increases rapidly, accompanied by a rapid decrease in water content (Mitchell and Scholz, 2001). That suggests a verification of ground and dissected mineral values in growing pigs at various stages along the growth curve needs to be conducted. Additionally, in the associations among body protein, water, and ash, most of the variation in chemical body composition between different groups of pigs can be attributed to variation in body lipid content. In a similar manner, body fat tissue content is the main contributor to variation in physical body composition (de Lange et al., 2001), with sex and genetic background the two main factors along with diet that affect lean and fat content in the carcass. Consequently, more studies should be addressed to evaluate differences in mineral content between different genders and breeds. For a deeper understanding of the procedures to estimate the whole-body mineral composition in pigs the reader is referred to ARC (1981), Rymarz (1986), and Hendriks and Moughan (1993).

Regarding potential changes with whole-body mineral distribution across different body weights, Jongbloed (1987) assumed a constant Ca/P ratio existed in all weights of growing pigs and settled on the value of 1.62, which is exactly the value calculated by Just Nielsen (1992) from slaughter investigations. Unlike them, Hendriks and Moughan (1993) presented an average Ca/P ratio of 1.52 (range 1.46 - 1.66). Ratios of Ca to P might differ among studies due to variables such as pig genotype, sex, collection procedures, and analytical techniques. However, it appears from each of these studies that Ca/P ratio changes with increasing body weight, apparently in correlation with increased mineralization of skeletal tissue with age (Pettey, 2004). In fact, according to Rymarz et al. (1982), as the live weight increases from 20 – 100 kg or as the content of crude protein in the body grows from 3 – 15 kg, Ca/P ratio increases from about 1.60 – 1.73.

Additionally, work that has been conducted to study mineral composition of pigs is somewhat dated and may not reflect modern pig genotypes, which do differ from older genotypes not only in regards to compositional aspects like protein deposition, but also in voluntary feed intake capacity, which may affect total mineral intake and dietary need. Pettey (2004) chose a few select studies from the literature representing experiments conducted in the

last 20 years to evaluate this aspect; because the mathematical representation of the data differed between authors, the provided means of P content (g) for each weight group studied were plotted against the average empty body weight (kg) of each group for the purpose of discussion and comparison only. Simple linear relationships were discovered in all data and the slopes of each derived equation were considered to be the amount of P accreted per kg increase in empty body weight. In the investigations of Rymarz et al. (1982), pigs from three different breeds were analyzed and the relationship of P content and empty body weight was: 5.51 (Landrace), 5.42 (Large White), and 4.79 (Hampshire) mg P/kg EBW. From this simple comparison it appeared that there may be an influence of breed (or body composition differences as defined by breed) on the P retention in growing pigs. Although in gilts of a similar age the actual contents of ash, Ca and P in the body differed significantly between the breeds, the differences considerably diminished when the contents of individual components were expressed as their ratio to the EBW and were non-significant for their ratio to the content of crude protein. Rymarz (1986) conducted another study involving a larger data set of Landrace gilts where the relationship of P accretion to empty body weight gain was 5.0 g/kg. Comparison of the Landrace response in the two Rymarz studies would indicate that even though breed differences may exist, other factors exist which may alter the within breed response as much as the across breed response. In the most recently published study (Mahan and Shields, 1998), data were collected from Hampshire-Yorkshire-Duroc x Duroc pigs and the mineral content of the whole empty body was determined. The relationship of P content to empty body weight gain was 4.37 in pigs from birth to 145 kg. In the final study reviewed, total mineral content of 36 Large White x Landrace-Large White boars and gilts was evaluated (Hendriks and Moughan, 1993). For boars, 4.18 g of P was gained for every kilogram increase in EBW, while the accretion rate in gilts was slightly higher at 4.40 g.

Rymarz et al. (1982) demonstrated that there is a correlation between whole-body mineral retention and protein deposition and that the retention of minerals per unit body protein appears to vary with the rate of protein retention. They also claimed that estimates of the storage of minerals based on protein deposition data would be preferable to those based on the live-weight gain. The relationship of P accretion to protein gain was estimated to be 35 g of P for every kg increase in whole body protein by Jongbloed (1987). Bertram et al. (1995) showed that pigs with a capacity for moderate or high lean growth had greater lean muscle deposition when increasing available P levels were fed, yet total body growth rate was maximized at a lower

dietary intake of available P (0.22% vs. 0.32%) for pigs from a high lean growth genotype. Furthermore, finishing pigs treated with porcine somatotropin require higher dietary percentages of P to maximize carcass lean deposition as compared with untreated pigs (Carter and Cromwell, 1998). Whether genetically high lean growth pigs have a greater need for P is still unclear and further studies on P accretion are needed. Concerning potential differences between gender, Hendriks and Moughan (1993) found no significant ($P>0.05$) interactions between EBW or fat-free body weight and sex, nor an effect of sex of animal for any of the minerals.

There is a great need to verify P requirement levels so that diet formulations can more closely match pig requirements at all stages of production. However, it is not clear whether the requirements for certain minerals by pigs possessing a high lean growth rate, due to superior genetics, may be higher than the levels shown in the NRC (1998) tables. The present thesis will try to respond to that question.

Conclusion

Waste issues are going to be a continuous societal concern. Present and future good agricultural practices have to encourage a more rational use of feed supplements, including minerals, in our farms. Problems related to the addition of trace minerals to the diets will be addressed by limiting their inclusion and/or by using organic forms, but more information is needed on whether organics are in fact different in digestibility or bioavailability and tissue supply. In addition, estimation of adequate phosphorus requirements would be best addressed by using a factorial approach, yet more data regarding potential genetic and gender differences have to be obtained.

Table 1.1 – Pig density for selected countries[1].

Country or Region	Population	Pig Inventory	Pigs / Hectare
Europe			
Germany	82,431,000	25,958,000	2.20
Spain	40,341,000	23,858,000	1.83
France	60,656,000	15,290,000	0.83
Netherlands	16,407,000	13,000,000	14.36
Denmark	5,432,000	12,990,000	5.67
Belgium	10,364,000	6,851,000	8.14
United Kingdom	60,441,000	5,588,000	0.99
Asia			
Japan	127,417,000	9,612,000	2.16
China	1,303,313,000	464,695,000	3.24
United States			
Iowa	2,926,000	15,000,000	1.38
North Carolina	8,049,000	9,600,000	4.23
Minnesota	4,919,000	6,100,000	0.70
Canada			
Quebec	7,598,000	4,280,000	2.31
Ontario	12,541,000	3,700,000	1.01

[1]Adapted from Saskatchewan Agricultural Food and Rural Revitalization, Statistics Canada, 2003.

Table 1.2 – Composition of trace mineral premixes in mg/kg of feed for grow-finish pigs in Spain[1].

	Mean	Mode	CV %	NRC 1998[2]
Fe	94	80	35.4	60
Cu[3]	99	90	46.7	4
Zn	109	110	29.6	60
Mn	46	50	18.3	2
Se	0.19	0.10	46.9	0.15
I	0.77	1.00	44.2	0.14
Co	0.27	0.40	61.5	-

[1]Adapted from Mateos et al. (2004).
[2]20 to 50 kg BW.
[3]Cu used as a growth promoter.

Table 1.3 – Absorption and Retention of Cu, Zn and Fe in nursery pigs fed different Cu sources.

	Control	Organic[1] Cu		Cu Sulfate
Cu (ppm)	0	50	100	250
ADG, g	563	518	565	562
G:F, g/kg	577	561	566	590
Copper				
Intake, mg/d[a,b,c]	28.3	80.6	135.7	394.7
Retention, mg/d[a,b,c]	0.2	5.2	8.1	64.8
Fecal Cu, mg/d[a,b,c]	27.3	72.9	123.6	325.5
Absorption, %[b,c]	3.7	8.9	8.8	17.6
Retention, %[b,c]	0.6	6.2	5.8	16.4
Zinc				
Absorption, %[a,c]	13.7	19.6	22.0	14.5
Retention, %[a,c]	12.2	18.2	20.6	13.5
Iron				
Absorption, %[d]	23.4	21.1	22.0	20.6
Retention, %[d]	22.6	20.2	21.0	20.1

Adapted from Wu et al. (2001)
[1]Bioplex™, Alltech, Inc.
[a]Linear increase ($P<0.01$) in response to Cu as Cu proteinate.
[b]Contrast between $CuSO_4$ and basal diet ($P<0.01$).
[c]Contrast between $CuSO_4$ and 50 + 100 ppm Cu as Cu proteinate ($P<0.01$).
[d]Contrast between $CuSO_4$ and basal diet ($P<0.05$).

Table 1.4 – Absorption and Retention of Zn, Cu and Fe in nursery pigs fed different Zn sources.

	Control	Organic[1] Zn		ZnO
Zn (ppm)	0	200	400	2000
ADG, g[a]	563	518	538	591
G:F, g/kg	577	571	578	567
Zinc				
Intake, mg/d[b,c,d]	226	337	536	2543
Retention, mg/d[b,c,d]	28	38	93	390
Fecal Zn, mg/d[b,c,d]	195	294	436	2109
Absorption, %[b]	13.8	12.9	18.7	17.0
Retention, %[b]	12.2	11.5	17.4	15.2
Copper				
Absorption, %[b,c,d]	3.7	16.9	16.1	21.3
Retention, %[b,c,d]	0.6	14.5	13.6	18.0
Iron				
Absorption, %[c,d]	23.4	26.1	24.5	37.0
Retention, %[c,d]	22.6	25.4	23.9	36.0

Adapted from Wu et al. (2001)
[1]Bioplex™, Alltech, Inc.
[a]Contrast between ZnO and 200 + 400 ppm Zn as Zn proteinate ($P<0.05$).
[b]Linear increase ($P<0.05$) in response to Zn as Zn proteinate.
[c]Contrast between ZnO and basal diet ($P<0.01$).
[d]Contrast between ZnO and 200 + 400 ppm Zn as Zn proteinate ($P<0.01$).

Figure 1.1 - Comparison of estimated daily phosphorus requirements by (1) use of factorial estimation (Jongbloed and Everts, 1992), (2) use of NRC (1998) model, and (3) use of factorial estimation (Jongbloed and Everts, 1992) with NRC (1998) estimated growth rates. Excerpted from Pettey (2004).

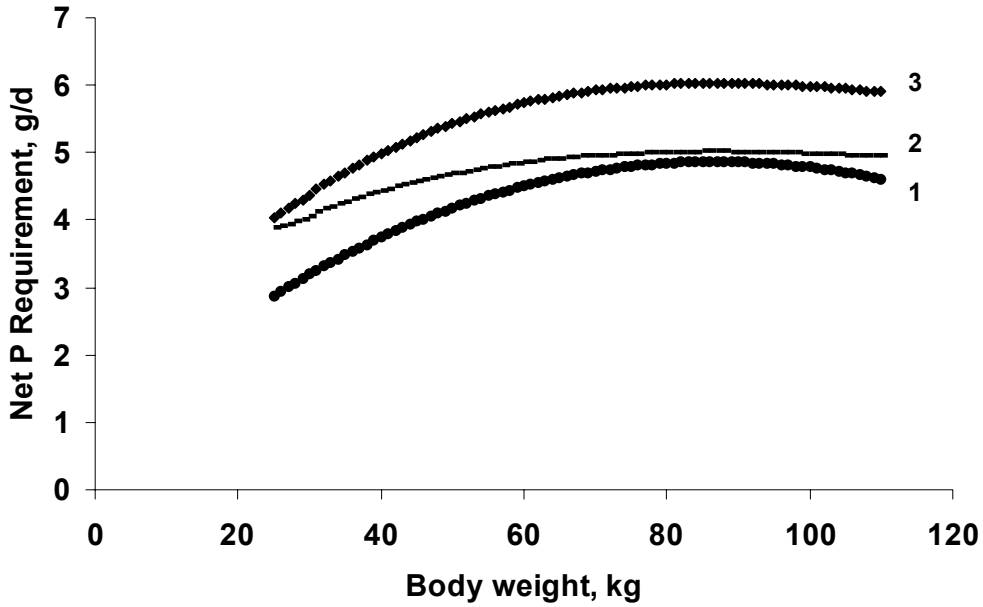
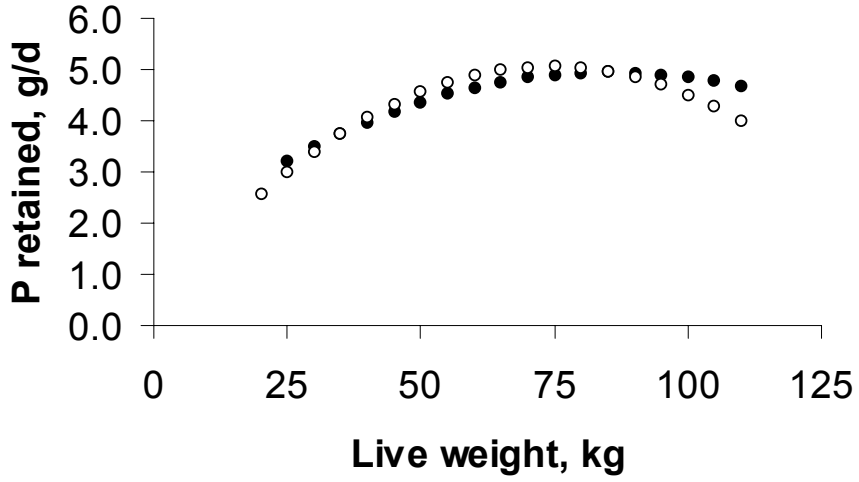


Figure 1.2 - Comparison of P accretion in WEB as estimated from body weight gain of pigs (Pettey, 2004) (○) and predicted growth rates from NRC, 1998 (●). Excerpted from Pettey (2004).



CHAPTER II

Digestibility and Retention of Organic Trace Minerals in Finishing Pigs

Introduction

Most of the research related to trace mineral requirements was carried out more than 30 years ago and may not apply to modern pigs. Traditionally, trace mineral supplementation has been achieved by the addition of inorganic salts. However, a few years ago organic trace mineral complexes were developed based on the theory that they are more bioavailable, or more similar to naturally-occurring forms in the body than inorganic trace minerals, and exhibit improved metabolic utilization resulting in enhanced performance responses and less nutrient excretion (Wedekind et al., 1994).

Wu et al. (2001) suggested that the use of certain mineral proteinates may reduce mineral waste with no negative effects on performance during the nursery period. Grower and finisher pigs require, however, the largest proportion of feed and produce the largest amount of waste. Whether organic sources of minerals can substitute greater amounts of inorganic sources with no negative effects on performance and body mineral status in finishing pigs is still unclear. In fact, it is possible that reduced mineral diets in the organic form had no negative effects in previous studies compared to the diet with standard levels of inorganic simply because there is not a real need for trace mineral supplementation in that stage of production.

The objectives of the present study were to assess the digestibility of reduced amounts of inorganic and organic (proteinates) trace minerals in finishing pigs as well as their long-term effects in body mineral status.

This experiment was developed in collaboration with the Animal Science Department (Burkett et al., 2006) of Iowa State University (ISU), where the pigs were fed the treatment diets prior to being shipped to UK for these evaluations.

Material and Methods

Pre Trial Information

A total of 560 crossbred pigs were used in the growth study at ISU. The pigs originated

from Wilson's Prairie View Farms, Burlington, WI, were initially blocked by weight, penned by sex and housed in totally slatted, environmentally controlled confinement facilities at the ISU Swine Unit with *ad libitum* access to feed and water. A four-phase grow-finish feeding program (Table 2.1) was utilized for all pigs according to the following regimen: Phase 1 (18 to 37 kg), Phase 2 (37 to 55 kg), Phase 3 (55 to 82 kg) and Phase 4 (82 to 118 kg). Each pig was provided 0.9 to 1.3 m^2 of floor space per pen. An antihelminthic (Ivermectin, Merial Inc., USA) was administered to pigs prior to initiation of the test period. Pigs that were unhealthy or injured during the experiments were removed from the test. Pigs delivered to UK were from Phase 4 of the ISU experiment.

Dietary treatments

A complete basal diet (meal form) was formulated and different sources (organic vs. inorganic) and concentrations of Fe, Zn, Cu, and Mn were used to develop the experimental dietary treatments (Table 2.2). Treatment (TRT) 1 was supplemented with 25% of a commercially recommended (Kent Feeds Inc., Muscatine, IA) level of Fe as $FeSO_4$, Zn (of which 25% was ZnO and 75% was $ZnSO_4$), Cu as $CuSO_4$, and Mn as $MnSO_4$. TRT 2 contained 50% of commercially recommended levels of Fe, Zn, Cu, and Mn from organic sources (Bioplex^{TM}, Alltech Inc., Nicholasville, KY). TRT 3 provided one half of the values of TRT 2. TRT 4 contained no supplemental microminerals and served as a negative control. Experimental trace mineral premixes were manufactured commercially (Kent Feeds Inc., Muscatine, IA) and the diets were mixed by a commercial feed manufacturer (Nevada Feed and Seed, Nevada, IA).

Treatment 1 was designed to provide 20.2, 27.5, 16.3 and 5.2 mg/kg of supplemental inorganic Fe, Zn, Cu and Mn, respectively; TRT 2 provided 30.0, 30.0, 6.2 and 1.5 mg/kg of supplemental organic Fe, Zn, Cu and Mn, respectively; TRT 3 contained one half of the values of TRT 2; TRT 4 contained no supplemental trace mineral premix and was formulated to provide 58.2, 25.9, 5.0 and 12.9 mg/kg of total Fe, Zn, Cu and Mn from the corn and soybean meal, which account for 145, 52, 167 and 645 % of the NRC (1998) requirements for finishing pigs. A description of the formulated total concentration of all diets is presented in Table 2.3.

During Phase 4, pigs were selected and transported to UK. Pigs remained on the same dietary treatment at UK as they had been on at ISU. The growing-finishing diets (Phase 4) were

shipped from ISU to Alltech Inc. (Nicholasville, KY), and transported from there to UK as needed.

Experimental Decisions and Conditions

The 24 barrows (95.6 kg initial body weight) used for this balance experiment were transported from the ISU facilities in February 2005. Pigs were divided into 2 groups according to their weight on the day prior to the trip: "heavy" and "light". Upon arrival in Lexington, they were randomly allotted (within the 2 groups) into 8 pens (3 pigs/pen) and an intramuscular injection of antibiotic (Naxcel® - Ceftiofur sodium, 10 ml/pig; Pfizer Inc., Cambridge, MA) was administered on d 1, 3, and 5 in order to avoid possible secondary infections or complications because of the transport stress. Pigs were weighed the morning after arrival to check weight loss during transit. Six days after arrival, the 12 pigs belonging to the heavy group were moved into the metabolic room and randomly allocated to individual metabolic crates. All of them were gaining weight and exhibited no adverse effects from the trip. The heaviest pigs were used first in order to allow the second group to reach a relatively similar body weight for the balance study. The second group of pigs was moved to the metabolic room when they reached an approximately similar BW, which occurred the day after the first group finished the balance trial.

This experiment was 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 UK campus. During the experiment, the rooms were cleaned daily. Room temperature, water availability, and animal well being were checked at least twice per day. The experiment was conducted under protocols approved by the University of Kentucky Institutional Animal Care and Use Committee (IACUC).

Balance Trial

Pigs were individually confined in metabolism crates (two collection groups, 12 pigs each). 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.

Pigs were weighed and confined in the crates for 7 days to adapt to the crates and the level of feed offered, which corresponded to 2% of BW. Movement was restricted during the first 24 h by adjusting the sides and top of the crates. As pigs became accustomed to the crates, they were gradually allowed more space. The interior space of the crates was adjusted to restrict pigs from turning around, thereby 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. At the end of each adaptation period, pigs were weighed again to obtain the current BW in order to determine the feed allowance for the collection period.

During the collection period, pigs were also fed the equivalent of 2% of their body weight per day. The daily feed allowance was split into two equal meals, fed at 0900 and 1600. 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). Orts were collected 40 minutes after the meal was offered. 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 (120 hours later) 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 0900 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 maintain pH low enough to limit microbial growth and prevent 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. The pigs were

weighed before and after the 5-d collection period to verify that they were in a positive nutrient balance.

Digestibility and retention calculations

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

App. digestibility, % =

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

App. retention, g/d = Nutrient intake, g/d – Total nutrient excretion (fecal + urinary), g/d

$$\text{Retention as a percent of intake, \%} = \left[\frac{\text{Nutrient retained per day}}{\text{Nutrient intake per day}}\right] \times 100$$

Retention as a percent of absorption, % =

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

Slaughter and Tissue Collection

After the metabolic trial, pigs were moved back to room B-6 where they stayed for 3 days. They were then moved to the meat lab the evening before the slaughter. All pigs were slaughtered by conventional methods and in accordance with a University of Kentucky Meat Lab procedures. Pigs were electrically stunned, hung by one leg by wrapping a chain around the hock, and then exsanguinated. Carcasses were scalded, mechanically dehaired, and then hung on the rail by the digital flexor tendons of both hind legs. All bleeding and evisceration procedures were conducted with a 15-cm boning knife.

During the evisceration process, heart, right kidney, liver, spleen and front feet were obtained and their weights recorded. Only one lobe (upper-left side, gall-bladder up) from each liver was kept for further analysis. Carcass was weighed and lightly rinsed with hot water and

then placed in a cooler (1° C) overnight. The following day, while still hanging on the rail, each carcass was weighed to determine moisture loss (drip loss). Then, a *longissimus* muscle sample at the tenth rib location was taken after its area was recorded. Additionally, back fat depth (at the same location) was also recorded.

Laboratory Analyses

All frozen feces were dried in a forced-air oven (Tru-Temp, Hotpack Corp., Philadelphia, PA) at 55^{o}C for 7 days, 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 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). 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.

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 a coefficient of variation higher than 5% 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. Apparent digestibility coefficients were calculated on a DM basis by using the equations already detailed.

