REASSESSMENT OF BONE PARAMETERS AND EVALUATION OF A
BONE BIOPSY TECHNIQUE FOR DETERMINING CALCIUM AND
PHOSPHORUS STATUS OF SWINE FROM WEANING TO MARKET

by

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(ABSTRACT)

Three trials, involving 300 pigs were conducted to
evaluate the effect of feeding 70 to 130% of the NRC
recommended Ca/P levels from weaning to market weight on
the relationships between measures of Ca and P status of
postweaning swine, and to derive response surfaces relating
diet and time effects to observed performance and bone
characteristics. Pigs were slaughtered every 4 wk
following start of the trials to obtain third and fourth
metacarpals and metatarsals. Asymptotic response surfaces,
relating the effects of dietary Ca/P level and time on test
to the observed performance or bone characteristics were
derived. The estimated lack of fit was significant for
many criteria, although the magnitude of difference in fit
appeared to be very small; therefore, the asymptotic
response surfaces were found to reflect well the response
of performance and bone criteria to dietary Ca/P levels of
70 to 130% of NRC recommendations over the period from
weaning to market. The Ca/P level associated with 95 and
98% of maximum bone length, wet weight, radius and dry fat-

free ash percentage of bones appeared to be the same or lower than that required to maximize body weight, average daily gain and feed intake; the performance criteria reached near maximum for Ca/P levels approximating the NRC recommendations. Bone wall thickness, cross-sectional area, bending and shear force, bending and shear stress, extracted weight of bone, and dry fat-free ash weight appeared to require higher Ca/P levels than recommended by NRC to reach 95 or 98% of maximum. Seventy-five pigs were biopsied at 4 wk intervals, and a biopsy sample was also taken from 225 pigs at slaughter, to evaluate a bone biopsy procedure for use as a live-animal sampling method in swine nutrition studies. Biopsy cores from the live and the slaughter pigs were similar, indicating that repeated sampling of the live animal did not significantly alter the composition of the biopsy core. Biopsy core measures were significantly correlated with intact third and fourth metacarpal and metatarsal bone measures. Comparisons of the least squares means and standard errors of biopsy core and bone dry fat-free ash percentage indicated that the biopsy procedure may be more useful when NRC recommended or higher Ca/P levels are fed. There was strong indications, however, that use of the biopsy procedure warrants further consideration.

, my fellow graduate students, and the undergraduate student helpers were the backbone of the Blacksburg phases of the project. Additionally, I would like to thank those special fellows at the Tidewater Research Station who filled this same role there:

,   ,   ,   and

.

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To my "adopted grandma",   , I would like to be able to give more than just a simple thank you. You have been there for me no matter what or when; you have forgiven me so many times when I have gotten so wrapped up in myself that I seemed to have forgotten you; in short, you are a very special person and I hope you know that you mean a lot to me.

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I would like to dedicate this work to my family,   ,   and to the memory of my grandparents, and   , and my "adopted grandpa",   .

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CHAPTER 1

INTRODUCTION

The calcium (Ca) and phosphorus (P) requirements of weanling, growing and finishing swine, as suggested by the NRC (1979), have been challenged in recent years as inadequate for maximization of bone development in the animals (Guèguen and Perez, 1981; Maxson and Mahan, 1983; Nimmo et al., 1981; Reinhard et al., 1976; van Kempen et al., 1976). However, maximization of growth and feed efficiency has been shown to occur at levels of Ca and P in the diet lower than those required for maximization of bone parameters (Crenshaw, 1986; Cromwell, 1979; Miller and Kornegay, 1983). Indeed, to date there has been no conclusive evidence that maximization of bone development is necessary, particularly in the case of animals fed to slaughter (Guèguen and Perez, 1981; Kornegay and Thomas, 1984; Miller and Kornegay, 1983). Levels of Ca and P which maximize performance in growing-finishing swine appear to be adequate for normal mobility in animals maintained for slaughter.

The Ca and P status of growing-finishing animals in recent studies, however, has been evaluated only on the basis of Ca and P levels that result in maximization of

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bone development. Mineral availabilities, particularly for
P, have also been determined on the basis of maximization
of bone parameters. In the case of evaluation of the
availability of P in a mineral supplement, for example, the
determination is made at levels above those where growth
has maximized (Figure 1). This procedure results in
evaluation of the availability of P levels in excess of
those required to obtain optimal performance.

The values obtained may not, therefore, reflect
results which would be obtained at the lower levels of Ca
and P associated with optimal performance. Therefore, it
would be desirable to establish a data base of the values
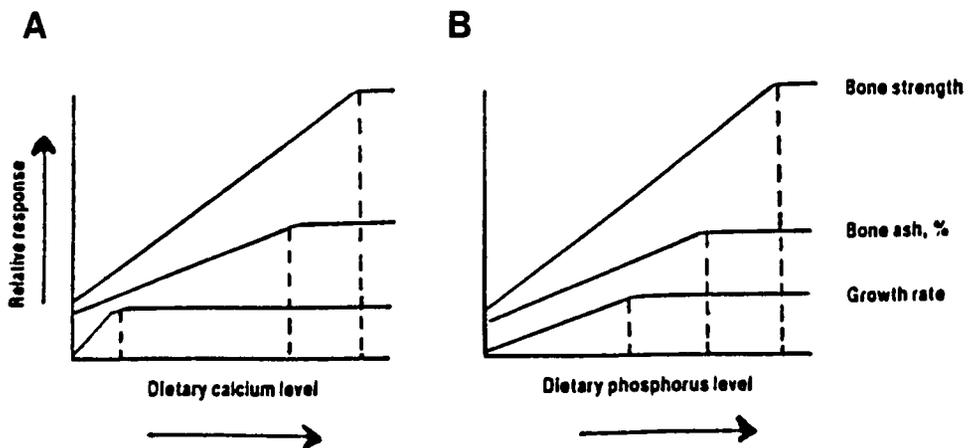
Figure 1. Relative effects of Ca (A) and P (B) on growth
   rate, bone ash and bone strength of growing-finishing
   pigs. (Adapted from Cromwell, 1979).

for bone characteristics at dietary levels of Ca and P that more closely reflect the levels required for optimal performance.

Additionally, measuring the Ca and P status of swine, or determining the Ca or P availability in feedstuffs or diets, depends heavily on techniques involving sacrifice of the animal to obtain bone samples for analysis. Ash, Ca and/or P content of bone, or breaking strengths of the metacarpals, metatarsals, femurs or humeri are often used for these determinations. The great variability inherent in the values obtained for these bone parameters necessitate use of large numbers of animals in order to detect true differences in dietary treatments or availability of Ca or P in a feedstuff or mineral supplement. Consequently, there is a high cost associated with obtaining and maintaining the necessary animals, each of which only provides one data point and whose carcasses have little or no salvage value if the measurements are obtained before the animal reaches market weight.

Therefore, a more desirable technique for determining the Ca and P status of swine would be one that (1) does not interfere with the growth or marketability of the animal; (2) allows repeated measures of the same animal, thereby reducing the number of animals required for growth studies of market animals; and (3) provides a reliable, repeatable

measure of the Ca and P status of the animal which is comparable to those obtained by conventional techniques.

Bone biopsy is a technique that has not been used in this type of research, but on preliminary consideration may provide a more desirable technique for evaluating Ca and P in growing-finishing swine. The use of the bone biopsy technique for this application must be validated in terms of the characteristics listed above. Validation would allow this technique to be used for purposes of determining requirements of the animal for maximum growth or bone development or for use in assessing the availability of Ca or P in an organic or inorganic compound for use in swine rations.

Based on these considerations, the objectives of this study were three-fold. The first objective was to determine relationships among measures of the Ca and P status of animals receiving various levels of Ca and P in the diet. The second objective was to establish response curves for use in evaluation of Ca and P status of postweaning swine over a wide range of dietary Ca and P levels during the period from weaning to market. The final objective was to evaluate bone biopsy techniques as a means of assessing the Ca and P status of the pig, at any stage of growth.

CHAPTER 2

REVIEW OF LITERATURE

Introduction

A good deal of research has been published regarding
the function, requirements and interactions of Ca and P in
swine nutrition (Kornegay, 1986). Yet, some questions
still persist, particularly in regard to the Ca and P
requirements of swine at different levels of production.