Tissue samples were ground in a small food processor (HC 3000, Black & Decker Inc., Shelton, CT) prior to and after being lyophilized in a freeze-dryer (Botanique model 18DX48SA, Botanique Preservation Equipment, Inc., Peoria, Arizona).

Front feet were dissected to obtain the metacarpals. Collecting the third and fourth metacarpal 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, the skin was longitudinally cut to allow faster heat release, 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. Bones were oven-dried overnight at 105ºC to remove moisture, 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 3 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

All ground, dried tissue samples along with feces, urine, ashed metacarpals and diets were analyzed for dry matter, ash, Ca, P, Fe, Zn, Cu and Mn. Inductively coupled plasma (ICP) spectrophotometry (model Vista-mpx CCD simultaneous ICP-OES, Varian, Inc., Palo Alto, CA) was used for the mineral determinations. Prior to ICP analysis, described samples were digested with nitric acid in a pressurized microwave (MDS-2000, CEM Corporation, Matthews, NC), and appropriately diluted.

Statistical Analysis

Experiment 1 was designed as a randomized complete block design. The analysis of variance (ANOVA) was conducted by using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS, 1998), with individual pig as the experimental unit. The model for analysis included the effects of collection group, diet, and diet by collection group interaction. Mean separation tests were conducted by Least Significant Difference (LSD). A $P \leq 0.05$ or 0.10 was considered significant or a tendency to be significant, respectively. In addition, responses with a significant difference among treatments were graphically (Microsoft Excel®) evaluated by plotting them against the daily mineral intake in order to obtain a visual separation

between a source and a dietary inclusion level effect. A linear, quadratic, or cubic regression curve was built for each response with quadratic and cubic functions added based on the magnitude of improvement in the R^2. Treatment responses located clearly above or below the regression line were considered to differ because of their source instead of their addition level.

Results

During the growing and early finishing period, pigs were at the ISU facilities (Burkett et al., 2006); the ones that showed signs of parakeratosis, listlessness, and weight loss were removed from the experiment. The number of pigs removed from test by treatment was not different for TRT 1 (n = 1), TRT 2 (n = 3), and TRT 3 (n = 4), however, all three were different ($P < 0.01$) from TRT 4 (n = 38).

While at UK, one of the pigs (from TRT 1) became injured while moving it into the metabolic room and was returned to its pen, and the wound was sutured and disinfected. Therefore, there are only 5 observations of TRT 1 for the digestibility and balance responses. This pig recovered and was subsequently slaughtered along with the rest of the pigs, so there was no missing data for the tissue response measures.

Analyzed Ca and P levels in Phase 4 are lower than the expected (0.55 and 0.48% for Ca and P, respectively), especially in the case of Ca in TRT 3 (0.44%). A comparison between the expected and the apparently added mineral concentration in Phase 4 is also shown in Table 2.2. There is a close agreement for Zn and Cu, yet apparently added Fe and Mn levels differ substantially from the expected supplemental values.

Digestibility and Balance Evaluations

Feed intake was similar during the 5-d collection period of the balance trials averaging 1.83, 1.83, 1.81, and 1.77 kg DM/d for treatments 1-4, respectively ($P = 0.71$). Pigs started the collection period averaging 102.6 ± 2.9 kg BW for the first group and 106.1 ± 6.3 kg BW for the second group. All pigs gained weight during the collection period (ADG averaged 1.08 kg/d). Fecal and urinary excretion did not differ among treatments. Consequently, dry matter digestibility was not different averaging 90.0%.

A balance report for all macro and microminerals studied in this experiment is shown in

Table 2.4. Mineral digestibility for the unsupplemented group (TRT 4) was high for all minerals. A treatment effect was detected for the apparent digestibility of Fe, Cu and Mn ($P < 0.001$). Cu digestibility versus Cu dietary intake (Figure 2.1) showed a clear inverse relationship independent of the source of Cu. In fact, Cu digestibility was reduced by 6.1% for every 10 mg of extra daily intake of dietary Cu above 5 mg/d ($R^2 = 0.80$; $P < 0.001$). Fe and Mn digestibilities exhibited, however, a random behavior. Zn retention was directly proportional to Zn supplementation and, furthermore, to Zn intake (Figure 2.2; $R^2 = 0.92$), averaging 34 mg retained per 100 mg of intake. Mineral retention as percentage of intake averaged 40.7, 34.3, 12.3, and 7.5% for Fe, Zn, Cu, and Mn, respectively, while retention as percentage of the absorbed was 99.9, 94.7, 88.5, and 71.3% on the average for the four minerals.

Regarding fecal daily excretion of minerals, the four trace minerals showed a treatment effect ($P < 0.001$), although in the cases of Fe (Figure 2.3) and Cu (Figure 2.4) only reflected an increase of excretion according to the increasing mineral intake ($R^2 = 0.65$, $R^2 = 1.0$, respectively) with no benefit for either organic or inorganic sources. Zn excretion (Figure 2.5) also showed a linear regression ($R^2 = 0.98$) to Zn ntake, and the single-degree of freedom comparison between TRT 1 and 2 for excretion as a percentage of the intake showed a slight reduction in favor of the inorganic sources ($P = 0.08$). TRT 1 and 2 provided the same amount of Mn, however TRT 2 (organic form) reduced the fecal excretion of Mn ($P < 0.001$) from 29.2 to 24.9 mg/d (14.7% reduction). On the other hand, the control diet (TRT 4) showed a reduction of the excretion by 9.8% ($P < 0.001$) in comparison with TRT 3 (reduced organics). Figure 2.6 illustrates the Mn excretion data.

Slaughter Responses

Pigs were slaughtered at 110.2 ± 4.7 kg BW with no significant differences among treatments. Carcass weight, *longissimus* muscle area, or back fat depth (Table 2.5) were not affected by treatment ($P > 0.05$). Absolute and relative weights of heart, kidney, liver, spleen and metacarpal bones are shown in Table 2.6. Pigs on TRT 3 exhibited higher absolute and relative weights of their heart and liver ($P < 0.05$) than those belonging to TRT 2.

Tissue Mineral Content

Copper and Mn content (Table 2.7) in heart from pigs fed TRT 3 was higher ($P < 0.05$) than in those fed TRT 2, but their concentration (Table 2.8) in the mentioned tissue was not different. Consequently, the heavier weight of the heart is the reason. Figure 2.7 gives a visual illustration of the differences among treatments in kidney content of Cu. Pigs from TRT 2 and 3 deposited 0.36 and 0.26 mg of Cu in kidney vs. 0.22 mg in pigs from TRT 1, while the daily intake was clearly less (18.2 and 12.7 vs. 39.0 mg/d), demonstrating the higher retention of organic Cu in kidney. Even though zinc content in liver was also linearly dependent on the daily intake ($R^2 = 0.56$), organic zinc was shown to be superior to the inorganic form in terms of liver deposition in this experiment. Pigs on TRT 2 had higher Zn content in liver ($P < 0.05$) than pigs on TRT 1 (64.2 vs. 48.0 mg) and pigs on TRT 3 reached the same liver content (48.6 mg) even though they were provided with 80 mg of dietary Zn per day on the average, in comparison with the 99 mg/d received by pigs on TRT 1 (Figure 2.8). Iron content in the spleen from pigs on TRT 2 has been also demonstrated to be superior ($P < 0.05$) to pigs from TRT 1, but that may correspond to an increasing Fe intake (495 vs. 449 mg/d). Concentration of trace minerals in *longissimus* muscle are shown in Table 2.8. Pigs on TRT 1 exhibited a higher Zn concentration than the unsupplemented pigs ($P < 0.05$). The rest of treatments did not show any difference from TRT 4 for any of the trace minerals.

Bone responses are shown in Table 2.9. Bone ash content and bone strength remained constant across treatments. Treatment differences were detected ($P < 0.001$) for ashed bone zinc concentration (Figure 2.9), but it is again a clear dose response and a real difference between the two sources cannot be stated. Cu content in ashed bone showed a negative correlation with Cu intake (Figure 2.10) with no difference between sources detected.

Discussion

Diets were manufactured commercially which may have facilitated differences between the formulated and analyzed values to happen for some of the minerals. Deviation of Fe from expected value may be due to Fe contamination of other dietary ingredients, especially calcium carbonate and dicalcium phosphate (Mateos et al., 2004).

The fact that diets for different treatments were formulated as a percentage of the

commercially recommended levels instead of as an absolute value makes it difficult to distinguish for some comparisons whether a treatment effect was caused by differences in the source of the mineral or simply by an increasing addition of it. In order to address that issue, data were evaluated graphically so that all the responses were expressed in comparison with the daily mineral intake instead of with the dietary mineral concentration.

Feed intake during the 12-d balance trial was low in comparison to a similar pig with *ad libitum* intake (2,770 g DM/d; NRC (1998) for a 100 kg BW barrow) which may have an influence on the interpretation of the balance results. Daily feed allowance was initially set at 3% BW during the adaptation period but many pigs refused part of the feed offered, probably due to the stress generated by the metabolism crate environment and, after 3 days, feed allowance was reduced to 2% BW (value reported in the Material and Methods section).

Mineral digestibility for the unsupplemented group (TRT 4) was high for all minerals demonstrating the ability of the body to up-regulate absorption in periods of need. Moreover, a large number of pigs from this treatment were removed across the four phases because of health and growth problems caused by mineral deficiency. This may have actually biased the results because the rest of the pigs from TRT 4 that remained in the trial for the entire grow-finish period (which were used in the balance study at UK) may have had a superior ability to digest and/or retain the small amount of trace minerals that they were provided. In addition, bioavailability of Zn may be limited by high dietary Ca. When Ca levels are increased in a diet with low dietary Zn, the incidence of parakeratosis is increased dramatically (Lewis et al., 1956). In the current study, Ca was supplied in the diets at concentrations higher than NRC (1998) estimated requirements. The relatively high Ca levels may have contributed to increased signs of parakeratosis in pigs from TRT 4 through an adverse effect on Zn bioavailability.

Several studies have shown reduction in trace mineral supplementation in weanling pig diets can be accomplished when organic trace minerals are utilized for the inorganic form of the mineral (Carlson et al., 2004; Veum et al., 2004). However, very few studies have compared the use of inorganic and organic forms of different trace minerals at differing concentrations supplemented during the entire grow-finish period.

Apparent digestibility of Zn was improved in the current experiment for pigs fed diets containing increasing amounts of Zn, regardless of their origin, which is in agreement with Wedekind et al. (1994), who reported that neither inorganic Zn as ZnO nor organic Zn as Zn-methionine or Zn-lysine (amino acid complexes) provided more bioavailable Zn than $ZnSO_4$

when three different concentrations of each source were fed to pigs from 25 to 90 kg BW. In contrast, Wu et al. (2001) reported that pigs fed 2000 ppm Zn from ZnO numerically retained the most Zn, but the proportion of Zn absorbed and retained in relation to Zn intake was less than that for the pigs fed either 200 or 400 ppm Bioplex^{TM} Zn. In fact, the clear digestibility and retention responses to Zn supplementation in our experiment responds to the fact that the four treatments provided an amount of Zn (99, 113, 80, and 40 mg/d for TRT 1-4, respectively) far below from the NRC (1998) requirements (154 mg/d for a 100 kg BW pig).

According to Hahn and Baker (1993), Cu-amino acids and Cu-proteinates show somewhat greater Cu absorption than Cu sulfate when used at pharmacological levels in nursery pigs, which was not found in this experiment with finishing pigs. Studies by Carlson (2001) and Wu et al. (2001) reported that increasing the dietary content of Cu increased both the rate of absorption and retention of Cu in nursery pigs, which differs from our findings for finishing pigs. In fact, this might be proof of the limited effect of high dietary Cu in fattening pigs, as stated by Bradley et al. (1983).

Carlson et al. (2004) and Creech et al. (2004) reported that fecal excretion of Zn (mg/d) in nursery pigs fed pharmacological doses was directly related to the quantity of Zn consumed (mg/d), regardless of the Zn source. This statement is in agreement with our results. Additionally, in our study, pigs fed diets containing organic trace minerals excreted 56% to 72% less Cu when compared to pigs fed diets containing inorganic trace mineral supplementation. That agrees with Burkett et al. (2006) who reported a reduction of 49% to 77% for the same treatments when assessed by an indirect collection method. Veum et al. (2004) reported similar reductions in fecal Cu excretion in their balance experiment in which they found that feeding 50 or 100 ppm organic Cu reduced fecal Cu excretion in swine manure by 77% and 61%, respectively, compared with feeding 250 ppm of inorganic $CuSO_4$. The comparison of these reductions in excretion with the reduction in dietary mineral supplementation clearly shows that the improvement is caused by a decreasing mineral supplementation rather than because of the use of an organic form of the mineral. In our study, and in Burkett et al. (2006), diets containing organic trace minerals had supplementation levels reduced by 56% to 72% compared to diets containing inorganic forms. Likewise, Cu content in diets using organic forms in the study by Veum et al. (2004) were reduced by 80% to 60%, which is in close agreement with the reduction in fecal excretion (77% and 61%, respectively), and corroborates our results (Figure 2.4). Organic Mn in TRT 2 showed an advantage in fecal excretion from TRT 1, but the comparison

between TRT 3 and 4 resulting in the opposite effect makes any affirmation in favor of the organic form uncertain. As a result, there is no evidence to say that this particular source of trace mineral may have an effect on mineral digestibility or retention in growing-finishing pigs at these levels of supplementation for any of the four minerals studied.

For all minerals, the tissue content and concentration varied by tissue. There were no patterns that were distinct for all tissues. The lack of patterns may be related to mineral form but also to the relative priority of different tissues for different minerals. There were several mineral/tissue combinations that appear to be decent models for further bioavailability evaluations. The use of organic Cu and Zn resulted in significantly higher content of these minerals in kidney and liver, respectively, compared to the inorganic forms. Whether these parameters should be considered as a measurement of bioavailability or not needs to be further investigated. It should be also kept in mind that the effects for these tissues are cumulative effects and reflect the entire set of treatments over the entire growing-finishing period and not just the four dietary treatments in Phase 4. The most accurate understanding of the effects of organic vs. inorganic minerals would incorporate estimates of mineral intake over the entire period.

The clearest observation would be that finishing barrows do not have a high trace mineral need. The need can apparently be satisfied with an unsupplemented diet with the exception of Zn which responds to supplementation in all treatments above the control supplementation level.

Implications

The real need for trace mineral supplementation in growing-finishing pigs is in doubt. Only zinc appears to be deficient in a common corn-soybean meal diet, but some commercial conditions might require small additions of a trace mineral premix. Fecal trace mineral excretion has been proved to respond quantitatively to mineral intake independently of the source, when working with a dietary trace mineral inclusion lower than the commercially used; in addition, mineral digestibility was similar for organic and inorganic trace minerals. Furthermore, if the need for a trace mineral premix in grow-finish pigs exists, this may be accomplished at a lower cost by using inorganic sources with no extra detriment of the environment.

Table 2.1 - Composition (as-fed basis) of the basal diets (TRT 4) in Experiment 1.

Body Weight	Phase 1 18 – 37 kg	Phase 2 37 - 55 kg	Phase 3 55 - 82 kg	Phase 4 82 - 118 kg
Ingredient composition, %				
Ground yellow dent corn	67.25	69.50	73.50	78.75
Soybean meal (47.5% CP)	26.75	24.50	21.00	15.75
Trace mineral mix [a]	3.00	3.00	2.50	2.50
Choice white grease	2.00	2.00	2.00	2.00
Celite	1.00	1.00	1.00	1.00
Formulated Content, %				
Crude fat	4.91	4.97	5.09	5.24
Lysine	1.12	1.05	0.93	0.79
Tryptophan	0.22	0.21	0.19	0.15
Threonine	0.75	0.71	0.66	0.59
Methionine	0.32	0.31	0.29	0.27
Ash	5.00	4.90	4.34	4.11
NaCl	0.52	0.52	0.45	0.45
Analyzed content[b]				
DM, %	87.50	86.56	85.66	83.83
CP, %	17.23	17.93	15.33	13.88
Ca, %	0.72	0.71	0.68	0.64
P, %	0.55	0.55	0.53	0.51
Mg, %	0.14	0.14	0.13	0.12
K, %	0.83	0.85	0.72	0.64
Na, %	0.20	0.20	0.21	0.20
ME, kcal/kg	3,410	3,420	3,380	3,340

[a]Inorganic trace minerals were supplemented from a commercially available trace mineral premix at recommended levels of Cu as $CuSO_4$, Fe as $FeSO_4$, and Zn (of which 25% was ZnO and 75% was $ZnSO_4$) and Mn as $MnSO_4$. Organic trace minerals (Cu, Fe, Zn and Mn) were supplemented in the form of Bioplex™ products (Alltech Inc., Nicholasville, KY). Data provided by ISU.
[b]Values reported by ISU.

Table 2.2 - Dietary mineral concentration (as-fed basis) of Phase 4 diets in Experiment 1.

		Total levels, %		Supplemental levels, ppm			
	TRT[a]	Ca	P	Fe	Zn	Cu	Mn
Expected	1	0.55	0.48	20.2	27.5	16.3	5.2
	2	0.55	0.48	30.0	30.0	6.2	1.5
	3	0.55	0.48	15.0	15.0	3.1	0.7
	4	0.55	0.48	0.0	0.0	0.0	0.0
Analyzed (Total)	1	0.51	0.43	214.5	47.0	18.6	14.3
	2	0.53	0.46	236.4	53.8	8.7	14.6
	3	0.44	0.41	189.7	39.0	6.2	11.4
	4	0.55	0.44	203.2	20.2	3.3	11.7
Apparently added[b]	1			11.3	26.8	15.3	2.6
	2			33.3	33.6	5.5	2.9
	3			-13.5	18.8	2.9	-0.3
	4			0.0	0.0	0.0	0.0

[a]Treatment (TRT) 1 was supplemented with 25% of the commercially recommended levels of Fe as $FeSO_4$, Zn (of which 25% was ZnO and 75% was $ZnSO_4$), Cu as $CuSO_4$, and Mn as $MnSO_4$. TRT 2 contained 50% of commercially recommended levels of Fe, Zn, Cu, and Mn from organic sources (Bioplex™, Alltech Inc., Nicholasville, KY). TRT 3 provided one half of the values of TRT 2. TRT 4 contained no supplemental microminerals and served as a negative control.

[b]Iron, zinc, copper, and manganese values are calculated by subtraction of TRT 4 from TRT 1, 2, and 3, respectively. Values analyzed at UK; they might differ from ISU results.

Table 2.3 - Formulated total concentration (as-fed basis) of Fe, Zn, Cu and Mn in the diets in Experiment 1.

		Phase[a]			
Mineral Conc.	Treatment[b]	1	2	3	4
Fe, ppm	1	95.42	93.22	83.59	78.44
	2	101.12	98.91	93.33	88.19
	3	86.13	83.93	78.34	73.20
	4	71.14	68.94	63.36	58.21
	NRC[c]	67.4	55.8	47.5	40.0
Zn, ppm	1	63.45	62.60	55.46	53.46
	2	60.40	59.54	57.91	55.92
	3	45.41	44.55	42.92	40.92
	4	30.41	29.56	27.92	25.93
	NRC	67.9	58.2	52.3	46.9
Cu, ppm	1	25.99	25.70	21.98	21.30
	2	12.72	12.43	11.96	11.27
	3	9.59	9.30	8.83	8.15
	4	6.47	6.17	5.70	5.02
	NRC	4.4	3.8	3.4	3.0
Mn, ppm	1	23.0	22.3	19.7	18.1
	2	18.3	17.6	16.0	14.4
	3	17.6	16.9	15.3	13.6
	4	16.8	16.1	14.5	12.9
	NRC	2.4	2.2	2.0	1.9

[a]Phase 1 - diet fed from 18-37 kg BW; Phase 2 - diet fed from 37-55 kg BW; Phase 3 - fed from 55-82 kg BW; Phase 4 - diet fed from 82-118 kg BW. Data of Phases 1, 2 and 3 provided by ISU.

[b]Treatment (TRT) 1 was supplemented with 25% of the commercially recommended (feed mill supplier) levels of Fe as $FeSO_4$, Zn (of which 25% was ZnO and 75% was $ZnSO_4$), Cu as $CuSO_4$, and Mn as $MnSO_4$. TRT 2 contained 50% of commercially recommended levels of Fe, Zn, Cu, and Mn from organic sources (Bioplex™, Alltech Inc., Nicholasville, KY). TRT 3 provided one half of the values of TRT 2. TRT 4 contained no supplemental microminerals and served as a negative control.

[c]NRC – values are extrapolated using their computer model program for predicting nutrient requirements NRC (1998) based on the average weight for the phase in standard conditions (barrows/gilts: 1/1).

Table 2.4 – Mineral balance in Experiment 1.