Brown et al. (1972) demonstrated that maximum bone
formation in swine occurs during the first 12 weeks of
life. These first weeks of life are also a period of
maximum rate of muscle development. Bone is the earliest
developing tissue in the young animal, followed by muscle,
then fat tissue. Inappropriate levels, or use of
unavailable sources, of the minerals and protein required
for the formation of bone or muscle tissues in the young
pig can result in malformation of either or both tissues.
Inadequate Ca and P in the diet of the young pig results in
poor performance and poorly mineralized (osteoporetic) bone
tissue. Kornegay (1986) summarized early research results
where it was established that a deficiency of P does not
alter the P content of the soft tissue, with P needs for
soft tissue development being met at the expense of bone

mineralization. Inclusion of recommended levels of Ca and P in the diet of the young pig, accompanied by high protein levels, results in continued muscle growth but osteoporetic bone tissue; i.e. soft tissue formation is occurring at the expense of bone mineralization. As the animal ages, the rate of bone formation declines, while the rate of muscle and fat accretion remain at high levels, suggesting the Ca and P requirements may be relatively less critical later in life than they are with young swine. In fact, compensation in bone mineralization has been shown to occur in later growth periods when inadequate levels of Ca and P were provided in earlier growth periods (Fammatre et al., 1977). Other effects on Ca and P utilization in the pig have also been identified.

In light of the many and varied aspects of Ca and P nutrition in swine, a review of the literature on a number of topics will be undertaken. The more current research regarding the requirement of weanling, growing and finishing swine will be reviewed first. Then, a section reviewing the function and metabolism of Ca and P, hormonal regulation of Ca and P, and factors affecting Ca and P metabolism and requirement will be presented. The effects of Ca and P on other nutrients and physiological conditions in swine will then be reviewed. The next section will summarize the availability of Ca and P in feedstuffs and

inorganic mineral supplements. Following that, the effects of Ca and P, and time, on performance (gain and feed efficiency) will be reviewed. The next sections will deal with the effects of time, Ca and P on bone criteria, including bone ash, bone ash Ca and P contents, and bone strength. Alternatives to slaughter, for purposes of recovering bone data, will be summarized in the final section of this literature review.

Requirement of Weanling, Growing and Finishing Pigs for Calcium and Phosphorus

The requirement of the pig for Ca and P are closely related because of the need to maintain the ratio of Ca to P within very narrow limits, at approximately 1.3:1 (Moser, 1978). The absolute requirement of Ca, however, is considered by many to be less critical than the requirement for P due to availability and cost concerns with P (Kornegay, 1986). Requirement estimates in recent years are higher than those obtained by early workers, due in large part to the better definition of requirements for all minerals today; i.e. growth was likely limited by nutrients other than Ca and P in the early work, therefore, researchers concluded the Ca and P requirements were lower than is the case when performance is allowed to come closer to its potential (Hays, 1976). Therefore, this review will consider only estimates of requirements made within the

last 25 years.

The NRC recommendations (1979) for dietary Ca and P levels for growing-finishing swine are based primarily on adequate dietary levels to attain optimum weight gain and not maximum skeletal mineralization (Maxson and Mahan, 1983). In 1981, Kornegay and Thomas concluded that although there was probably no need to maximize bone development in market hogs, the dietary levels of Ca and P required for development of an adequate skeleton for market hogs was still unclear.

The phosphorus requirement of growing-finishing swine, in particular, has received considerable attention over the years. This is due in part to the high cost of supplementing P in the diet as compared to the cost of providing Ca (Kornegay, 1986). In addition, the levels of phosphorus that were adequate for pigs in the past may not be sufficient for today's pig (Cromwell, 1985). Due to the low levels of available phosphorus in the typical corn-soybean meal diet (Beeson, 1960), supplementation of inorganic phosphorus to the ration is necessary. NRC (1979) recognized the issue of differing P availability in feedstuffs; in part their recommendations were designed to assure adequate levels of inorganic P in the corn-soybean meal diet. However, many additional trials since then have been devoted to evaluating the level of supplemental

inorganic phosphorus required to obtain desirable performance in swine, both on corn-soybean meal diets and when other cereal grains are used.

The NRC (1979) recommendations for weanling, growing and finishing swine list suggested levels of Ca and P in the diet according to the weight of the animal. The absolute amount of Ca and P required per pig per day increases as the animal grows. The percentage Ca and P in the diet needed by the pig, however, decreases at heavier body weights; the increased feed intake associated with heavier body weights results in an increase in absolute intake of Ca and P, even though the percentage in the diet is decreasing. The dietary levels recommended (NRC, 1979) are .80% Ca and .60% P for the period from 5 to 10 kg; .65% Ca and .55% P for pigs weighing 10 to 20 kg; .60% Ca and .50% P for weights of 20 to 35 kg; .55% Ca and .45% P for the period from 35 to 60 kg; and .50% Ca and .40% P fed from a weight of 60 kg until market weight.