Response (LS Means[1])	Treatment 1	2	3	4	SEM[2]	P-Value
Feed intake[3,4], g/d	1,838	1,832	1,805	1,769	45	0.71
Fecal excretion[4], g/d	184	189	186	166	7	0.36
Urinary excretion, g/d	3,474	3,930	5,204	2,037	1,145	0.31
DM digestibility, %	90.03	89.72	89.72	90.61	0.37	0.31
Calcium						
Intake, mg/d	10,736^{ab}	11,059^{a}	9,139^{c}	10,840^{ab}	265	<0.001
Excreted (feces), mg/d	7,489^{ab}	7,495^{a}	6,667^{b}	6,714^{ab}	302	0.13
Excreted (urine), mg/d	142	192	306	130	74	0.36
Absorption, mg/d	3,247^{bc}	3,564^{ab}	2,472^{c}	4,126^{a}	267	0.005
Retention, mg/d	3,106^{bc}	3,372^{ab}	2,167^{c}	3,996^{a}	264	0.002
Digestibility (app.), %	30.4^{b}	32.4^{ab}	26.9^{b}	37.9^{a}	2.4	0.03
Retention as % intake	29.2^{bc}	30.7^{ab}	23.6^{c}	36.7^{a}	2.4	0.01
Retention as % absorpt.	95.1^{ab}	94.5^{ab}	87.9^{b}	96.9^{a}	2.5	0.11
Phosphorus						
Intake, mg/d	8,962^{ab}	9,665^{a}	8,502^{b}	8,846^{b}	223	0.016
Excreted (feces), mg/d	5,225^{ab}	5,391^{a}	4,863^{ab}	4,576^{b}	249	0.14
Excreted (urine), mg/d	792^{b}	896^{ab}	1,041^{ab}	1,178^{a}	118	0.18
Absorption, mg/d	3,737^{b}	4,274^{a}	3,639^{b}	4,270^{a}	139	0.008
Retention, mg/d	2,945^{ab}	3,377^{a}	2,598^{b}	3,092^{a}	159	0.010
Digestibility (app.), %	41.9^{b}	44.5^{ab}	42.8^{b}	48.4^{a}	1.7	0.08
Retention as % intake	33.0	35.2	30.5	35.1	1.7	0.21
Retention as % absorpt.	78.9	78.6	71.3	72.4	2.8	0.16
Magnesium						
Intake, mg/d	1,805	1,797	1,724	1,710	44	0.34
Excreted (feces), mg/d	1,140	1,186	1,109	1,061	50	0.39
Excreted (urine), mg/d	243	203	259	244	29	0.58
Absorption, mg/d	665	611	615	649	30	0.57
Retention, mg/d	423^{a}	408^{ab}	356^{b}	404^{ab}	20	0.14
Digestibility (app.), %	36.9	34.1	35.6	38.2	1.3	<0.001
Retention as % intake	23.4^{ab}	22.7^{ab}	20.6^{c}	23.7^{a}	1.0	0.18
Retention as % absorpt.	64.0	67.1	58.2	62.5	3.3	0.33

LS Means without a common superscript within a row differ ($P < 0.05$).
[1]Each mean represents 6 individually penned pigs (5 for TRT 1).
[2]Standard error of the mean.
[3]Net ADFI: offered (2% BW) minus refused or spilled; measured during the 5-d collection period.
[4]Dry matter basis.

Table 2.4 – Mineral balance in Experiment 1 (cont.).

Response (LS Means[1])	Treatment 1	2	3	4	SEM[2]	P-Value
Iron						
Intake, mg/d	449[b]	495[a]	392[c]	404[c]	10	<0.001
Excreted (feces), mg/d	274[ab]	290[a]	251[b]	214[c]	8	<0.001
Excreted (urine), mg/d	0.2[ab]	0.2[ab]	0.3[a]	0.1[b]	0.0	0.12
Absorption, mg/d	175[b]	205[a]	141[c]	190[ab]	7	<0.001
Retention, mg/d	174[b]	205[a]	140[c]	190[b]	7	<0.001
Digestibility (app.), %	38.9[bc]	41.5[b]	35.7[c]	47.1[a]	1.3	<0.001
Retention as % intake	38.9[bc]	41.5[b]	35.6[c]	47.1[a]	1.3	<0.001
Retention as % absorpt.	99.9[a]	99.9[a]	99.8[b]	99.9[a]	0.0	0.024
Zinc						
Intake, mg/d	99[b]	113[a]	80[c]	40[d]	1.7	<0.001
Excreted (feces), mg/d	61[b]	75[a]	50[c]	26[d]	2	<0.001
Excreted (urine), mg/d	1.3	1.4	1.7	1.2	0.2	0.45
Absorption, mg/d	38[a]	38[a]	30[b]	14[c]	1	<0.001
Retention, mg/d	35.8[a]	37.2[a]	28.5[b]	12.9[c]	1.2	<0.001
Digestibility (app.), %	37.7	34.1	37.3	35.4	1.1	0.12
Retention as % intake	36.4	32.9	35.3	32.4	1.2	0.12
Retention as % absorpt.	96.5[a]	96.3[a]	94.5[ab]	91.4[b]	1.0	0.014
Copper						
Intake, mg/d	39.0[a]	18.2[b]	12.7[c]	6.6[d]	3.9	<0.001
Excreted (feces), mg/d	37.9[a]	16.6[b]	10.6[c]	5.0[d]	0.4	<0.001
Excreted (urine), mg/d	0.14[a]	0.07[cd]	0.10[bc]	0.06[d]	0.01	0.008
Absorption, mg/d	1.1[b]	1.6[ab]	2.1[a]	1.5[ab]	0.3	0.11
Retention, mg/d	0.92[b]	1.56[ab]	2.04[a]	1.46[ab]	0.27	0.095
Digestibility (app.), %	2.7[d]	9.1[c]	16.7[b]	23.3[a]	1.6	<0.001
Retention as % intake	2.4[d]	8.7[c]	15.9[b]	22.3[a]	1.6	<0.001
Retention as % absorpt.	74.8[b]	88.9[ab]	94.8[a]	95.4[a]	5.1	0.06
Manganese						
Intake, mg/d	30.0[a]	30.4[a]	23.5[b]	23.3[b]	0.6	<0.001
Excreted (feces), mg/d	29.2[a]	24.9[b]	22.4[c]	20.2[d]	0.7	<0.001
Excreted (urine), mg/d	0.63[ab]	0.50[ab]	0.79[a]	0.42[b]	0.11	0.13
Absorption, mg/d	0.8[c]	5.5[a]	1.1[c]	3.1[b]	0.4	<0.001
Retention, mg/d	0.16[c]	5.0[a]	0.27[c]	2.66[b]	0.44	<0.001
Digestibility (app.), %	2.7[c]	18.2[a]	4.4[c]	13.3[b]	1.5	<0.001
Retention as % intake	0.6[c]	16.6[a]	1.1[c]	11.5[b]	1.6	<0.001
Retention as % absorpt.	70.2[a]	89.9[a]	38.8[a]	86.4[a]	31.2	0.65

LS Means without a common superscript within a row differ ($P < 0.05$).
[1]Each mean represents 6 individually penned pigs (5 for TRT 1).
[2]Standard error of the mean.
[3]Net ADFI: offered (2% BW) – refused or spilled; measured during the 5-d collection period.
[4]Dry matter basis.

Table 2.5 - Carcass measurements[1] in Experiment 1.

	Treatment					P-
Response	1	2	3	4	SEM[2]	Value
BW, kg	112.93	110.99	108.36	108.40	4.20	0.29
HCWT, kg	85.71	83.13	82.31	83.13	3.05	0.35
CCWT, kg	82.49	80.23	79.00	80.09	2.93	0.33
LEA, cm^2	40.2	37.5	40.8	40.6	1.7	0.53
BF, cm	2.34	2.62	2.24	2.84	0.23	0.29

[1]Each value represents 6 pigs. Means without a common superscript within a row differ ($P < 0.05$).
[2]Standard error of the mean.
BW: body weight at slaughter, HCWT: hot carcass weight, CCWT: cold carcass weight,
LEA: longissimus muscle area, BF: Back fat.

Table 2.6 - Tissue weights[1] in Experiment 1.

	Treatment					P-
Item	1	2	3	4	SEM[2]	Value
Absolute						
Heart, g	412^{a}	360^{b}	427^{a}	399^{ab}	16	0.09
Kidney, g	137	143	150	148	8	0.67
Liver, g	1459^{ab}	1287^{b}	1502^{a}	1333^{ab}	65	0.10
Spleen, g	203	179	204	191	10	0.26
Metacarpals[3],g	56.8	54.2	53.4	54.7	1.7	0.56
Relative[4]						
Heart, %	0.365^{ab}	0.324^{b}	0.394^{a}	0.368^{ab}	0.015	0.08
Kidney, %	0.121	0.129	0.138	0.136	0.007	0.37
Liver, %	1.292^{ab}	1.160^{b}	1.387^{a}	1.230^{ab}	0.053	0.055
Spleen, %	0.179	0.161	0.188	0.177	0.009	0.27
Metacarpals,%	0.050	0.049	0.050	0.051	0.002	0.94

[1]Each mean represents 6 pigs. Means without a common superscript within a row differ ($P < 0.05$).
[2]Standard error of the mean.
[3]Forced air oven dried and defatted weights.
[4]As a percentage of body weight.

Table 2.7 – Absolute trace mineral content (mg) in tissues[1] in Experiment 1.

		Treatment					P-
Tissue	Mineral	1	2	3	4	SEM[2]	Value
Heart	Fe	11.98	10.78	13.22	12.65	0.85	0.22
	Zn	4.65	4.38	4.97	4.47	0.25	0.23
	Cu	0.55^{ab}	0.49^{b}	0.59^{a}	0.54^{ab}	0.02	0.06
	Mn	0.21^{ab}	0.19^{b}	0.23^{a}	0.22^{ab}	0.01	0.16
Kidney	Fe	5.7	5.79	6.73	7.86	1.2	0.54
	Zn	2.08^{ab}	2.46^{a}	2.08^{ab}	1.78^{b}	0.14	0.02
	Cu	0.22^{b}	0.36^{a}	0.26^{ab}	0.19^{b}	0.04	0.17
	Mn	0.13^{ab}	0.14^{a}	0.12^{b}	0.13^{ab}	0.01	0.04
Liver	Fe	272.7^{a}	259.2^{a}	236.8^{ab}	175.4^{b}	23.5	0.05
	Zn	48.0^{b}	64.2^{a}	48.6^{b}	31.6^{c}	3.5	<0.001
	Cu	18.2	9.2	14.6	11.6	3.6	0.36
	Mn	2.81^{b}	2.62^{b}	2.84^{b}	3.42^{a}	0.25	0.03
Spleen	Fe	21.9^{b}	38.4^{a}	29.4^{ab}	27.7^{b}	3.4	0.02
	Zn	3.16	3.03	3.68	3.14	0.26	0.31
	Cu	0.13	0.13	0.15	0.13	0.02	0.61
	Mn	0.11	0.11	0.12	0.11	0.01	0.47

[1]Each mean represents 6 pigs. Means without a common superscript within a row differ ($P < 0.05$).
[2]Standard error of the mean.

Table 2.8 – Trace mineral concentration (ppm) in tissues[1] in Experiment 1.

		Treatment					P-
Tissue	Mineral	1	2	3	4	SEM[2]	Value
Heart	Fe	126.9	126.8	136.2	136.0	5.7	0.47
	Zn	49.35^{ab}	51.48^{a}	51.24^{a}	48.33^{b}	0.95	0.08
	Cu	5.84	5.84	6.06	5.82	0.14	0.60
	Mn	2.22	2.29	2.34	2.34	0.05	0.28
Kidney	Fe	182.6	175.1	197.0	225.0	28.4	0.62
	Zn	66.39^{ab}	73.76^{a}	62.66^{bc}	53.43^{c}	3.55	0.007
	Cu	6.98^{b}	10.55^{a}	8.01^{ab}	5.67^{b}	0.96	0.027
	Mn	4.05	4.15	3.66	4.00	0.17	0.24
Liver	Fe	663.5^{a}	705.9^{a}	564.9^{ab}	462.6^{b}	51.8	0.02
	Zn	118.0^{b}	176.7^{a}	117.1^{b}	83.8^{c}	9.8	<0.001
	Cu	44.12	25.60	34.66	31.33	8.05	0.45
	Mn	6.79^{b}	7.19^{b}	6.83^{b}	9.11^{a}	0.54	0.01
Spleen	Fe	406.0	597.8	473.3	459.7	74.2	0.34
	Zn	50.84^{ab}	50.89^{b}	58.87^{a}	49.53^{b}	2.56	0.07
	Cu	2.26	2.21	2.47	1.97	0.27	0.32
	Mn	1.86	1.89	1.98	1.74	0.12	0.58
Loin	Fe	14.51	13.75	14.39	15.18	0.63	0.49
muscle	Zn	33.69^{a}	30.34^{ab}	31.98^{ab}	29.06^{b}	1.45	0.17
	Cu	1.01^{ab}	0.86^{b}	1.18^{a}	1.04^{ab}	0.10	0.19
	Mn	2.40	2.26	2.26	2.26	0.11	0.72

[1]Each mean represents 6 pigs. Means without a common superscript within a row differ ($P < 0.05$). Dry matter basis.
[2]Standard error of the mean.

Table 2.9 – Bone responses[1] in Experiment 1.

Parameter	Mineral	Treatment 1	2	3	4	SEM[2]	P-Value
Bone ash, %		40.1	40.1	40.6	40.3	0.3	0.63
Bone strength, kg		179.0	182.5	182.3	178.1	10.3	0.99
Ashed bone Concentration, ppm	Fe	23.9	25.1	23.5	20.3	1.7	0.28
	Zn	103.7[b]	125.8[a]	86.5[c]	30.4[d]	5.2	<0.001
	Cu	0.98	1.17	1.77	1.80	0.28	0.11
	Mn	23.02	22.75	22.95	22.37	0.32	0.49

[1]Each mean represents 6 pigs. Means without a common superscript within a row differ ($P < 0.05$).
[2]Standard error of the mean.

Figure 2.1 – Change in copper apparent digestibility in response to copper daily intake in Experiment 1.

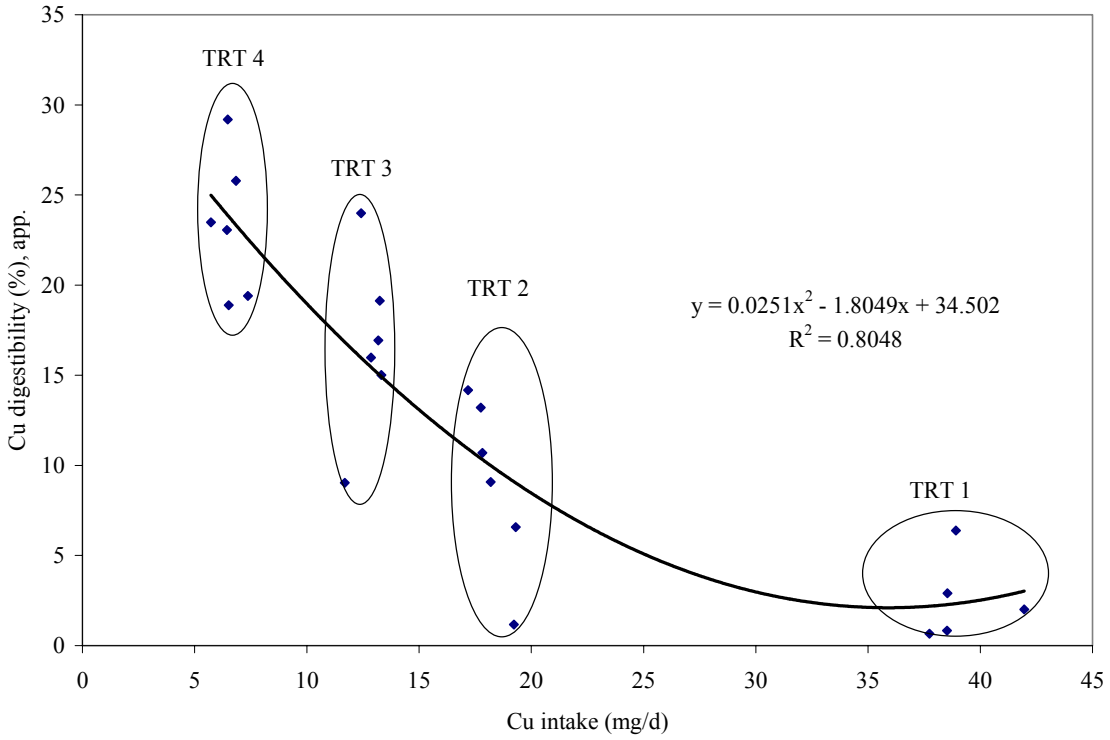


Figure 2.2 – Change in zinc retention in response to zinc daily intake in Experiment 1.

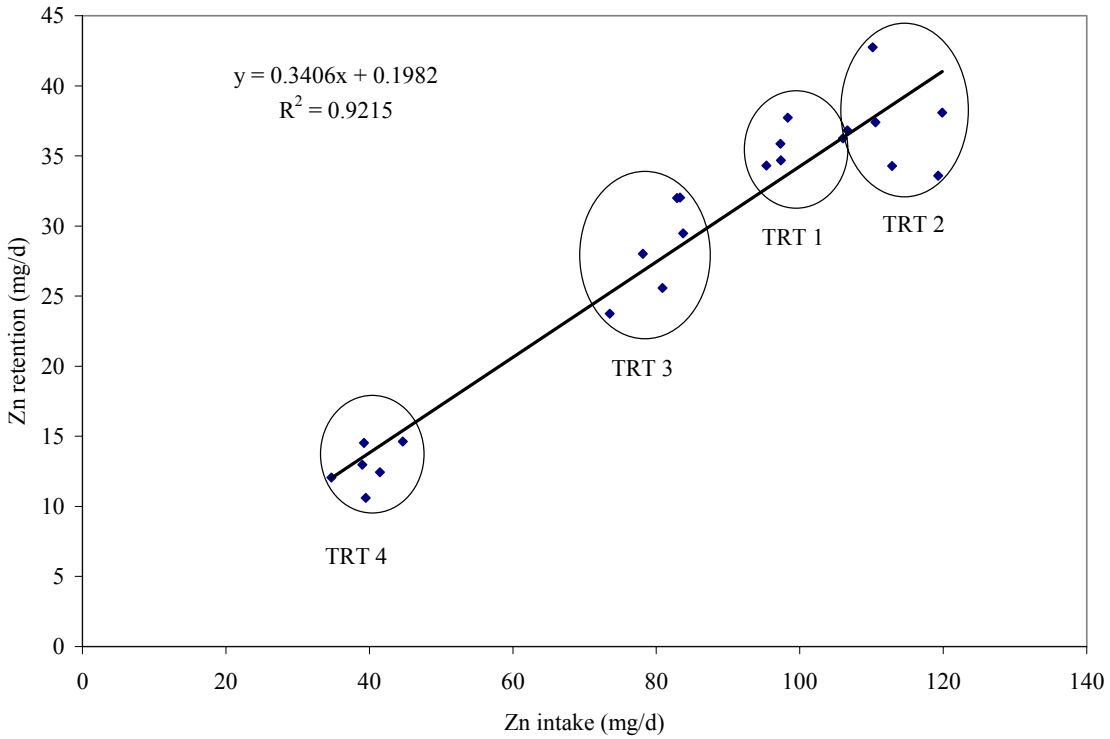


Figure 2.3 – Change in iron fecal excretion in response to iron daily intake in Experiment 1.

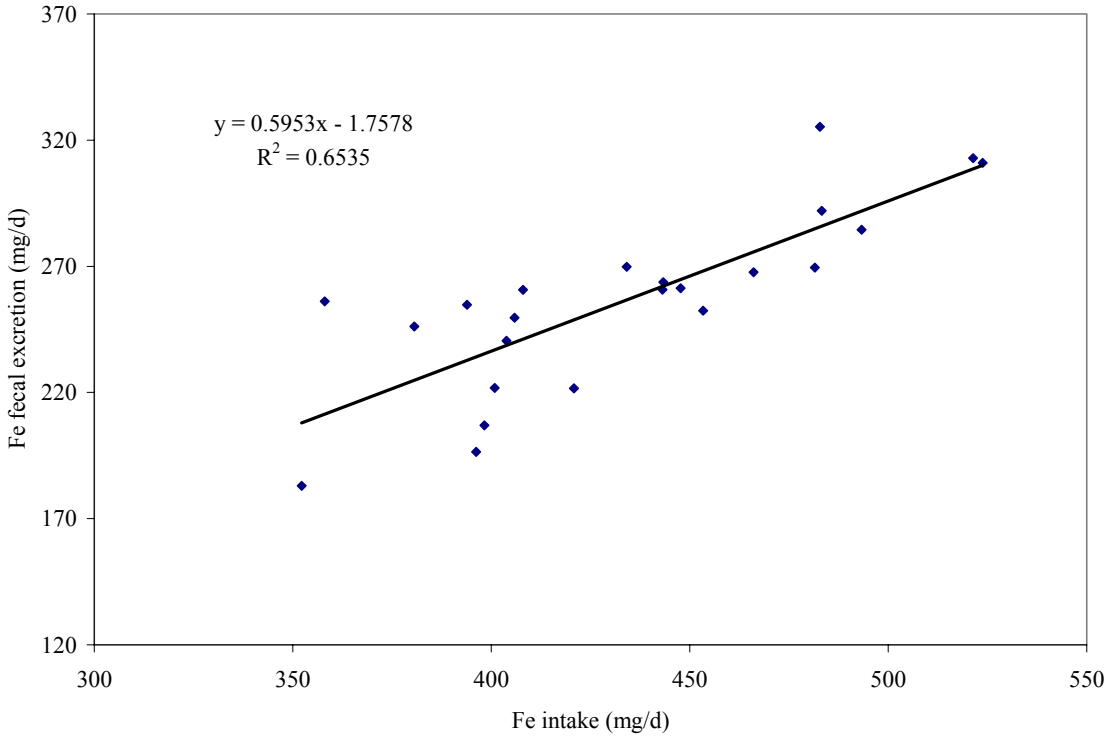


Figure 2.4 – Change in copper fecal excretion in response to copper intake in Experiment 1.