These values are generally considered minimums, and the actual level fed can be influenced by a number of factors, including: ingredient quality; availability of Ca and P in the ingredients fed; the performance potential of the animal; protein and energy content of the feed; stress due to disease, overcrowding, poor ventilation or inadequate temperature control; compensatory growth;

ingredient and/or nutrient interactions; and variability in animal performance and in management. Many of these factors will be discussed later in regard to their effects on Ca and P metabolism. A brief review here, however, of research concerning the adequacy of NRC-recommendations for swine is in order.

Changes in NRC-recommendations for Ca and P from 1968 to 1988

The NRC-recommendations for Ca and P in swine diets have changed very little over the last 20 plus years. Ca and P recommendations were the same for the 1968 and 1973 editions (Table 1). For the 1979 edition, the P recommendations were increased from .50 to .55% of the diet for the 10 to 20 kg pig, and from .40 to .45% of the diet for the 35 to 60 kg BW class. Ca recommendations were decreased from .65 to .60% of the diet for 20 to 35 kg

Table 1. Changes in NRC Ca and P recommendations from 1968 to 1988, expressed as the percentage of Ca/P in the diet by body weight range.

	Body weight, kg						
Year	5	10	20	35	50	60	100
1968	.80/.60	----- .65/.50 -----		------ .50/.40 -----			
1973	.80/.60	----- .65/.50 -----		------ .50/.40 -----			
1979	.80/.60	.65/.55	.60/.50	- .55/.45 -		.50/.40	
1988	.80/.65	.70/.60	--- .60/.50 ---		--- .50/.40 ---		

pigs, and increased from .50 to .55% of the diet for the 35 to 60 kg BW class (NRC 1968, 1973, 1979).

In the newest <u>Nutrient Requirements of Swine</u> (NRC, 1988), the P recommendations for 5 to 10 kg, and the Ca and P recommendations for the 10 to 20 kg pig were increased by .05 percentage points (Table 1). The weight classes used to define Ca and P recommendations for swine over 20 kg were also redefined in the 1988 edition. Dietary levels of .60% of the diet are recommended for 20 to 50 kg pigs, and .50% Ca in the diet is recommended for pigs from 50 to 100 kg; the corresponding P recommendations are .50 and .40% P in the diet, respectively

For purposes of the following discussion on the adequacy of the NRC-recommendations for weanling, growing and finishing swine, the results of the research summarized have been considered on the basis of the levels used in comparison to the 1979 NRC Nutrient Requirements of Swine. In light of the foregoing discussion, the conclusions drawn by the individual researchers in regard to the adequacy of the NRC-recommendations in use at the time the research was conducted would be very similar to the conclusions they would have made if the 1979 NRC Nutrient Requirements of Swine were used. Therefore, unless otherwise indicated, references to NRC-recommendations refer to the 1979 NRC-

recommendations.

Ca and P Requirements of Weanling Swine

Coalson et al. (1972), Mahan et al. (1976b) and
Schiefelbein et al. (1979) suggested that higher than NRC-
recommended dietary Ca and P levels are required for
weanling swine, particularly to enhance bone
mineralization. Coalson et al. (1974) and Guéguen and
Perez (1981) have both suggested that the Ca level in the
diet of the weanling pig should be higher than NRC-
recommendations. Others have found the NRC-recommendations
provide for optimal or near optimal performance (Chapman et
al., 1962; Mahan, 1982; Mahan et al., 1980; Reinhart and
Mahan, 1985; Rutledge et al., 1961; Zimmerman et al.,
1963). From these studies, it would appear that the NRC-
recommendations are adequate for performance, and will
provide for good bone mineralization, although higher
levels of Ca and P would be required to maximize bone
measures.

Ca and P Requirements of Growing-Finishing Pigs

Most researchers report that adequate performance is
obtained when NRC-recommended Ca and P levels are fed for
the entire growing-finishing period (Allee et al., 1974;
Crenshaw et al., 1981b; Cromwell et al., 1970, 1972; Doige
et al., 1975; Fammatre et al., 1977; Greer et al., 1977;
Harmon et al., 1970b; Hays et al., 1970; Hines et al.,

1979; Irlam et al., 1974; Kornegay et al., 1981a; Lepine et al., 1985a; Maxson and Mahan, 1983; Miller et al., 1981; Moser, 1978; Nimmo et al., 1980a,b; Pierce et al., 1977; Pond et al., 1978a; Reinhart and Mahan, 1985; Scherer et al., 1970; Schroeder et al., 1974). Many of these researchers concluded that higher levels of Ca and are required to maximize bone strength and mineralization (Crenshaw et al., 1981b; Cromwell et al., 1979; Fammatre et al., 1977; Hines et al., 1979; Kornegay and Thomas, 1981; Kornegay et al., 1981a; Lepine et al., 1985b; Maxson and Mahan, 1983; Moser, 1978; Reinhard et al., 1974, 1976; Ross et al., 1984; Schroeder et al., 1974).

Kornegay (1986) used data sets of performance and bone criteria (daily gain, gain:feed ratios, bone breaking strength, and dry, fat-free bone ash) from a number of research reports, since 1969, to fit asymptotic response curves for the effects of dietary Ca/P level on these criteria. Dietary Ca/P levels were expressed as a percentage of the NRC (1973) recommendation, and the Ca/P level associated with 97, 98 and 99% of maximum response for each criteria was estimated. Using the combined data from many studies, he found that 97 to 98% of maximum response for daily gain, efficiency and bone ash could be obtained with the NRC-recommended Ca and P levels, but that maximization of bone strength required about 128% of the

NRC-recommended dietary Ca and P levels.

Some reports in recent years have indicated that the
NRC Ca and P recommendations for growing-finishing swine
are not high enough, particularly for developing boars,
which are fed according to the recommendations for growing-
finishing animals. Based on suggestions of higher
requirements of boars and/or gilts for Ca and P, the
enhanced mineralization and strength of bones on higher Ca
and P diets, and the desire to increase structural
soundness in animals maintained for the breeding herd,
several researchers have suggested that the requirements of
developing boars and gilts need to be increased somewhat
(Cline, 1981; Cromwell, 1985; Cromwell et al., 1979; Kesel
et al., 1983; Kornegay and Thomas, 1981; Moser, 1978; Nimmo
et al., 1981; Schroeder et al., 1974). In studies where
enhanced bone mineralization in developing boars due to
higher than NRC-recommended levels of Ca and P was noted,
and where structural soundness was scored, researchers did
not find that feeding high Ca and P levels resulted in
improvement in soundness scores (Cline, 1981; Crenshaw et
al., 1981b; Kornegay and Thomas, 1981; Kornegay et al.,
1981a, 1983; Lepine et al., 1985a,b; Miller et al., 1981;
Nimmo et al., 1980a,b).

Others have suggested that the NRC P recommendations,
in particular, were not high enough for breeding herd

replacements (Cline, 1981; Newman and Elliot, 1974; van Kempen et al., 1976). Hays (1976), in discussing estimation of the P requirement for growing-finishing pigs, concluded that those researchers that start with lighter weight pigs estimate the requirement to be higher than expected. The dietary P allowances he recommended were, in general, higher than the minimum requirements estimated by the NRC committee. He attributed this to the need for a safety factor to account for differences in biological availability and the variability in the estimates on which the recommendations were made.

If, in fact, we do need to increase the Ca and P recommendations for developing boars and/or gilts, it poses the question as to how that should be accomplished. Recommendations for growing-finishing swine are currently used as guidelines for developing boars and gilts. This arises from the fact that many producers raise animals destined for the breeding herd with animals destined for slaughter due to space limitations. Additionally, final selection of gilts for the breeding herd is often not made until the gilts are nearing the end of the finishing period. As indicated earlier, maximization of bone mineralization is probably not necessary in growing-finishing swine. Therefore, it appears the most efficient solution would be to develop nutrient recommendations for

animals that will be retained for the breeding herd
separately from those animals being raised for slaughter.
In response to this need, the latest revised NRC-
recommendations (1988) list separate Ca and P
recommendations for breeding herd replacements, as well as
recommended levels for energy, crude protein and lysine for
these animals.

In conclusion, there appears to be little evidence
that the Ca and P levels recommended by the National
Research Council (1979) are inadequate for weanling,
growing and finishing pigs raised for slaughter.