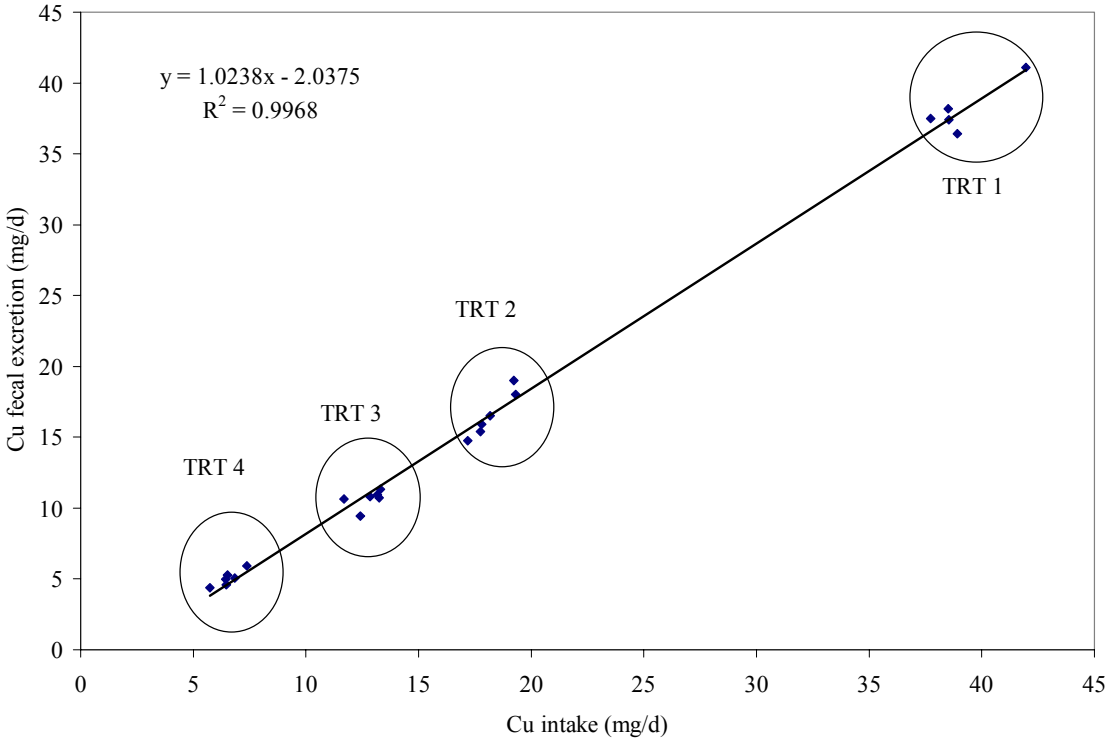


Figure 2.5 – Change in zinc fecal excretion in response to zinc daily intake and source in Experiment 1.

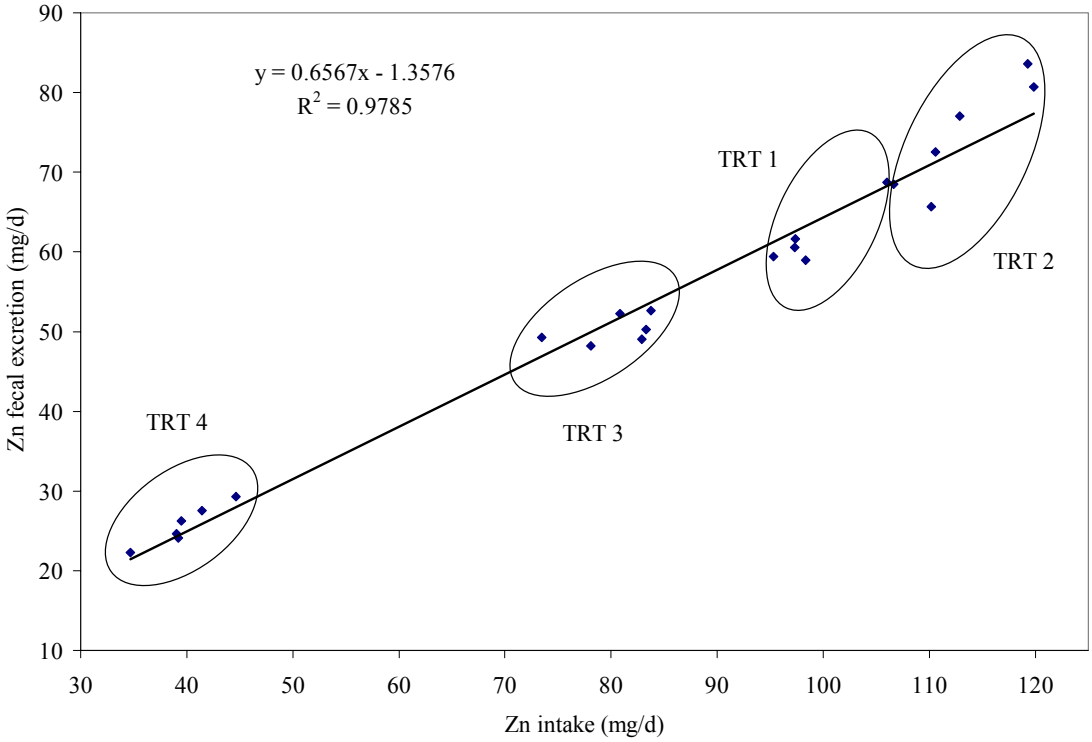


Figure 2.6 – Change in manganese fecal excretion in response to manganese daily intake and source in Experiment 1.

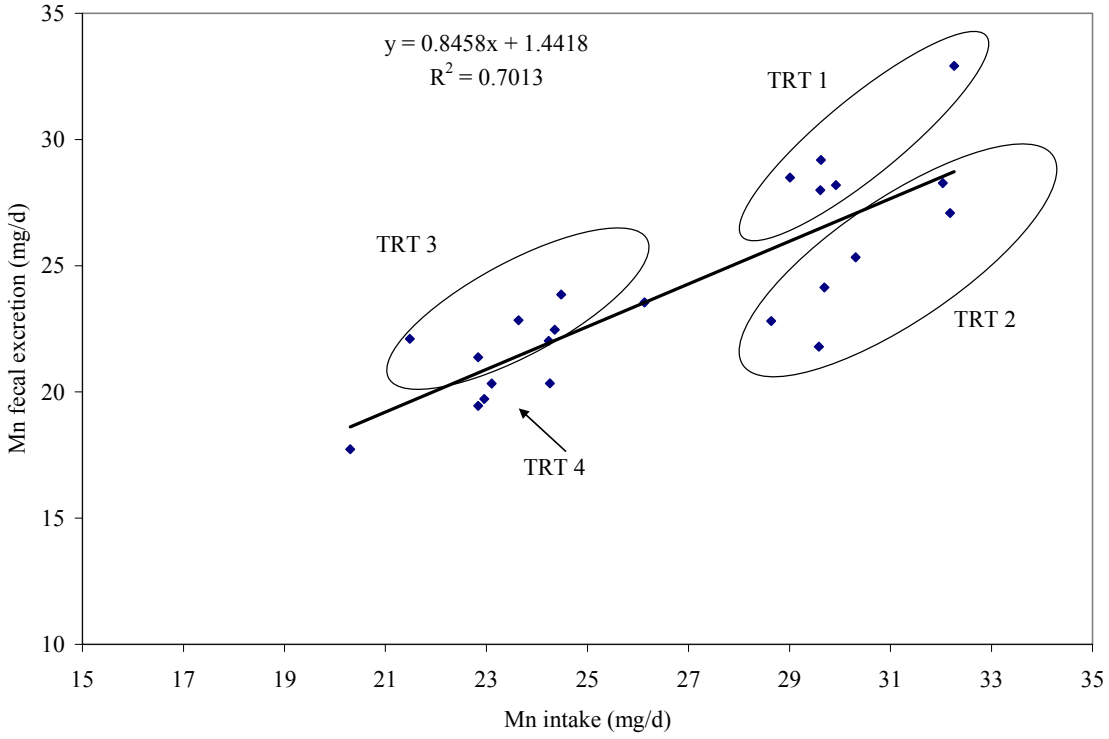


Figure 2.7 – Change in copper kidney content in response to copper daily intake and source in Experiment 1.

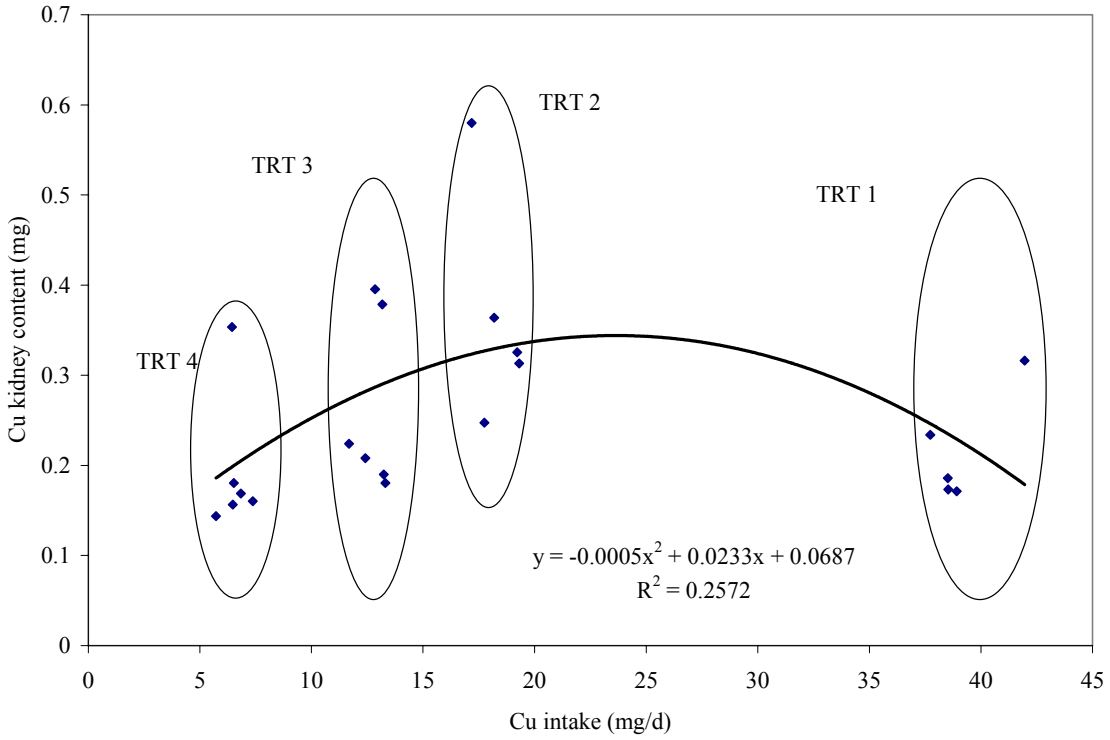


Figure 2.8 – Change in zinc liver content in response to zinc daily intake and source in Experiment 1.

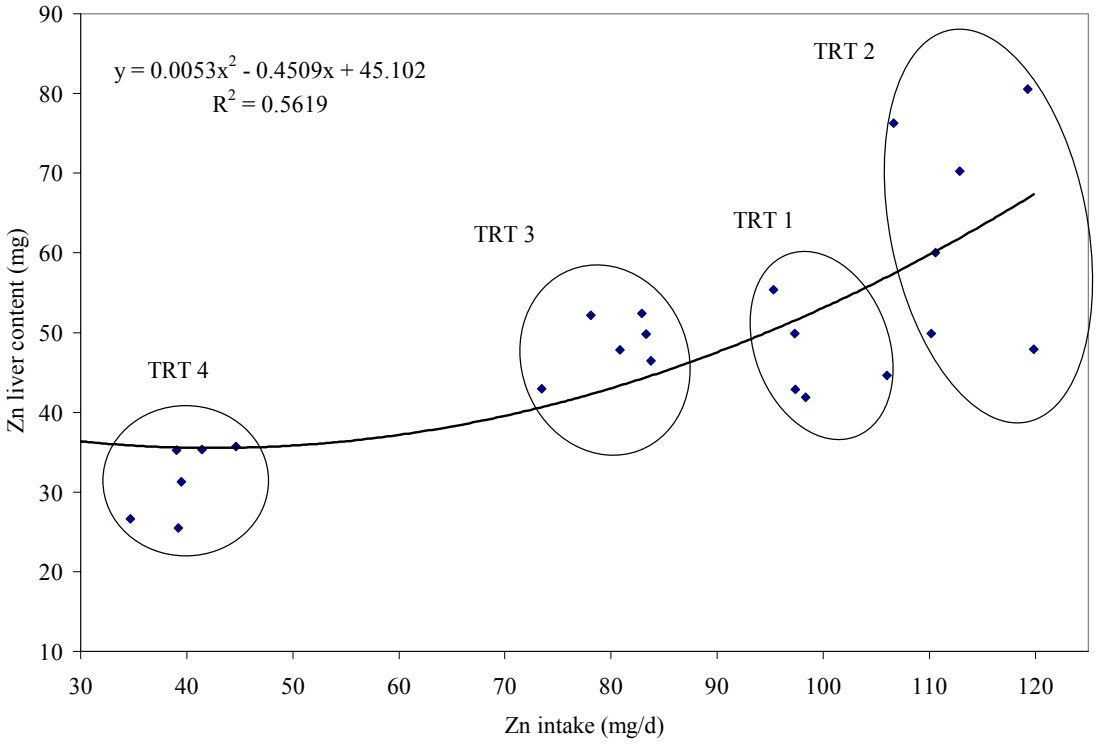


Figure 2.9 – Change in ashed bone zinc concentration in response to zinc daily intake and source in Experiment 1.

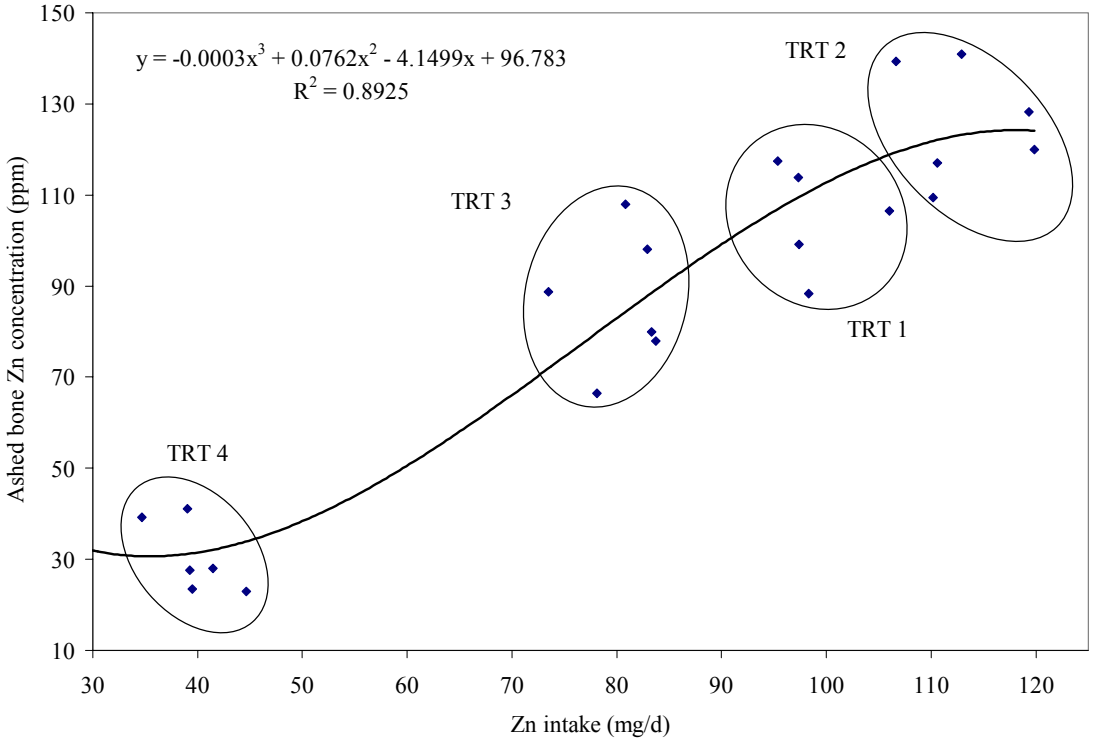
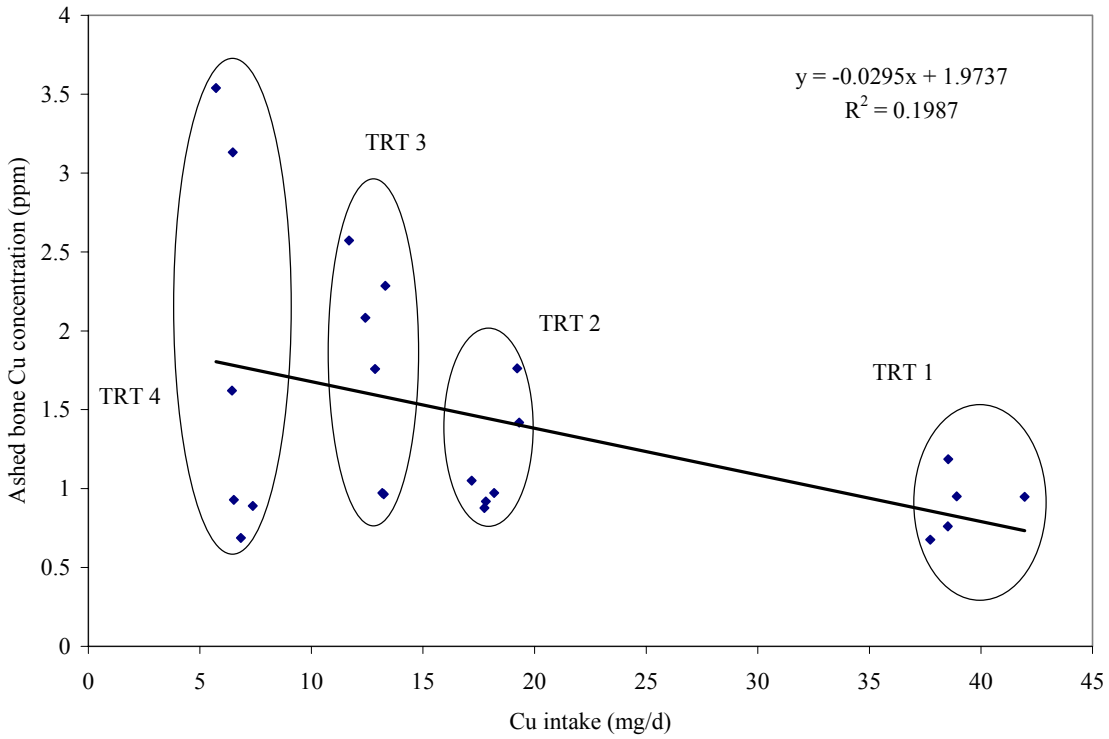


Figure 2.10 – Change in ashed bone copper concentration in response to copper daily intake and source in Experiment 1.



CHAPTER III

Retention and Distribution of Phosphorus in Grow-Finish Pigs

Introduction

Overfeeding of P has led to an increase of P excreted by the pig into the environment. If there is not a corresponding increase in the rate of P extraction by crops grown in these fields, the excess mineral may result in a threat to the bodies of water in the farm's areas of influence (Hollis and Curtis, 2001). 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). There is therefore a great need to verify P requirement levels so that diet formulations can more closely match pig requirements at all stages of growth. Moreover, the requirements for certain minerals by pigs possessing a high lean growth rate, due to superior genetics, may be higher than the levels shown in the NRC (1998) tables.

Additionally, work that has been conducted to study mineral composition of pigs is somewhat dated and may not reflect modern pig genotypes, which do differ from older genotypes not only in regards to compositional aspects like protein deposition, but also in voluntary feed intake capacity, which may affect total mineral intake.

The objective of the present study was to further test the impact of lean growth potential on P body accretion beyond that already generated at UK. Gilts and barrows from two genetic backgrounds were used to determine the potential difference due to lean growth potential.

Material and Methods

The procedures used in the management of pigs, collection of whole body tissue samples, and the handling of these samples as the whole body components were further processed either through grinding or physical separation are described in this section. In most of the procedures, the methodology previously developed by Pettey (2004) was followed. Some of the more detailed "step-by-step" procedures can also be found in the appendices.

Experimental Decisions and Conditions

Experiment 2 was conducted from July 6 to November 8, 2005, in an environmentally-controlled room at the Animal Laboratory of the Animal and Food Sciences building (W. P. Garrigus Building) located on the University of Kentucky campus.

The "high" lean growth rate group was represented by 27 PIC pigs (Line 65, Pietrain and Duroc origin; Pig Improvement Company, Franklin, KY) while 17 crossbred pigs (Duroc by Yorkshire-Landrace, or Duroc by Yorkshire) represented the "moderate" lean growth rate group. The moderate lean pigs were crossbred pigs from the University of Kentucky swine facilities at the Animal Research Center (ARC) in Versailles, KY. Animals were selected and allotted so that littermates were equally split into each experimental group and resulting group weights were similar. Animals with health problems or visible conformation conditions (e.g., leg problems) were not used.

The 27 PIC pigs, 17 barrows and 10 gilts (initial body weight 18.8 kg and 19.9 kg, respectively), were assigned to one of five pre-determined slaughter weight groups of 30, 50, 70, 90, and 110 kg BW according to Table 3.1. Likewise, the 17 crossbred pigs, 11 barrows and 6 gilts (initial body weight 16.3 kg and 16.1 kg, respectively), were assigned to one of three pre-determined slaughter weight groups of 30, 70, and 110 kg. UK crossbred pigs were used by Pettey (2004) in a similar experiment, which allows us to use a lower number of pigs from this genetic background. Pigs were penned individually in 1.2 x 1.2 m pens. When pigs assigned to the heavier slaughter weights reached approximately 85 kg body weight, pens were increased in size to 1.2 x 2.4 m. All pigs were allowed *ad libitum* access to feed from a stainless steel one-hole feeder and water from a nipple waterer. Pigs were weighed weekly with an electronic scale (WayPig 13, Raytec Mfg., Ephrata, PA) to monitor growth. Feed intake was measured when pigs reached the next phase of diets or when pigs were slaughtered by recording all feed offered and subtracting feed removed from the feeder.

The word "breed" will be commonly used during this chapter in regard to each of the two genetic backgrounds even though "genetic line", "composite" or "strain" would more accurately describe the real nature or origin of PIC and crossbred pigs.

Dietary Treatments

A common corn-soybean meal diet was formulated for all pigs for four dietary phases (Phase 1: 20 to 40 kg; Phase 2: 40 to 60 kg; Phase 3: 60 to 80 kg; Phase 4: 80 to 110 kg BW). The composition and nutrient analysis of diets are shown in Table 3.2. All diets were fortified to exceed NRC (1998) requirements for lysine, calcium, and P (with a Ca:P ratio of 1.1) in order to maximize growth potential and to allow the maximum deposition of Ca and P. A broad-spectrum antibiotic (Tylan-40® - Tylosin phosphate; Elanco, Inc., Greenfield, IN) was added to the diet at 0.05% in all phases to avoid any health-related issue. Diets were mixed at the University of Kentucky feed mill in a 1000-kg capacity horizontal mixer and were fed in meal form. Analyses of diets were conducted for dry matter, crude protein (N × 6.25), fat, ash, Ca, and P.

Whole Body Tissue Collection Procedures

All pigs were slaughtered by conventional methods after a fasting period of 16-20 hours. Pigs were electrically stunned, hung by one leg by wrapping a chain around the hock, and then exsanguinated. Carcasses were scalded, mechanically dehaired, and then hung on the rail by the digital flexor tendons of both hind legs. All bleeding and evisceration procedures were conducted with a 15-cm boning knife. The following paragraphs describe the procedures for the collection of specific body components.

Head

The head was removed from the carcass by inserting the knife into the neck, caudal to the ear, and separating the spinal column distal to the axis vertebrae. The separating cut continued horizontally through the ventral portion of the neck. The jowl was removed with the head. The head was immediately weighed and placed in a plastic bag for storage at -20° C.

Viscera

The viscera were removed beginning with the large intestine and the other digestive organs (small intestine, stomach, liver, pancreas, and spleen) contained in the abdominal body cavity. The diaphragm was removed along with the heart, lungs, esophagus, and trachea contained in the thoracic cavity. The kidneys and all leaf fat were removed from the carcass and

combined with the other visceral organs. A weight was collectively taken of all visceral components. The small and large intestine was unfurled by removing all mesentery and connective tissues. The elongated intestines were then split from end to end, so that the inner intestinal lining could be exposed and rinsed of all digesta and digestive secretions. Then the stomach, bladder, and gall bladder were opened longitudinally with scissors and rinsed clean of all contents. All excess water was squeezed from the rinsed organs prior to obtaining an empty visceral weight. The empty viscera were placed in a plastic bag for storage at -20° C.

Carcass

After dehairing and removal of the hooves, the carcass was further scraped with a knife to remove all visible hair stubble. The head and jowl was removed from the carcass as previously described. The carcass was then split with a mechanically operated meat saw along the dorsal midline. The ventral portion of the vertebrae served as a visual marker to split the vertebral column in the middle of each spinal process, so that the carcass was equally represented by a right and left half. Each carcass was weighed and lightly rinsed with hot water and then placed in a cooler (1° C) overnight. The following day, while still hanging on the rail, each carcass was weighed to determine moisture loss (drip loss), and then wrapped in plastic, and placed into a cardboard box for storage at -20° C.

Sample Processing

After whole body tissues were collected on the slaughter floor, samples were further processed to obtain a homogenized sample for lab analyses. The left half of each carcass, the entire viscera, and the head were each ground individually in a large meat grinder (Model 801, Autio Co., Astoria, OR). Fifteen right half carcasses were kept for further dissection while the remaining 29 were ground as previously described. The whole body components were taken directly from the freezer, ground to pass through a 6 mm plate, and were collected in a large plastic tub. A generous portion of dry ice was added to the once-ground tissue and mixed into the sample by hand. The entire tub of ground tissue was ground twice more with dry ice added between grindings. A sub-sample of each tissue group was taken from the tub by randomly sampling 500 to 1200 g (depending on the sample type and size) from different portions of the mixture.

Samples of ground carcass, viscera, and head were dried to a constant weight by

lyophilization (General purpose freeze dryer 36D x 66 series, Virtis, Inc., Gardiner, NY). Freshly ground samples of tissue were weighed into plastic containers and placed into the freeze dryer for 10 to12 days. All samples were removed from the dryer and allowed to air equilibrate for 24 h prior to reweighing to determine moisture loss.

Dried, ground carcass, viscera, and head samples were homogenized in a small food processor (HC 3000, Black & Decker Inc., Shelton, CT). In carcass samples from the pigs whose both halves of the carcass were ground, the percentage that each half comprised of the whole carcass weight (as taken prior to splitting) was used to determine a multiplicative factor in order to make a composited sample for further analysis. All ground tissue samples were analyzed for dry matter, nitrogen, ether extract, ash, and P.

Physical Separation of Carcass Tissues

The right half carcass of 15 pigs (8 from TRT 1 – 30 kg BW; 7 from TRT 5 – 110 kg BW) was physically separated into bone and soft tissue (skin, lean, fat, and connective tissues). After being removed from the freezer, each right carcass half was weighed to measure moisture loss, and placed in a cool (~15° C) room to partially thaw overnight. All soft tissue was removed from the bone using sharp poultry shears and placed in an air-tight, plastic container. The periosteum associated with each bone was left intact if possible. Both separated tissues (bone and soft tissue) were stored in covered plastic containers, refrozen and then weighed prior to being ground through a large meat grinder (Model 801, Autio Co., Astoria, OR) similar to the left carcass half. Care was taken to reduce moisture loss by only separating tissue from small sections of carcass at a time, and refreezing any soft tissue that over-thawed and was dripping moisture. Dissected bone was refrozen, and then weighed collectively. Bones were then smashed with a hammer and coarsely ground through a small meat grinder (Model TCA-22, Lasar Manufacturing Co. Inc., Los Angeles, CA) fitted with a 9-mm plate. All of the ground bone was placed in 25 x 40 cm stainless steel pan and oven-dried (55° C) for 96 h. For bone from 90 to 110 kg pigs, two pans were used so that the ground bone tissue could be spread to an even depth of 5 cm within the pan. After drying, ground bone was air equilibrated overnight, and then weighed to determine moisture loss. At least two-thirds of the bone was further ground in the same small meat grinder to reduce particle size to pass through a 3-mm screen and kept for analysis. Bone was analyzed for moisture, nitrogen, ash, P, and ether extract.

Laboratory Analyses

Phosphorus (dry samples)

From the subsamples taken at mixing, a 2.5-g (feed, feces), 3.5-g (bone), or 4.0-g (carcass, soft tissue, viscera, head) aliquot was measured into a quartz crucible and dry-ashed overnight in a muffle furnace at 600° C. Following cooling, 40 ml of 3N HCl were added to the ashed sample and the solution was heated to boiling for 15 min. The solution was quantitatively transferred to a volumetric flask and diluted with distilled, deionized water to 250 ml. The sample solution stood overnight to allow any heavy particles to settle. A 50-ml (feces, feed, viscera, carcass, soft tissue, blood, skin) or 25-ml (bone) aliquot was taken by volumetric pipette from this solution and placed into a 250-ml Erlenmeyer flask, heated, and mixed with 50 ml of Quimociac solution. A yellow precipitate was formed after boiling for 2 minutes. This precipitate was gravimetrically filtered into a porcelain crucible and dried overnight at 105° C. The weight of the precipitate was taken and the percentage P in the sample was calculated as follows:

$$\text{Total P (\%)} = \left[\frac{\text{(precipitate wt. x 250 ml)}}{\text{aliquot, ml}} \times \frac{\text{(0.013997 x 100)}}{\text{sample wt}} \right]$$

All P analyses were conducted in duplicate with an acceptable coefficient of variation of 7%. For high variable samples (those with many bone chips), a CV up to 10% was accepted whenever 3 or more analytical values were available.

Nitrogen

Samples were sent to University of Missouri, Experiment Station Chemical Laboratories, Columbia, MO, for nitrogen analysis. They were conducted using a gas combustion method (FP-2000, Leco Corp., St. Joseph, MI). All nitrogen analyses were conducted at least in duplicate with an acceptable coefficient of variation of 7%.

Lipid

A 2.5 to 4-g portion of sample was weighed onto a tared 12.5-cm sheet of filter paper (P8, Fisher Scientific, Pittsburgh, PA). Lipid was extracted from the sample using ethyl ether in

a fat extraction unit (Tecator 1043, Foss, Silver Spring, MD). Extracted fat was collected in a cup, heated for 15 minutes at 105°C to expel all residual ethyl ether, and weighed to calculate the percentage lipid in each sample. All fat analyses were conducted at least in duplicate with an acceptable coefficient of variation of 7%.

Water

Total water content was determined in ground carcass, viscera, head, soft tissue and bone by first lyophilizing the samples as described above. To determine the lab dry matter of all samples, 1.5 to 2 g of sample was weighed into a pre-weighed aluminum pan and placed in a forced-air oven at 105° C overnight. The samples were removed from the oven, allowed to cool for 10-15 minutes in a dessicator and reweighed. All dry matter analyses were conducted in duplicate with an acceptable coefficient of variation of 1%.

Ash

The percentage inorganic content of whole body tissues was determined by weighing 2 to 4 g of sample into a dried and pre-weighed porcelain crucible. Samples were placed into a muffle furnace and heated to 600° C overnight. Samples were removed from the furnace at 150° C and allowed to cool for 10-15 minutes in a dessicator prior to weighing. Ashes from this assay were used for further P analysis. All ash analyses were conducted in duplicate with an acceptable coefficient of variation of 7%. For high variable samples (those with many bone chips), a CV up to 10% was accepted whenever 3 or more analytical values were available.

Calculations

The weight difference between collected viscera and empty viscera (which represents gut fill) was subtracted from live weight at slaughter to calculate empty body weight. Blood, hair and hooves weight was calculated by subtracting the weight of all collected samples (carcass, head, viscera) from the live weight at slaughter.

Chemical composition of whole body components were calculated by multiplying the analyzed percentage of the nutrient, as determined in a dry sample, by the dry weight of the component. Concentrations (g/kg) of nutrients in each component were determined by dividing the weight of nutrient by the fresh weight of the whole body component. Analyzed results from

right dissected carcasses were summed with their respective left carcass' values to obtain the total amount of each parameter in the whole carcass.

Accretion rates of nutrient and tissue components were calculated by first regressing the weight of nutrient on live weight of pigs. All relationships were linear except for lipid accretion in gilts where a quadratic equation was used. These linear equations (quadratic in regards to lipid in gilts) were used to determine an "initial" and "final" composition for each pig. By "initial" it is meant the average one that pigs had at the set weight previous to its slaughter ("final") weight. A different equation was used for each gender within each breed whenever a significant difference between genders was determined for a body component. Weight ranges for accretion rates were 30 to 50, 50 to 70, 70 to 90, and 90 to 110 kg. The days for pigs to reach the final weight from the initial weight in each weight range were calculated by building a regression line with the recorded weights of the animals at different known times. Accretion rates were calculated by using the relationship of nutrient content to live weight of each pig. The accretion rates provided in each table are the average of five (or six) pigs from the initial weight in the range to the final slaughter weight for those respective pigs. Rate of gain was determined by dividing the gain for each pig by the calculated days.

Carcass lean accretion rate (g/d) for the standard growth period (20 – 120 kg BW) was calculated separately for gilts and barrows of each breed as follows:

$$\frac{\text{WEB N 110 kg BW} - \text{WEB N 30 kg BW (g)}}{\text{Recorded days from 30 to 110 kg BW}} \times 6.25 \times 2.55 \times 0.97$$

The daily amount of N deposited in WEB from 30-110 kg BW was multiplied by 6.25 to convert it into protein, then by 2.55 to convert it into carcass lean tissue (NRC, 1998) and, finally, by 0.97 to correct the growth range used in Experiment 2 for the standard range used by the NRC, 1998 (20-120 kg BW).

Statistical Analysis

Pigs were allotted to slaughter weight groups (Table 3.1) such that litter groups and weights were evenly divided across groups. Two females and three (or four) males were assigned to each group. Slaughter weight groups were considered treatments and individual pig was considered the experimental unit. Each treatment contained five (or six) observations. Initial

analyses contained pigs from the common slaughter weights of both breeds to determine if there was a breed effect. Each breed was then analyzed independently to determine sex effects and to obtain a regression curve. Linear, quadratic, and cubic effects of body weight, empty body weight, and individual nutrient weights in whole body on the mass and accretion of whole body nutrients were tested by orthogonal polynomials (Steel et al., 1997). An analysis of variance was conducted for the common treatments (30, 70, and 110 kg slaughter weights) in both genetic lines. A $P \leq 0.05$ or 0.10 was considered significant or a tendency to be significant, respectively.

Results

Calculated carcass lean accretion rate from 20 to 120 kg BW was 396, 387, 341, and 345 g/d for PIC gilts, PIC barrows, crossbred gilts, and crossbred barrows, respectively.

Initial body weight of pigs allotted to the final slaughter weights of 30, 50, 70, 90, and 110 kg (30, 70, and 110 kg for the UK pigs) was 30.4 ± 2.1 kg. Final body weights were 31.2, 50.1, 69.9, 93.5, and 110.1 kg for the PIC pigs and 30.4, 71.0, and 110.3 kg for the UK pigs. The weights of all body components are shown in Table 3.3. Carcasses of PIC pigs were lighter ($P < 0.05$) than those from UK pigs. PIC gilts exhibited lighter empty viscera and heavier carcass weights than PIC barrows across all the weight groups ($P < 0.05$). Empty body weight averaged 97.8 and 98.3% of live weight (after 17 hours of fasting) across all groups for PIC and UK pigs, respectively. Carcass, as a percentage of WEB, increased linearly ($P < 0.01$) as pigs grew from 30 to 110 kg; whereas, head and viscera decreased linearly ($P < 0.01$) and quadratically ($P < 0.05$), respectively, as a percentage of WEB (Table 3.4). Blood, hair and hoof mass was not measured directly but by difference from the live weight previous to slaughter.

Quantitatively, N, lipid, ash, and P (Tables 3.5, 3.6, 3.7, and 3.8, respectively) all increased linearly ($P < 0.01$) as body weight increased. Gilts accreted more N (Table 3.5) than barrows and the difference was greater as the live weight increased (Figure 3.1), which is denoted by the interaction between gender and weight group ($P < 0.06$). Logically, the carcass contributed the greatest percentage to the total for all chemical components. The carcass increased its percentage contribution, as body weight increased, to total N and lipid (Tables 3.5 and 3.6, respectively) in a quadratic fashion ($P < 0.05$) and to total P (Table 3.8) linearly ($P < 0.05$), while the same regression was not significant for ash (Table 3.7). The contribution of carcass fat to total WEB lipid was 3-4 percentage units higher for the PIC gilts than for the

barrows across all the slaughter weights. Additionally, PIC pigs (both genders) had lower ($P < 0.05$) lipid content in carcass and in WEB (Figure 3.2) than UK pigs. Empty viscera declined linearly ($P < 0.01$) in its contribution to WEB N, ash, and P. Head contributed increasingly less N and lipid to WEB contents of these components. P from the head as a percentage of WEB P did not significantly vary from 30 to 110 kg, yet empty viscera showed the clearest change, linearly decreasing by almost 4 percentage units for PIC pigs and up to 5 units for UK pigs from 30 to 110 kg. P accretion (Figure 3.3a) was very similar for both breeds and in both cases females retained more mineral ($P < 0.05$) than males, with an evident difference after 70 kg BW. The difference between genders was much more evident for PIC pigs than for UK pigs (1.13 vs. 0.27 g P/kg BW increase, respectively; Figure 3.3b). Even though accretion of P in viscera (Figure 3.4) appears to be very variable, PIC gilts clearly manifested a much higher rate ($P < 0.01$) than the rest (289 vs. 56-76 mg/kg BW); such a high difference in P accretion for the PIC gilts was not demonstrated for carcass (Figure 3.5) or head.

The relationship of WEB N to P is shown in Figure 3.6. The components were linearly ($P < 0.01$) related and the equation indicates that 1 g of P is deposited for every 5.14 g of N for pigs of both genders and genetic backgrounds ($P > 0.10$) from 30 to 110 kg BW. Similarly, ash was related to P (Figure 3.7) with a slope of the linear ($P < 0.01$) equation indicating that P comprises on average 16.5% of WEB ash. The fat-free concentration of ash and P in WEB, carcass, head, and viscera are shown in Table 3.9. PIC males accreted 5.6 g P per kg of fat-free empty body while the rest of the pigs accreted an average of 7.0 (Figure 3.8).

Concentrations of each nutrient in physically separated soft tissue (muscle, fat and skin) and subsequent whole body calculations are shown in Table 3.10. Because only two weight groups were used for dissection purposes, no linear or quadratic tests could be performed. The analysis of variance showed that UK pigs had a greater amount of soft tissue than PIC pigs ($P < 0.05$) as an absolute value but not as a percentage of the carcass weight. A gender effect and a gender by weight group interaction were detected for several N and P parameters in PIC pigs. As is commonly assumed, P in soft tissue accounted for approximately 25% of the total P in the carcass, remaining constant from 30 to 110 kg BW. Bone composition is defined in Table 3.11. As a percentage of fresh bone, water content decreased ($P < 0.01$) with increasing body weight, seemingly being replaced by ash and lipid, which both increased ($P < 0.01$). Lipid absolute and relative content in bone differed ($P < 0.05$) between both breeds in favor of UK pigs.

Table 3.12 shows the differences in whole carcass content of N, ash, and P depending on

the method used for carcass processing in the 15 pigs that were processed by both methods, grinding and dissection. Results from dissected right carcasses are higher than from ground left carcasses for the three responses in both weight groups except for carcass N content at 110 kg. The simple sum of the content in left and right carcass (method used for these pigs) appears to be an intermediate value in all the cases.

The live growth rates of PIC pigs from 30 to 50, 50 to 70, 70 to 90, and 90 to 110 kg were 0.97, 1.13, 1.14, and 0.99 kg/d (Table 3.13). Rate of body weight gain increased up to an average of 80 kg, but then declined in 100 kg pigs, however no significant overall effect was observed. Rate of WEB gain responded accordingly, but the peak was detected in 60 kg pigs. Feed intake increased linearly ($P < 0.01$) across all weight groups studied, and consequently P intake increased in a linear ($P < 0.01$) fashion as well. The efficiency of P retention in WEB was calculated as a percentage of total P intake. Efficiency remained fairly constant (34%) in pigs from 30 to 70 kg, but it decreased down to 30% and 23% during the last two periods, respectively, but no linear or quadratic response was found. N and ash showed variable accretion rates and lipid exhibited a slight increasing retention rate with a peak around 80 kg BW and then a rapid decline during the last growth period.

Table 3.14 illustrates a comparison between PIC and UK pigs used in the current study, and between these and the pigs used by Pettey (2004), which were of a similar genetic background. PIC pigs exhibited a superior gain to feed ratio ($P < 0.01$), and reached the target slaughter weight within a shorter period of time ($P = 0.07$) than UK pigs. UK pigs, on the other hand, had a greater amount of dissectable soft tissue ($P < 0.01$) and fat in carcass ($P = 0.03$). Females needed more days to reach the slaughter weight ($P = 0.02$) than males, with a 10 day difference for 110 kg pigs. PIC gilts showed a higher amount of N in WEB ($P < 0.01$) than PIC barrows. The contribution of carcass to WEB was higher for the pigs used by Pettey (2004), and the amount of N in WEB as well as the fat content in carcass was closer to the PIC pigs than to the current UK pigs at 110 kg.

Discussion

The initial hypothesis of having PIC as a leaner breed was partially accomplished because they had a higher carcass lean accretion rate and a smaller amount of fat in carcass than

UK pigs; yet they failed to have less quantity of fat than the UK pigs used by Petty in 2004. Both breeds were leaner than a moderate lean accretion pig (325 g/d), as considered by the NRC (1998). Differences observed in the 30 kg group between current UK pigs and Pettey's ones (Table 3.13) are due to the higher slaughter weight (38.6 vs. 31.2 kg) used in his experiment for this weight group.

One potential source of error in analyzing body composition by grinding the carcass is the proper mixing of small bone and skin particles prior to sampling and analysis. In fact, de Lange (2001) recognized physical tissue separation as a superior method to analyzing carcass and whole body composition, however this technique requires a generous amount of time and labor work. Still, unlike Pettey (2004) and some
other previous studies, the whole carcass was ground in this experiment, which is the predominant method reported in the literature.

The vast difference in P accretion in viscera between PIC gilts and the rest seems to be validated by the high correlation coefficient ($R^2 = 0.86$), but it is difficult to explain biologically. Moreover, this difference does not constitute the major contribution to WEB P accretion.

The average P concentration in WEB found in this study (4.87 and 4.12 g/kg for females and males, respectively, as an average from both breeds) differs from the one stated by Pettey (2004), 4.72 g/kg, and completes the spectrum of values previously reported. In the investigations of Rymarz et al. (1982), pigs from three different breeds were analyzed and the relationship of P content and empty body weight was: 5.51 (Landrace), 5.42 (Large White), and 4.79 (Hampshire). Rymarz (1986) conducted another study involving a larger data set of Landrace gilts where the relationship of P accretion to empty body weight gain was 5.0 g/kg. Hendriks and Moughan (1993) evaluated the total mineral content of 36 Large White x Landrace-Large White boars and gilts. For boars, 4.18 g of P was gained for every kilogram increase in EBW, while the accretion rate in gilts was slightly higher at 4.40 g. In the most recently published study (Mahan and Shields, 1998), data were collected from Hampshire-Yorkshire-Duroc x Duroc pigs and the relationship of P content to empty body weight gain was 4.37 in pigs from birth to 145 kg. From the data reported by Mahan and Shields (1998), the concentration of P was the highest in the suckling pig, decreased linearly until 75 kg, and then remained fairly constant to 145 kg. In contrast, Rymarz (1986) reported that P concentration of WEB gradually increased from 5.35 g/kg WEB in the young pig, maximized at 5.9 g/kg WEB at 100 kg body weight, and then gradually decreased before reaching a constant concentration

(around 5.0 g/kg) in pigs greater than 120 kg. Pigs from current experiment at UK showed a fairly constant concentration from 30 to 110 kg, with the gilts having a greater concentration than the barrows in both breeds ($P < 0.05$).

Rymarz et al. (1982) reported that in gilts of a similar age the contents of P in the body differed significantly between the breeds, but the differences considerably diminished when the contents of individual components were expressed as their ratio to the EBW and were non-significant for their ratio to the content of crude protein. Moreover, the relationship of P accretion to protein gain was estimated to be 35 g of P for every kg increase in whole body protein by Jongbloed (1987), which represents 4.57 g N per g of P (protein as N x 6.25). In our experiment, N/P ratio was 5.14, which is greater than Jongbloed's (1987) but smaller than the one found by Pettey (2004), 5.80, and may respond to an increasing lean accretion rate from the old genotypes to the actual high lean pigs. This ratio was similar ($P > 0.05$) for both breeds and genders in our experiment, in agreement with Rymarz et al. (1982).

Rymarz et al. (1982) stated that there is a correlation between whole-body mineral retention and protein deposition and that the retention of minerals per unit body protein appears to vary with the rate of protein retention. They also claimed that estimates of the storage of minerals based on protein deposition data would be preferable to those based on the live-weight gain because potential breed differences are diminished due to the elimination of the influence of fat content in the body. Our research seems to support this argument; it appears to be a greater need for P in breeds with a higher lean accretion rate, and this P requirement can be accurately calculated by using a factorial method based on lean accretion rate, allowing the possibility for imputing different lean accretion rates for gilts and barrows, like the current NRC (1998) model.

Implications

Information provided by this study allows for the quantification of WEB P and relationships of WEB P to other nutrients in the body. This information, along with data from Pettey (2004), will help to build an equation to predict P requirements based on protein accretion.

Table 3.1 – Allotment of pigs to slaughter weight groups in Experiment 2.

	Weight group, kg					
Breeding	30	50	70	90	110	Total
PIC						
Barrows, n	4	3	3	3	4	17
Gilts, n	2	2	2	2	2	10
Crossbred (UK)						
Barrows, n	4		3		4	11
Gilts, n	2		2		2	6

Table 3.2 - Composition and nutrient analysis of the diets in Experiment 2 (as-fed basis).

	Phase 1	Phase 2	Phase 3	Phase 4
Body Weight	20 - 40 kg	40 - 60 kg	60 - 80 kg	80 - 110 kg
Ingredient composition, %				
Corn, ground	67.675	73.045	77.205	80.385
Soybean meal, dehulled	28.000	23.000	19.000	16.000
Choice white grease	1.000	1.000	1.000	1.000
Lysine-HCl (78% lysine)	0.220	0.120	0.100	0.080
DL-methionine	0.140	0.070	0.050	0.010
Threonine	0.090	0.040	0.020	0.000
Dicalcium phosphate	1.500	1.400	1.300	1.200
Limestone, ground	0.800	0.750	0.750	0.750
Salt, iodized	0.350	0.350	0.350	0.350
Vitamin mix[a]	0.100	0.100	0.100	0.100
Trace mineral mix[b]	0.075	0.075	0.075	0.075
Antibiotic[c], Tylan®-40	0.050	0.050	0.050	0.050
Formulated content				
Ether extract, %	3.688	3.837	3.950	4.038
Crude protein, %	18.921	16.992	15.437	14.276
Lysine, %	1.193	1.000	0.853	0.755
Calcium, %	0.736	0.681	0.648	0.617
Phosphorus, %	0.660	0.622	0.588	0.558
Available phosphorus, %	0.354	0.329	0.306	0.283
ME, kcal/kg	3,355	3,369	3,377	3,384
Analyzed content				
Dry matter, %	89.234	89.390	89.610	88.97
Ether extract, %	3.10	3.55	3.60	3.49
Crude protein, %	19.625	17.305	15.770	13.605
Calcium, %	0.84	0.74	0.69	0.63
Phosphorus, %	0.69	0.65	0.60	0.58
Ash, %	5.15	4.61	4.35	3.91

[a]Provided per kg of diet: vitamin A, 6,600 IU; vitamin D_3, 880 IU; vitamin E, 44 IU; vitamin K (as menadione sodium bisulfite complex), 6.4 mg; riboflavin, 8.8 mg; pantothenic acid, 22.0 mg; niacin, 44.0 mg; folic acid, 1.32 mg; d-biotin, 0.22 mg; vitamin B_{12}, 33 µg; vitamin B_6, 4.4 mg.

[b]Provided per kg of diet: Fe, 135 mg; Zn, 135 mg; Mn, 45 mg; Cu, 13 mg; I, 1.5 mg, Se, 0.3 mg; Co, 0.23 mg.

[c]Provided 44 mg tylosin per kg of diet (Tylan-40).

Table 3.3 – Weight of body components in Experiment 2[1].

	Weight group, kg					
Item	30	50	70	90	110	SEM
PIC						
Live weight[2], kg[a,g]	31.2	50.1	69.9	93.5	110.1	1.2
Live weight[3], kg[a,g]	28.7	48.5	66.5	89.4	107.1	1.2
WEB, kg[a,g]	28.0	47.2	65.1	87.3	104.8	1.1
Carcass, kg[a,b,d,g]	19.3	34.1	48.8	66.6	80.8	1.0
Gilts	*19.7*	*35.5*	*50.1*	*68.9*	*81.4*	*1.6*
Barrows	*18.9*	*32.7*	*47.6*	*64.3*	*80.1*	*1.3*
Empty viscera, kg[a,b,g]	4.07	5.92	7.42	9.90	11.46	0.51
Gilts	*4.05*	*5.72*	*6.55*	*9.65*	*10.40*	*0.79*
Barrows	*4.09*	*6.13*	*8.30*	*10.14*	*12.53*	*0.64*
Head, kg[a,g]	2.33	3.58	4.55	5.74	6.57	0.23
Blood/hair/hoof[4], kg[a,g]	2.35	3.59	4.30	5.06	5.97	0.36
UK						
Live weight[2], kg[a,g]	30.4		71.0		110.3	0.8
Live weight[3], kg[a,g]	29.5		68.0		107.4	1.0
WEB, kg[a,g]	28.9		67.0		105.7	0.9
Carcass, kg[a,d,g]	20.5		51.3		81.9	0.8
Empty viscera, kg[a,g,h]	3.64		6.47		11.24	0.38
Head, kg[a,g,h]	2.44		4.92		6.59	0.14
Blood/hair/hoof[4], kg[a,g]	2.31		4.28		6.02	0.37

[1]PIC: Pig Improvement Company; UK: University of Kentucky; WEB: whole empty body; SEM: Standard error of the mean, pooled error from 3 (or 4) barrows and 2 gilts.
[2]Weight obtained 18 hours prior to slaughter (before fasting).
[3]Weight obtained 1 hour prior to slaughter (after 17 hours fasting).
[4]Weight obtained by difference.
[a]LS means among weight groups within breed differ ($P < 0.05$).
[b]LS means between genders within breed differ ($P < 0.05$).
[d]LS means between breeds differ ($P < 0.05$). Data from common weight groups of both breeds analyzed.
[g]Linear effect of live weight[2] ($P < 0.05$).
[h]Quadratic effect of live weight[2] ($P < 0.05$).

Table 3.4 – Weight of body components as percentage of WEB in Experiment 2[1].

	Weight group, kg					
Item	30	50	70	90	110	SEM
PIC						
WEB[2], kg[a,g]	28.7	48.5	66.5	89.4	107.1	1.2
WEB[2], % of LW[b,d]	97.8	97.3	97.9	97.6	97.8	0.2
Gilts	*97.86*	*97.72*	*98.26*	*97.81*	*98.33*	*0.38*
Barrows	*97.70*	*96.83*	*97.52*	*97.42*	*97.36*	*0.31*
Carcass, %[a,b,d,g,h]	68.8	72.2	75.0	76.2	77.1	0.8
Gilts	*68.8*	*72.5*	*76.8*	*77.5*	*78.1*	*1.2*
Barrows	*68.8*	*71.9*	*73.2*	*74.9*	*76.1*	*1.0*
Empty viscera, %[a,b,d,g,h]	14.5	12.6	11.4	11.4	10.9	0.6
Gilts	*14.1*	*11.7*	*10.1*	*10.9*	*10.0*	*0.9*
Barrows	*14.9*	*13.5*	*12.8*	*11.8*	*11.9*	*0.7*
Head, %[a,b,f,g]	8.3	7.6	7.0	6.6	6.3	0.3
Blood/hair/hoof[3], %[a,g]	8.4	7.6	6.6	5.8	5.7	0.7
UK						
WEB[2], kg[a,g]	28.9		67.0		105.7	0.9
WEB[2], % of LW[d]	98.1		98.6		98.4	0.2
Carcass, %[a,d,g,h]	70.9		76.6		77.5	0.9
Empty viscera, %[a,b,d,g,h]	12.6		9.7		10.6	0.6
Gilts	*11.3*		*9.0*		*10.7*	*0.7*
Barrows	*14.0*		*10.4*		*10.5*	*0.8*
Head, %[a,f,g]	8.5		7.3		6.2	0.3
Blood/hair/hoof[3], %[a,g]	8.0		6.4		5.7	0.7

[1]PIC: Pig Improvement Company; UK: University of Kentucky; WEB: whole empty body; SEM: Standard error of the mean, pooled error from 3 (or 4) barrows and 2 gilts.
[2]Weight obtained 1 hour prior to slaughter (after 17 hours fasting).
[3]Weight obtained by difference.
[a]LS means among weight groups within breed differ ($P < 0.05$).
[b]LS means between genders within breed differ ($P < 0.05$).
[d]LS means between breeds differ ($P < 0.05$). Data from common weight groups of both breeds analyzed.
[f]Breed by gender interaction ($P < 0.05$). Data from common weight groups of both breeds analyzed.
[g]Linear effect of live weight ($P < 0.05$).
[h]Quadratic effect of live weight ($P < 0.05$).

Table 3.5 – Nitrogen content, concentration and percentage of total body nitrogen of WEB components in Experiment 2[1].

	Weight group, kg					
Item	30	50	70	90	110	SEM
PIC	----- Nitrogen[2], g -----					
WEB[a,b,c,g]	721	1204	1703	2115	2646	37
Gilts	*734*	*1240*	*1732*	*2173*	*2809*	*57*
Barrows	*707*	*1169*	*1675*	*2056*	*2484*	*47*
Carcass[a,b,c,g]	524	923	1342	1666	2104	35
Gilts	*539*	*965*	*1381*	*1732*	*2270*	*53*
Barrows	*510*	*882*	*1302*	*1601*	*1938*	*44*
Viscera[a,d,g]	85	121	157	192	223	7.4
Head[a,g]	56	84	107	131	154	5.4
UK						
WEB[a,g]	739		1695		2526	51
Carcass[a,g]	553		1350		2016	51
Viscera[a,d,g]	74		137		201	5.5
Head[a,g]	56		109		145	4.6
PIC	----- Nitrogen, g/kg -----					
WEB[b,c]	25.7	25.5	26.2	24.2	25.3	0.46
Gilts	*25.65*	*25.34*	*25.59*	*24.47*	*26.96*	*0.71*
Barrows	*25.75*	*25.72*	*25.84*	*23.99*	*23.59*	*0.58*
Carcass[a,g]	27.2	27.1	27.5	25.0	26.0	0.54
Viscera[b,g]	21.0	20.5	21.2	19.5	19.7	0.55
Head[b,d]	24.0	23.4	23.5	22.9	23.4	0.38
UK						
WEB	25.6		25.3		23.9	0.61
Carcass[a,g]	27.1		26.3		24.6	0.66
Viscera[a,g,h]	20.5		21.2		17.9	0.44
Head[d]	23.0		22.3		21.9	0.60
PIC	----- Nitrogen[2], % of total N -----					
Carcass[a,b,d,g,h]	72.8	76.6	78.7	78.8	79.4	0.45
Gilts	*73.4*	*77.8*	*79.7*	*79.7*	*80.8*	*0.7*
Barrows	*72.1*	*75.4*	*77.8*	*77.9*	*78.0*	*0.6*
Viscera[a,b,d,g,h]	11.9	10.0	9.2	9.1	8.5	0.40
Head[a,b,g]	7.8	7.0	6.3	6.2	5.8	0.28
UK						
Carcass[a,d,g,h]	74.9		79.6		79.7	0.68
Viscera[a,d,g]	10.1		8.2		8.0	0.49
Head[a,g]	7.6		6.5		5.7	0.32

[1]PIC: Pig Improvement Company; UK: University of Kentucky; WEB: whole empty body; SEM: Standard error of the mean, pooled error from 3 (or 4) barrows and 2 gilts.
[2]Nitrogen content of blood, hair and hooves was inferred from Pettey (2004).
[a]LS means among weight groups within breed differ ($P < 0.05$).
[b]LS means between genders within breed differ ($P < 0.06$).
[c]Weight group by gender interaction within each breed ($P < 0.06$).
[d]LS means between breeds differ ($P < 0.05$). Data from common weight groups of both breeds analyzed.
[g]Linear effect of live weight ($P < 0.05$).
[h]Quadratic effect of live weight ($P < 0.05$).

Table 3.6 – Lipid content, concentration and percentage of total body lipid of WEB components in Experiment 2[1].

	Weight group, kg					
Item	30	50	70	90	110	SEM
PIC	----- Lipid, kg -----					
WEB[a,d,e,g,h]	4.43	11.00	16.64	24.47	26.30	0.94
Carcass[a,d,g]	3.63	9.60	14.74	21.73	22.99	1.12
Viscera[a,b,e,f,g]	0.40	0.70	0.94	1.51	1.85	0.26
Head[a,b,d,g]	0.40	0.71	0.95	1.24	1.46	0.06
UK						
WEB[a,d,e,g]	4.84		18.19		31.43	0.88
Carcass[a,d,g]	4.11		16.36		27.07	0.89
Viscera[a,e,f,g,h]	0.30		0.76		2.79	0.26
Head[a,d,g]	0.43		1.07		1.58	0.05
PIC	----- Lipid, g/kg -----					
WEB[a,d,g,h]	158	233	256	281	251	11
Carcass[a,g,h]	189	281	303	326	285	15
Viscera[b,e,f,g]	99	118	124	150	157	23
Head[a,g]	169	197	210	215	222	10
UK						
WEB[a,d,g,h]	168		271		298	12
Carcass[a,d,g,h]	201		319		331	14
Viscera[a,e,f,g]	81		113		246	24
Head[a,g]	175		218		240	11
PIC	----- Lipid, % of total lipid -----					
Carcass[a,b,d,g,h]	82.2	87.1	88.6	88.7	87.2	1.6
Gilts	*83.92*	*89.74*	*90.58*	*90.59*	*88.74*	*2.5*
Barrows	*80.40*	*84.39*	*86.69*	*86.88*	*85.69*	*2.1*
Viscera[b]	8.8	6.4	5.6	6.2	7.2	1.4
Gilts	*7.8*	*4.7*	*3.6*	*4.9*	*5.3*	*1.8*
Barrows	*9.9*	*8.1*	*7.7*	*7.5*	*9.1*	*1.6*
Head[a,g,h]	9.0	6.5	5.7	5.1	5.6	0.6
UK						
Carcass[d,h]	84.9		89.9		85.9	1.7
Viscera[h]	6.3		4.2		9.0	1.3
Head[a,g]	8.8		5.9		5.1	0.4

[1]PIC: Pig Improvement Company; UK: University of Kentucky; WEB: whole empty body; SEM: Standard error of the mean, pooled error from 3 (or 4) barrows and 2 gilts.
[a]LS means among weight groups within breed differ ($P < 0.05$).
[b]LS means between genders within breed differ ($P < 0.05$).
[d]LS means between breeds differ ($P < 0.05$). Data from common weight groups of both breeds analyzed.
[e]Breed by weight group interaction ($P < 0.08$). Data from common weight groups of both breeds analyzed.
[f]Breed by gender interaction ($P < 0.08$). Data from common weight groups of both breeds analyzed.
[g]Linear effect of live weight ($P < 0.05$).
[h]Quadratic effect of live weight ($P < 0.05$).

Table 3.7– Ash content, concentration and percentage of total body ash of WEB components in Experiment 2[1].

	Weight group, kg					
Item	30	50	70	90	110	SEM
PIC	----- Ash[2], g -----					
WEB[a,g]	728	1266	1678	2438	2837	171
Carcass[a,g]	549	972	1286	1913	2244	165
Viscera[a,d,g]	41	57	79	97	101	5.4
Head[a,g]	120	215	290	400	458	25
UK						
WEB[a,g]	759		1661		2672	118
Carcass[a,g]	576		1260		2077	105
Viscera[a,d,g]	37		69		93	2.7
Head[a,g]	128		308		467	22
PIC	----- Ash, g/kg -----					
WEB	25.9	26.8	25.9	27.9	27.0	1.8
Carcass	28.4	28.5	26.5	28.7	27.7	2.2
Viscera	10.0	9.7	10.8	10.0	9.0	0.8
Head[a,g]	51.5	60.5	63.8	70.1	69.7	4.3
UK						
WEB	26.3		24.8		25.3	1.1
Carcass	28.1		24.6		25.4	1.5
Viscera[a,b,g,h]	10.2		10.8		8.4	0.4
Head[a,g]	52.3		62.6		70.8	3.1
PIC	----- Ash[2], % of total ash -----					
Carcass	75.3	76.7	76.5	78.1	78.7	1.4
Viscera[a,g]	5.6	4.5	4.8	4.1	3.7	0.4
Head	16.6	17.1	17.4	16.6	16.4	1.3
UK						
Carcass	75.8		75.9		77.5	1.1
Viscera[a,g]	4.9		4.2		3.6	0.2
Head	16.9		18.5		17.6	1.0

[1]PIC: Pig Improvement Company; UK: University of Kentucky; WEB: whole empty body; SEM: Standard error of the mean, pooled error from 3 (or 4) barrows and 2 gilts.
[2]Ash content of blood was inferred from Pettey (2004).
[a]LS means among weight groups within breed differ ($P < 0.05$).
[d]LS means between breeds differ ($P < 0.05$). Data from common weight groups of both breeds analyzed.
[g]Linear effect of live weight ($P < 0.05$).
[h]Quadratic effect of live weight ($P < 0.05$).

Table 3.8– Phosphorus content, concentration and percentage of total body phosphorus of WEB components in Experiment 2[1].

	Weight group, kg					
Item	30	50	70	90	110	SEM
PIC	----- Phosphorus[2], g -----					
WEB[a,b,c,g]	134	231	302	439	473	13
Gilts	*136*	*241*	*305*	*448*	*522*	*20*
Barrows	*132*	*221*	*300*	*362*	*439*	*17*
Carcass[a,b,c,g]	100	178	230	310	376	11
Gilts	*103*	*187*	*227*	*354*	*410*	*18*
Barrows	*98*	*169*	*234*	*280*	*343*	*14*
Viscera[a,b,g]	12.0	18.1	22.8	24.0	22.4	3.1
Gilts	*12.2*	*21.3*	*27.5*	*31.5*	*34.2*	*4.8*
Barrows	*11.8*	*14.8*	*18.0*	*16.6*	*18.5*	*3.9*
Head[a,g]	21.1	33.5	48.3	62.6	76.4	2.8
UK						
WEB[a,b,g,h]	134		294		493	7
Gilts	*141*		*305*		*500*	*10*
Barrows	*126*		*283*		*486*	*7*
Carcass[a,b,g,h]	101		227		395	6
Gilts	*106*		*234*		*403*	*9*
Barrows	*96*		*221*		*386*	*8*
Viscera	12.0		15.1		19.8	2.9
Head[a,g]	20.5		50.6		77.2	3.6
PIC	----- Phosphorus, g/kg -----					
WEB[b]	4.78	4.88	4.66	4.50	4.59	0.19
Gilts	*4.74*	*4.91*	*4.68*	*5.04*	*4.94*	*0.26*
Barrows	*4.83*	*4.85*	*4.64*	*4.22*	*4.23*	*0.21*
Carcass[g]	5.20	5.22	4.73	4.90	4.66	0.22
Viscera[b]	2.96	3.07	3.21	2.45	1.95	0.37
Head[a,g]	9.02	9.44	10.71	10.96	11.62	0.50
UK						
WEB[a,b,h]	4.64		4.39		4.67	0.07
Gilts	*4.78*		*4.54*		*4.77*	*0.29*
Barrows	*4.49*		*4.24*		*4.56*	*0.22*
Carcass[h]	4.92		4.44		4.82	0.14
Viscera[a,g]	2.39		2.34		1.78	0.40
Head[a,g]	8.36		10.24		11.71	0.54

[1]PIC: Pig Improvement Company; UK: University of Kentucky; WEB: whole empty body; SEM: Standard error of the mean, pooled error from 3 (or 4) barrows and 2 gilts.
[2]Phosphorus content of blood was inferred from Pettey (2004).
[a]LS means among weight groups within breed differ ($P < 0.05$).
[b]LS means between genders within breed differ ($P < 0.05$).
[c]Weight group by gender interaction within breed ($P < 0.05$).
[g]Linear effect of live weight ($P < 0.05$).
[h]Quadratic effect of live weight ($P < 0.05$).

Table 3.8– Phosphorus content, concentration and percentage of total body phosphorus of WEB components in Experiment 2 (cont.)[1].

	Weight group, kg					
Item	30	50	70	90	110	SEM
PIC	----- Phosphorus[2], % of total P -----					
Carcass[g]	74.9	77.1	76.0	78.2	78.4	0.9
Viscera[g]	8.8	7.7	7.6	6.4	5.0	1.0
Head[b]	15.8	14.7	16.0	14.8	16.5	0.8
UK						
Carcass	75.2		77.4		80.0	1.9
Viscera[a,g]	9.0		5.1		4.0	0.9
Head	15.3		17.1		15.7	1.1

[1]PIC: Pig Improvement Company; UK: University of Kentucky; WEB: whole empty body; SEM: Standard error of the mean, pooled error from 3 (or 4) barrows and 2 gilts.
[2]Phosphorus content of blood was inferred from Pettey (2004).
[a]LS means among weight groups within breed differ ($P < 0.05$).
[b]LS means between genders within breed differ ($P < 0.05$).
[c]Weight group by gender interaction within breed ($P < 0.05$).
[g]Linear effect of live weight ($P < 0.05$).
[h]Quadratic effect of live weight ($P < 0.05$).

Table 3.9– Fat free concentration of ash and phosphorus in WEB components in Experiment 2[1].

	Weight group, kg					
Item	30	50	70	90	110	SEM
PIC	----- g/kg fat-free empty body -----					
Ash	30.81	34.92	34.81	38.78	36.39	2.75
Phosphorus[c,h]	5.69	6.36	6.27	6.33	6.13	0.30
UK						
Ash[g]	31.59		34.03		35.93	1.32
Phosphorus[a,g]	5.58		6.03		6.65	0.12
PIC	----- g/kg fat-free carcass -----					
Ash	35.01	39.62	37.98	42.54	39.34	3.68
Phosphorus[h]	6.42	7.26	6.80	6.85	6.54	0.35
UK						
Ash	35.25		36.05		37.85	1.98
Phosphorus[a,g]	6.16		6.51		7.21	0.16
PIC	----- g/kg fat-free viscera -----					
Ash	11.08	11.01	12.39	11.65	10.67	0.98
Phosphorus[b]	3.29	3.48	3.64	2.85	2.71	0.45
Gilts	*3.25*	*4.14*	*4.60*	*3.71*	*3.07*	*0.70*
Barrows	*3.32*	*2.82*	*2.67*	*2.00*	*2.35*	*0.50*
UK						
Ash[b,h]	11.13		12.19		11.14	0.33
Phosphorus[g]	3.69		2.65		2.33	0.43
PIC	----- g/kg fat-free head -----					
Ash[a,g]	61.98	75.20	80.67	89.73	89.70	5.68
Phosphorus[a,g]	10.87	11.75	13.56	13.99	14.94	0.64
UK						
Ash[a,g]	63.49		80.07		93.00	3.64
Phosphorus[a,g]	10.15		13.10		15.40	0.66

[1]PIC: Pig Improvement Company; UK: University of Kentucky; WEB: whole empty body; SEM: Standard error of the mean, pooled error from 3 (or 4) barrows and 2 gilts.
[a]LS means among weight groups within breed differ ($P < 0.05$).
[b]LS means between genders within breed differ ($P < 0.05$).
[c]Weight group by gender interaction within breed ($P < 0.05$).
[g]Linear effect of live weight ($P < 0.05$).
[h]Quadratic effect of live weight ($P < 0.05$).

Table 3.10 Soft tissue chemical composition and nutrient distribution in Experiment 2[1].

	Weight group, kg		
Item	30	110	SEM
PIC			
Soft tissue weight, kg[a,b,d]	14.38	68.91	0.54
Soft tissue % carcass weight[a]	85.87	89.64	0.62
Fat-free soft tissue weight, kg[a]	12.36	52.93	0.60
Nitrogen, g[a,b,c]	433	1751	23
Nitrogen, g/kg[a,b,c]	30.09	26.54	0.44
Nitrogen % total carcass N[a]	82.86	86.62	0.70
Ash, g[a]	142	517	25
Ash % fat-free soft tissue	1.15	1.05	0.05
Phosphorus, g[a,b,c]	26.9	101.8	0.8
Phosphorus % fat-free soft tissue[b]	0.218	0.210	0.003
Phosphorus % total carcass P[b,c]	25.35	24.90	0.39
UK			
Soft tissue weight, kg[a,d]	16.05	70.69	0.66
Soft tissue % carcass weight[a]	84.60	90.26	0.46
Fat-free soft tissue weight, kg[a]	13.46	51.60	0.69
Nitrogen, g[a]	466	1643	51
Nitrogen, g/kg[a]	29.03	23.21	0.80
Nitrogen % total carcass N	83.16	87.08	0.83
Ash, g[a]	148	548	9
Ash % fat-free soft tissue	1.10	1.16	0.05
Phosphorus, g[a]	28.1	95.8	2.8
Phosphorus % fat-free soft tissue	0.208	0.201	0.05
Phosphorus % total carcass P	25.71	24.18	0.56

[1]PIC: Pig Improvement Company; UK: University of Kentucky; WEB: whole empty body; SEM: Standard error of the mean, pooled error from 3 (or 4) barrows and 2 gilts.
[2]Pooled error from 8 pigs (4 males and 4 females).
[a]LS means among weight groups within breed differ ($P < 0.05$).
[b]LS means between genders within breed differ ($P < 0.05$).
[c]Weight group by gender interaction within breed ($P < 0.07$).
[d]LS means between breeds differ ($P < 0.05$). Data from common weight groups of both breeds analyzed.

Table 3.11 - Bone chemical composition and nutrient distribution in Experiment 2[1].

Item	Weight group, kg 30	110	SEM
	--- Bone weight, kg ---		
PIC[a]	2.765	8.005	0.144
UK[a]	2.923	8.075	0.233
PIC	--- Nutrient content, g ---		
Water[a]	1528	3109	95
Nitrogen[a]	90	276	7
Lipid[a,d]	199	1145	60
Ash[a]	450	1801	37
Phosphorus[a]	79	313	6
UK			
Water[a]	1587	3060	189
Nitrogen[a]	94	259	6
Lipid[a,d]	270	1400	86
Ash[a]	461	1749	26
Phosphorus[a]	81	306	4
PIC	--- % fresh bone ---		
Water[a]	55.22	38.85	0.68
Nitrogen	3.24	3.45	0.09
Lipid[a,d]	7.28	14.31	0.88
Ash[a]	16.32	22.50	0.39
Phosphorus[a]	2.87	3.91	0.07
UK			
Water[a]	54.18	37.86	1.80
Nitrogen	3.23	3.20	0.07
Lipid[a,d]	9.24	17.41	1.53
Ash[a]	15.89	21.70	0.94
Phosphorus[a]	2.80	3.80	0.18
PIC	--- % fat-free, dry bone ---		
Nitrogen[a]	8.64	7.36	0.16
Ash[a]	43.50	48.04	0.69
Phosphorus[a]	7.65	8.34	0.16
UK			
Nitrogen	8.86	7.17	0.55
Ash	43.39	48.58	1.52
Phosphorus	7.63	8.50	0.30
	--- % of P in bone ash ---		
PIC[a]	19.91	15.32	0.63
UK[a]	20.45	14.78	1.37

[1]PIC: Pig Improvement Company; UK: University of Kentucky; WEB: whole empty body; SEM: Standard error of the mean, pooled error from 3 (or 4) barrows and 2 gilts.
[a]LS means among weight groups within breed differ ($P < 0.05$).
[d]LS means between breeds differ ($P < 0.05$). Data from common weight groups of both breeds analyzed.

Table 3.12 – Comparison of results for whole carcass composition according to different tissue processing and calculation methods in Experiment 2[1].

	Weight group, kg	
Item	30	110
Carcass nitrogen, g		
Ground[2]	530	2036
Dissected[3]	541	2020
Ground + dissected[4]	535	2028
Carcass Ash, g		
Ground[2]	543	2004
Dissected[3]	601	2292
Ground + dissected[4]	571	2150
Carcass phosphorus, g		
Ground[2]	96	362
Dissected[3]	108	406
Ground + dissected[4]	102	384

[1]Average from 8 and 7 pigs (30 and 110 kg weight group, respectively).
[2]Value from analyzed ground left carcass and corrected by the proportional weight of the whole carcass.
[3]Value from analyzed dissected right carcass and corrected by the proportional weight of the whole carcass.
[4]Value from analyzed ground left carcass plus analyzed right dissected carcass. Method chosen for calculation of results of the 15 partially dissected pigs.

Table 3.13 – Daily intake and accretion rate of empty body and chemical components for PIC pigs at different live weight ranges in Experiment 2[1].

	Live weight range, kg				
Item	30-50	50-70	70-90	90-110	SEM
Body weight, kg/d	0.973	1.128	1.139	0.990	0.096
Empty body weight, kg/d	0.914	1.082	1.059	0.990	0.112
Feed intake, kg/d[a,c,g]	1.939	2.194	2.674	3.394	0.240
Gain:Feed[a,g,h]	0.507	0.523	0.431	0.303	0.030
Phosphorus intake, g/d[a,g]	13.38	13.91	15.70	19.50	1.50
P retention % of intake	33.70	34.21	30.00	22.46	5.30
Accretion					
Nitrogen, g/d	22.42	30.35	22.37	26.32	3.71
Lipid, g/d	317	326	360	193	65
Ash, g/d	24.45	27.74	22.90	26.16	6.04
Phosphorus, g/d	4.51	4.76	4.71	4.38	0.83

[1]SEM: Standard error of the mean, pooled error from 3 (or 4) barrows and 2 gilts.
[a]LS means among weight ranges differ ($P < 0.05$).
[c]Weight range by gender interaction ($P < 0.07$).
[g]Linear effect of live weight ($P < 0.05$).
[h]Quadratic effect of live weight ($P < 0.05$).

Table 3.14 – Comparison of growth performance and lean accretion between PIC and UK pigs in Experiment 2[1].

	Weight group, kg			
Item	30	70	110	SEM
PIC				
Average daily gain, kg/d[a,g,h,i]	0	1.113	1.059	0.077
Feed intake, kg/d[a,g,h,i]	0	2.291	2.544	0.161
Gain:Feed[a,d,f,g,h,i]	0	0.487	0.419	0.017
Days to reach slaughter weight[a,b,d,g]	0	35	76	2.0
Gilts	*0*	*37*	*81*	*3.2*
Barrows	*0*	*33*	*71*	*2.6*
Carcass % WEB[a,b,d,g,h]	68.8	75.0	77.1	0.8
Soft tissue weight, kg[a,b,d]	14.38		68.91	0.54
WEB N, g[a,b,c,g]	721	1703	2646	37
Carcass N as a % total N[a,b,d,g,h]	72.8	78.7	79.4	0.45
Fat in carcass, kg[a,d,g]	3.63	14.74	22.99	1.12
Fat in viscera, kg[a,b,e,f,g]	0.40	0.94	1.85	0.26
UK				
Average daily gain, kg/d[a,b,c,g,h]	0	1.023	1.005	0.013
Feed intake, kg/d[a,b,g,h]	0	2.258	2.725	0.084
Gain:Feed[a,d,f,g,h]	0	0.456	0.371	0.014
Days to reach slaughter weight[a,b,d,g]	0	40	81	1.8
Gilts	*0*	*41*	*85*	*2.7*
Barrows	*0*	*38*	*76*	*2.2*
Carcass % WEB[a,d,g,h]	70.9	76.6	77.5	0.9
Soft tissue weight, kg[a,d]	16.05		70.69	0.66
WEB N, g[a,g]	739	1695	2526	51
Carcass N as a % total N[a,d,g,h]	74.9	79.6	79.7	0.7
Fat in carcass, kg[a,d,g]	4.11	16.36	27.07	0.89
Fat in viscera, kg[a,e,f,g,h]	0.30	0.76	2.79	0.26
UK 2004[2]				
Carcass % WEB	74.6	77.9	80.7	
Soft tissue weight, kg	20.6		70.6	
WEB N, g	968	1878	2721	
Carcass N as % total N	76.7	79.6	81.0	
Fat in carcass, kg	3.55	10.29	21.80	
Fat in viscera,kg	0.26	1.06	2.36	

[1]PIC: Pig Improvement Company; UK: University of Kentucky; WEB: whole empty body; SEM: Standard error of the mean, pooled error from 3 (or 4) barrows and 2 gilts.
[2]Pigs used by Pettey (2004) with a genetic background similar to our UK pigs. WEB for these pigs was 35.5, 67.9, and 103.9 kg, respectively.
[a]LS means among weight ranges differ ($P < 0.05$).
[b]LS means between genders within breed differ ($P < 0.05$).
[c]Weight group by gender interaction within breed ($P < 0.07$).
[d]LS means between breeds differ ($P < 0.07$). Data from common weight groups of both breeds analyzed.
[e]Breed by weight group interaction ($P < 0.07$. Data from common weight groups of both breeds analyzed.
[f]Breed by gender interaction ($P < 0.07$). Data from common weight groups of both breeds analyzed.
[g]Linear effect of live weight ($P < 0.05$).
[h]Quadratic effect of live weight ($P < 0.05$).
[i]Cubic effect of live weight ($P < 0.05$).

Figure 3.1 – Change in WEB nitrogen content in response to increasing live weight in Experiment 2.

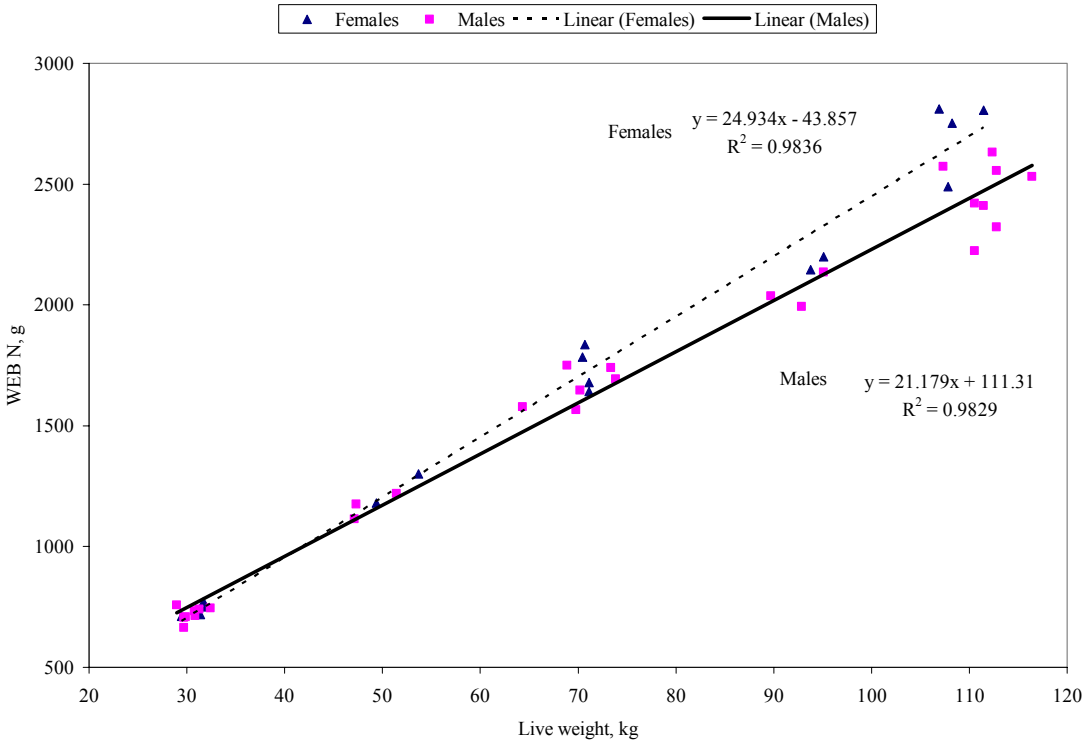


Figure 3.2 – Change in WEB fat content in response to increasing live weight in Experiment 2.

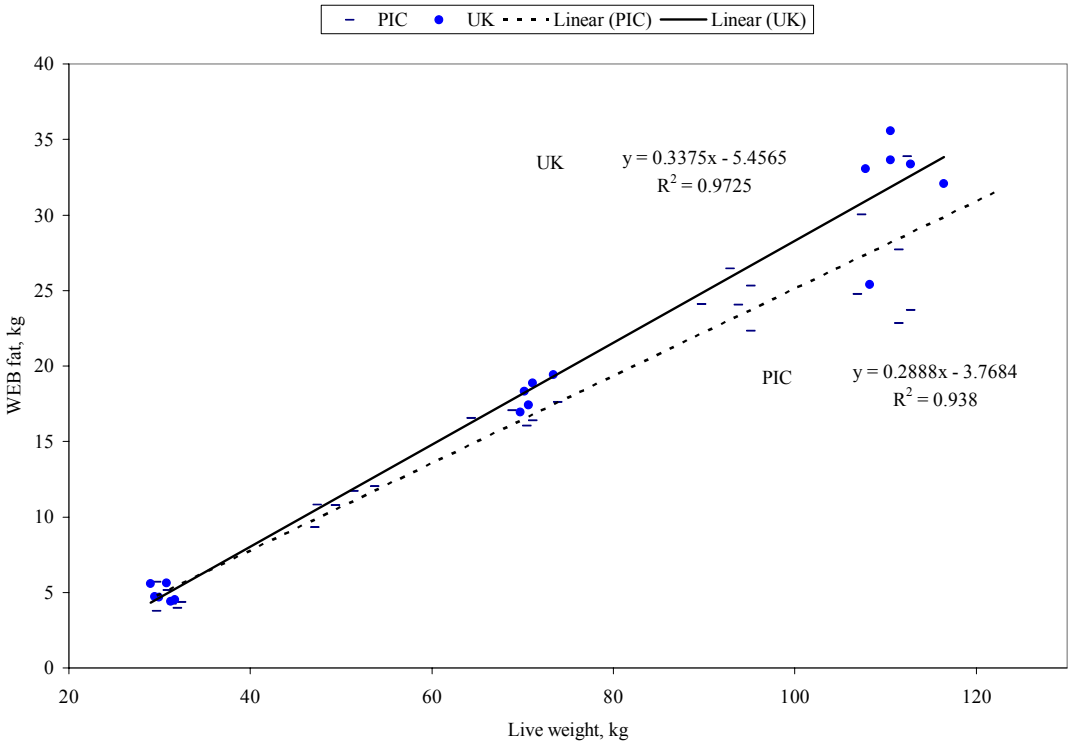


Figure 3.3a – Change in WEB P content in response to increasing live weight in Experiment 2.

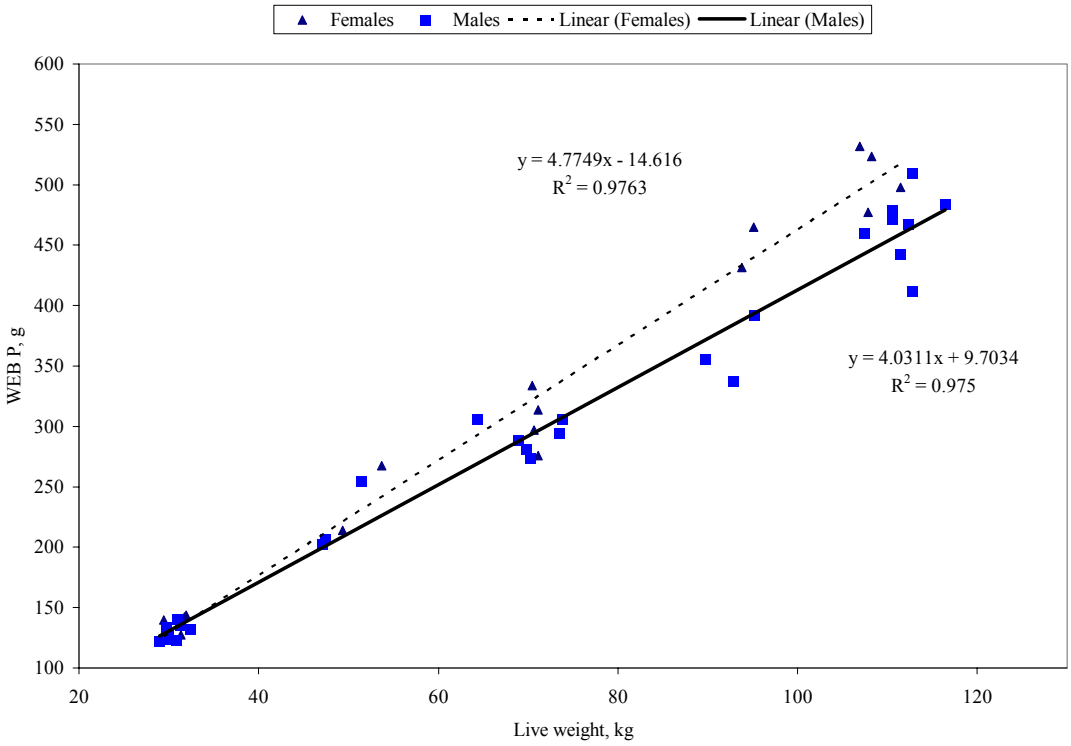


Figure 3.3b – Change in WEB P content in response to increasing live weight in Experiment 2.

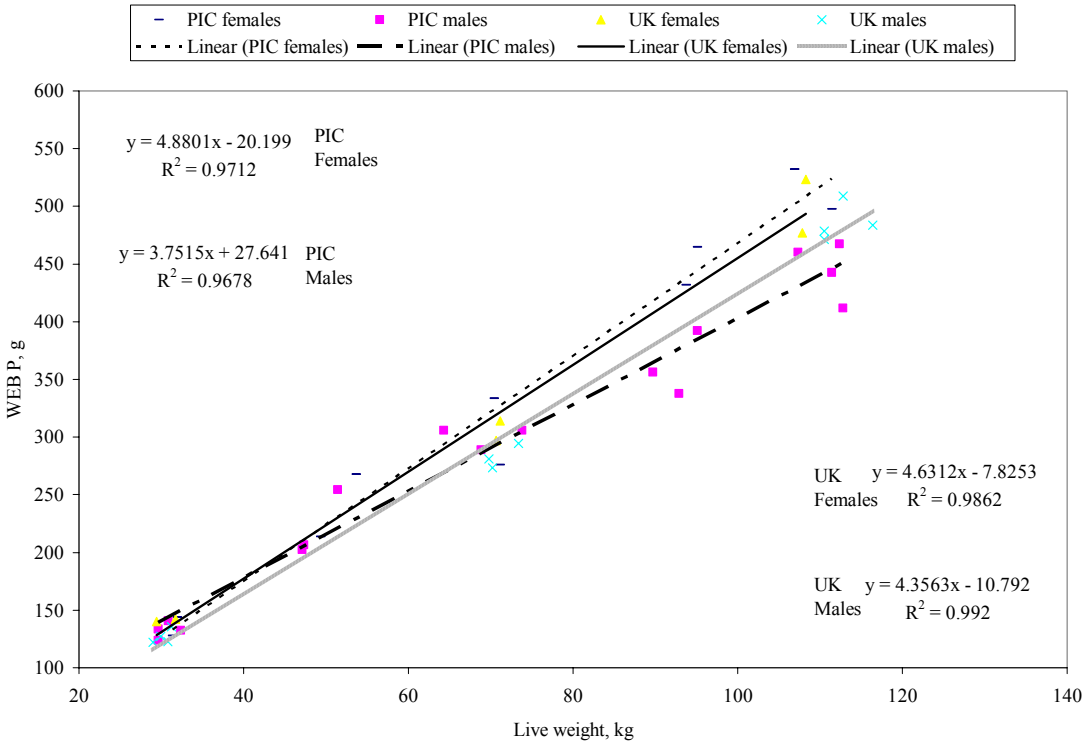


Figure 3.4 – Change in viscera P content in response to increasing live weight in Experiment 2.

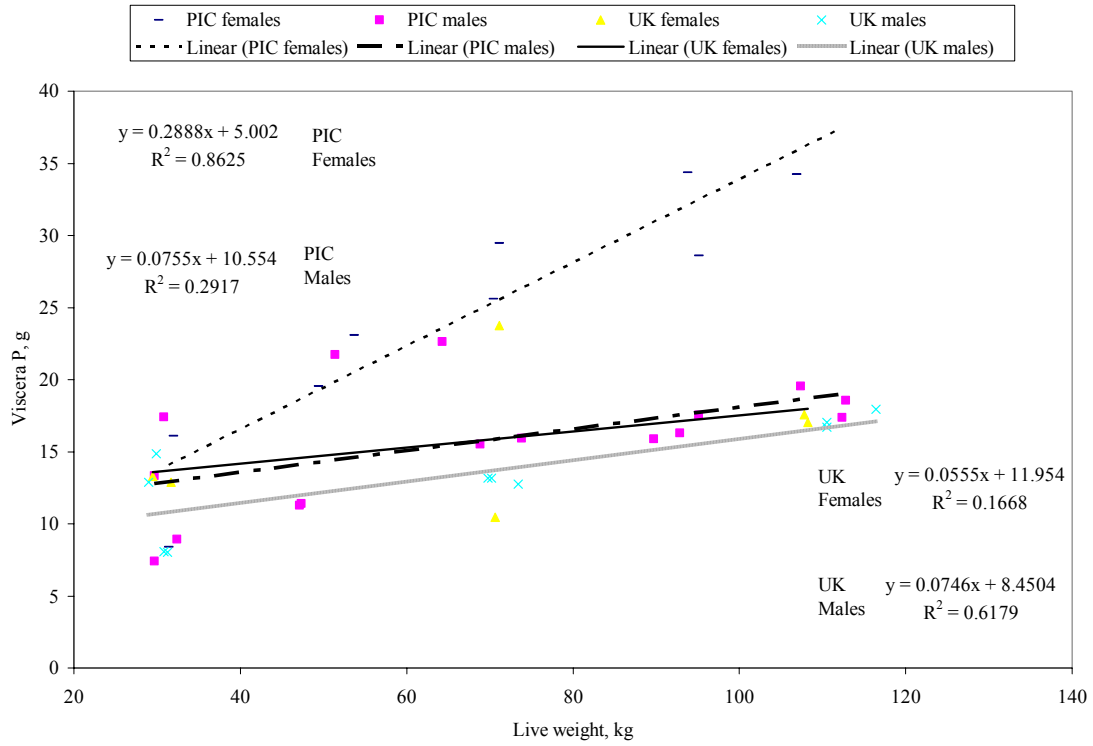


Figure 3.5 – Change in carcass P content in response to increasing live weight in Experiment 2.

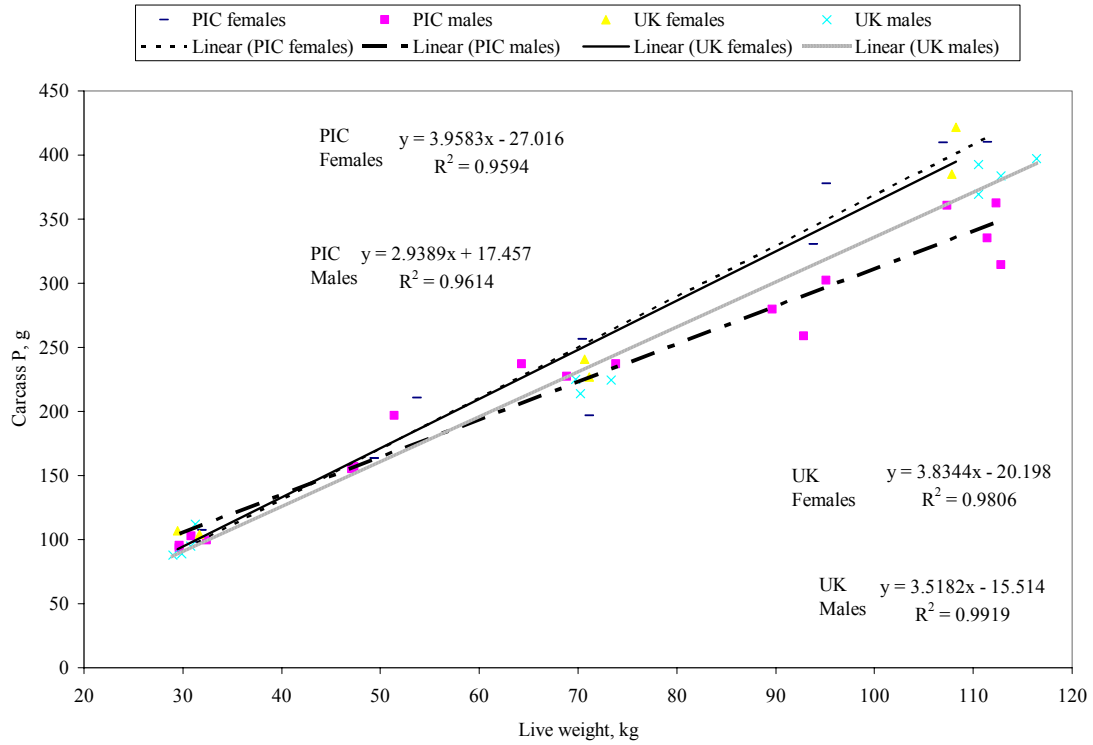


Figure 3.6 – Relationship of WEB N content to WEB P content in Experiment 2.

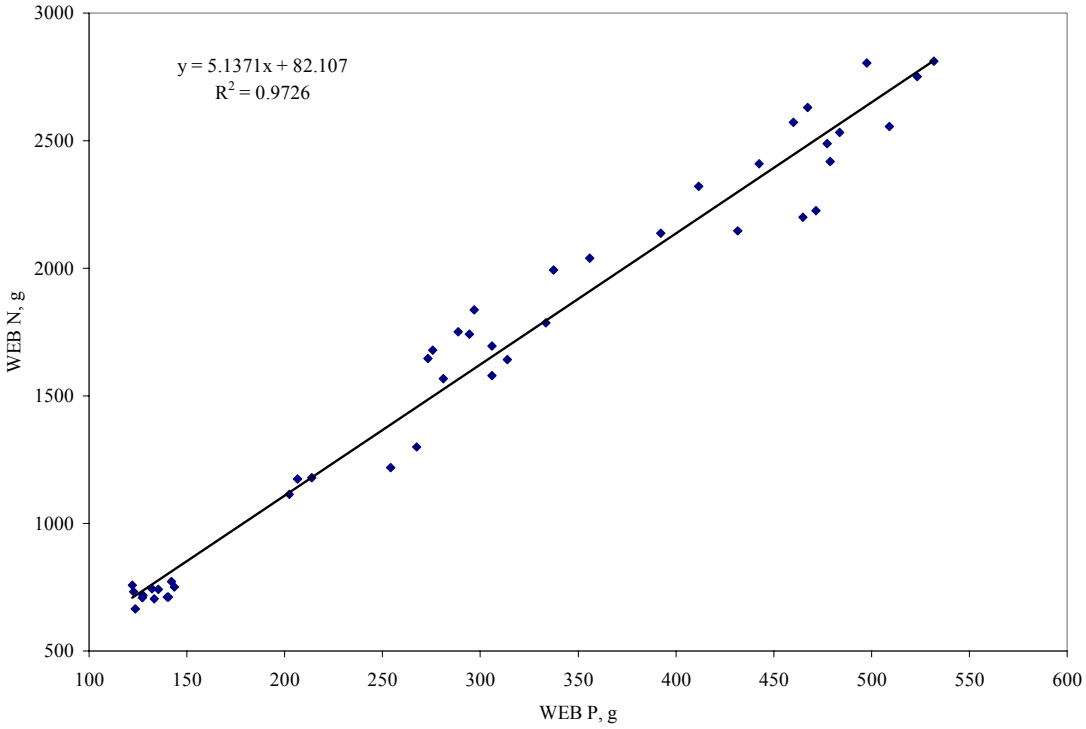


Figure 3.7 – Relationship of WEB P content to WEB ash content in Experiment 2.

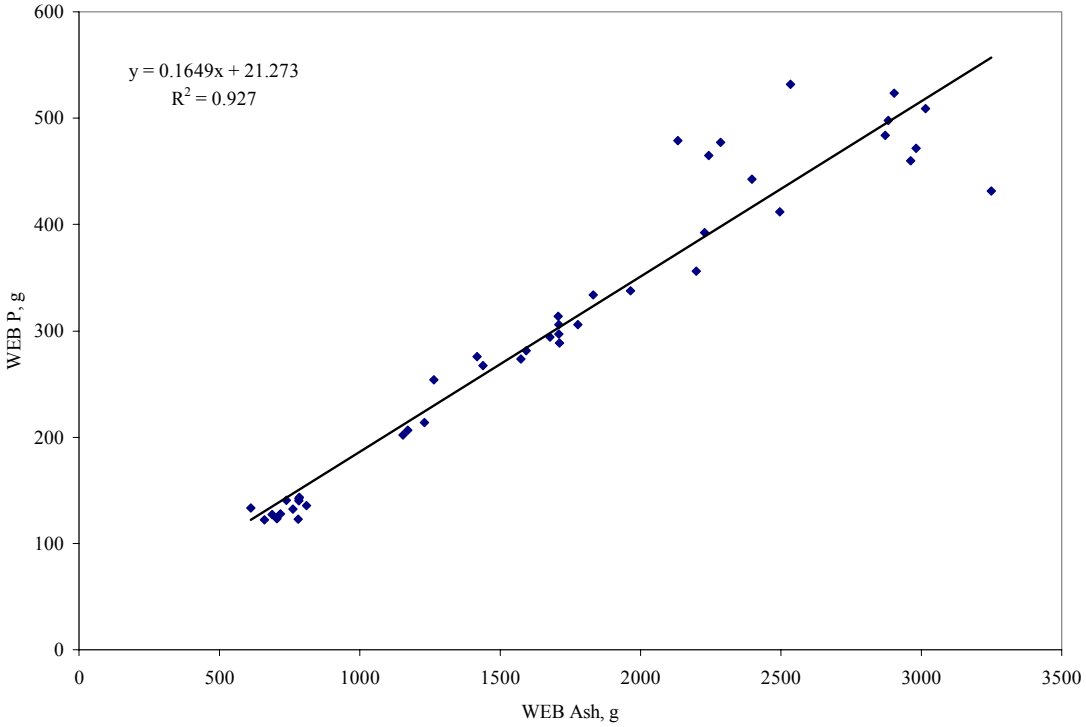
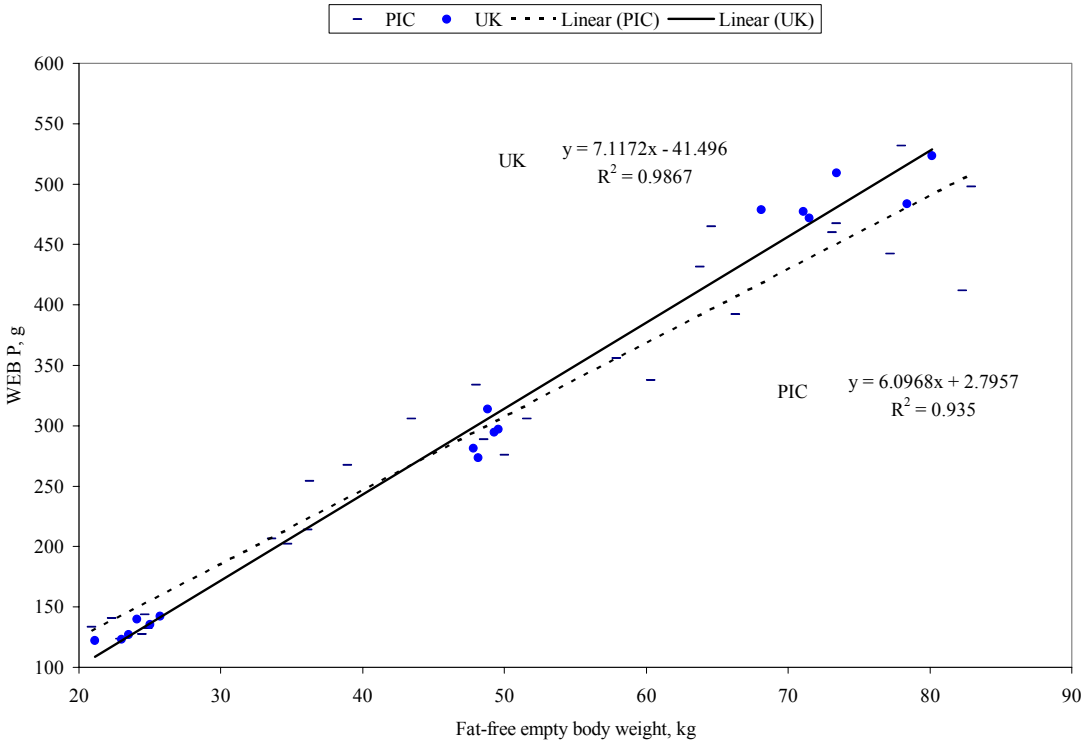


Figure 3.8 – Change in WEB P content in response to increasing fat-free empty body weight in Experiment 2.



APPENDIX I - Description of pig and treatment designations in Experiment 1

ID	Pig #	Collection	Treatment	BW on arrival	Final BW, kg
1	080	1	1	97.4	108.3
2	172	2	1	86.5	115.1
3	176	2	1	87.4	113.7
4	177	1	1	97.4	113.7
5	182	2	1	89.2	109.2
6	258	1	1	98.3	117.8
7	094	2	2	89.2	113.7
8	151	1	2	96.5	104.2
9	209	1	2	99.2	115.5
10	214	1	2	94.7	111.0
11	278	2	2	88.3	113.7
12	281	2	2	87.4	107.8
13	076	2	3	86.5	113.7
14	158	1	3	95.1	108.7
15	218	1	3	92.4	106.0
16	250	1	3	92.4	106.9
17	264	2	3	83.4	101.5
18	275	2	3	88.3	113.3
19	083	2	4	86.1	104.6
20	095	2	4	93.3	114.6
21	184	1	4	92.9	102.8
22	266	1	4	96.9	109.6
23	301	2	4	91.5	115.1
24	303	1	4	93.3	103.7

APPENDIX II - Analyzed total concentration (DM basis) of Fe, Zn, Cu and Mn in the diets in Experiment 1

		Phase[a]			
Mineral Conc.	Treatment[b]	1	2	3	**4[d]**
Fe, mg/kg	1	263	306	264	241
	2	321	337	278	268
	3	251	302	249	215
	4	274	259	291	226
	NRC[c]	67.4	55.8	47.5	40.0
Zn, mg/kg	1	64	77	77	53
	2	67	86	79	61
	3	81	72	51	44
	4	36	68	33	22
	NRC	67.9	58.2	52.3	46.9
Cu, mg/kg	1	23	21	14	20.9
	2	12	14	12	9.9
	3	10	11	8	7.0
	4	7	8	6	3.7
	NRC	4.4	3.8	3.4	3.0
Mn, mg/kg	1				16.1
	2				16.6
	3				12.9
	4				13.0
	NRC	2.4	2.2	2.0	1.9

[a]Phase 1 - diet fed from 18-37 kg BW; Phase 2 - diet fed from 37-55 kg BW; Phase 3 - fed from 55-82 kg BW; Phase 4 - diet fed from 82-118 kg BW. Data of Phases 1, 2 and 3 provided by ISU.

[b]Treatment (TRT) 1 was supplemented with 25% of the commercially recommended (feed mill supplier) levels of Fe as $FeSO_4$, Zn (of which 25% was ZnO and 75% was $ZnSO_4$), Cu as $CuSO_4$, and Mn as $MnSO_4$. TRT 2 contained 50% of commercially recommended levels of Fe, Zn, Cu, and Mn from organic sources (Bioplex™, Alltech Inc., Nicholasville, KY). TRT 3 provided one half of the values of TRT 2. TRT 4 contained no supplemental microminerals and served as a negative control.

[c]NRC – values are extrapolated using their computer model program for predicting nutrient requirements NRC (1998) based on the average weight for the phase in standard conditions (barrows/gilts: 1/1).

[d]Dry matter content was 88.8, 88.1, 88.3, and 89.7% for TRT 1-4, respectively.

APPENDIX III - Description of pig and treatment designations in Experiment 2

ID	Pig #	Sex	Breed	Weight group
1	3003	F	PIC	1
2	3140	F	PIC	1
3	3127	M	PIC	1
4	4154	M	PIC	1
5	4163	M	PIC	1
6	4169	M	PIC	1
7	3117	F	PIC	2
8	3134	F	PIC	2
9	4153	M	PIC	2
10	4165	M	PIC	2
11	4166	M	PIC	2
12	3122	F	PIC	3
13	3131	F	PIC	3
14	3119	M	PIC	3
15	4155	M	PIC	3
16	4162	M	PIC	3
17	3084	F	PIC	4
18	3143	F	PIC	4
19	3180	M	PIC	4
20	4158	M	PIC	4
21	4159	M	PIC	4
22	3128	F	PIC	5
23	3130	F	PIC	5
24	4152	M	PIC	5
25	4160	M	PIC	5
26	4161	M	PIC	5
27	4164	M	PIC	5
28	3111	F	UK	1
29	3174	F	UK	1
30	3117	M	UK	1
31	3158	M	UK	1
32	3176	M	UK	1
33	3225	M	UK	1
34	3173	F	UK	3
35	3225	F	UK	3
36	3159	M	UK	3
37	3174	M	UK	3
38	3222	M	UK	3
39	3172	F	UK	5
40	3221	F	UK	5
41	3116	M	UK	5
42	3157	M	UK	5
43	3172	M	UK	5
44	3221	M	UK	5

APPENDIX IV – Physical Separation of Carcass Tissues

1. Remove carcass from freezer and allow to cool overnight. Keep carcass wrapped in plastic and placed on a metal pan so that all drip loss can be quantitatively collected the next day. The room for thawing should be no warmer than 10-15 °C.
2. Use a standard, sharp, deboning knife for most dissection. Protection (metal glove) on the hand opposite the cutting hand should be worn, as slipping of the knife during skinning and deboning is common.
3. Remove large muscle groups from the carcass if possible. Large cuts of tissue are preferred to prevent water loss during the separation and in the freezer prior to grinding.
4. Bones should be separated from each other at the joints. Care should be taken to prevent removal of bone processes, especially of the vertebrae. Cartilage should be kept with bone, with particular care of the costal cartilage of the ribs, the scapular cartilage, and the cartilage of the manubrium and xiphoid process. To remove all soft tissue from each bone, scissor can be used, which allow a close cut to be made, but not allow cuts or gashes into the bone. All tissue should be removed between the processes of the vertebrae.

APPENDIX V - Quimociac Reagent Preparation

The following steps should be followed to prepare 1 L of reagent:

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

APPENDIX VI – Weight group averages and equations used to calculate initial values of WEB components in PIC pigs in Experiment 2

	Weight group Averages				
Sex	30	50	70	90	110
Gilts, kg	31.65	51.53	70.78	94.45	109.17
Barrows, kg	30.65	48.62	69.00	92.56	110.99
Component / Sex	Equations				R^2
EBW, gilts and barrows	$y = 0.9588x - 1.5716$				0.9944
N, gilts	$y = 25.46x - 83.273$				0.9835
N, barrows	$y = 21.567x + 96.636$				0.9731
Fat, gilts	$y = -0.0021x^2 + 0.5613x - 11.891$				0.9721
Fat, barrows	$y = 0.3009x - 4.1207$				0.9544
Ash, gilts and barrows	$y = 23.652x + 23.387$				0.9526
P, gilts	$y = 4.8801x - 20.199$				0.9712
P, barrows	$y = 3.7448x + 28.117$				0.9644

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VITA

The author was born in Zaragoza, Spain on March 15, 1979 and raised in La Puebla de Hijar, Teruel, Spain. In 1997 he enrolled at University of Zaragoza, where he received his degree in Veterinary Science in 2002, majoring in Animal Production and Economy. He worked as a veterinarian at a beef cattle farm before enrolling the University of Kentucky in 2004, to pursue a M.S. with a focus in swine nutrition. He has authored or coauthored 1 peer-reviewed journal article and 3 abstracts presented at regional or national scientific meetings.

Aitor Balfagón-Romeo