Function and Metabolism of Calcium and Phosphorus

It is beyond the scope of this review to completely
summarize what is known about the functions and metabolism
of Ca and P. Therefore, only a brief summary of the
function and metabolism of these minerals will be included.
Following this, short summaries on hormonal controls on Ca
and P, and the factors affecting Ca and P metabolism will
be presented.

<u>Functions of Ca and P</u>

Ca is the most abundant mineral in the body,
accounting for 1 to 2% of total body weight. P is a close
second, making up .7 to 1.2% of total body weight. Ca and
P are closely linked due to the joint role they play in

development and maintenance of the skeletal system.
Approximately 99% of the total body Ca and 75 to 80% of the
total body P is located in the skeleton, with the balance
of these two minerals located in the soft tissues. The
effects of dietary Ca and P levels are quantified on the
basis of changes in bone characteristics in most studies.
The ratio of Ca and P in the bone tissue remains very
constant, at approximately 2:1, while the total body Ca to
P ratio is approximately 1.66:1. In addition to the
critical role bone tissue plays in the skeletal support of
the animal, bone tissue also serves as a reservoir of Ca
and P, and of other nutrients as well, making normal bone
development a priority for the animal's growth (Cromwell,
1982; Harrison, 1984; Hays, 1976; Kornegay, 1986; Schuette
and Linkswiler, 1984; Wasserman, 1960). Although Ca and P
are closely interrelated in structural support of the body,
both minerals also have very critical functions by
themselves.

Functions of Ca. Ca is required for the conversion of
prothrombin to thrombin; therefore, it plays a critical
role in blood clotting. Additionally, Ca serves to
activate or stabilize the activity of several enzymes. Ca
is required for promoting normal contraction of smooth,
skeletal and cardiac muscle. Ca also functions to maintain
normal excitability in nervous tissue and aid in

transmission of nerve impulses. Ca is also thought to be
necessary for the maintenance of a normal intracellular
environment; to aid in the development of bioelectrical
potential at the cell surface; for maintenance of selective
permeability in various membranes; and for the integrity of
intracellular cement substances. Along with K and Na, Ca
helps to regulate the heartbeat. Ca is known to be
involved in the secretion of a number of hormones and
hormone-releasing factors. In addition to these specific
roles, Ca is required for milk production, weight gain,
feed utilization and all other types of livestock
productivity (Cromwell, 1982; Peo, 1976; Schuette and
Linkswiler, 1984; Wasserman, 1960).

Functions of P. According to Hays (1976), P has more known
physiological functions than any other mineral element. P
is involved in most, if not all, metabolic reactions in the
body. In soft tissues, P is a component of many organic
compounds, such as phospholipid, nucleic acids,
phosphorylated energy transfer compounds (acetyl phosphate,
creatine phosphate, adenosine di- and triphosphates,
nicotinamide-adenine dinucleotide phosphate, etc.), and
phosphorylated metabolic intermediates (glucose-6-
phosphate, glycerol phosphate, 3-phosphoglyceric acid,
etc.). Through these compounds and others, P functions in
energy transfer, carbohydrate metabolism, protein

metabolism, fat metabolism, genetic transmission, control
of cellular metabolism, formation of cell membranes,
maintenance of cell permeability, and maintenance of
osmotic pressure and acid-base balance. In situations
where P is deficient, synthesis of protein, fat and
carbohydrates are limited and bone and teeth development is
depressed, leading to an overall reduction in the
performance of an animal; the reduction is probably more
severe than can be produced by deficiency of any other
individual mineral (Cromwell, 1982; Harrison, 1984; Hays,
1976; Wasserman, 1960).

Metabolism of Ca and P

Besancon and Guéguen (1969) monitored the metabolism
of Ca in 30 to 40 kg pigs by isotope dilution techniques,
following a single intravenous injection of 2 to 3 μCi
^{45}Ca. The true utilization of feed Ca was 45%, while
retention was 31% of the amount ingested. Endogenous Ca
was found to account for only 17% of the fecal Ca
excretion, while only negligible amounts were excreted via
the urine. The methods they used allowed them to estimate
that resorption of bone tissue was 68% of the level of
total accretion, with 46% of the accretion due to Ca of
endogenous origin. From these components, they were able
to estimate that the total exchangeable Ca pool was 24 g.

Hansard and coworkers (1961) evaluated the absorption,

excretion and utilization of Ca in pigs from 10 days to 72 mo of age. They found that total fecal Ca excretion increased with increasing age and weight. They associated the increased fecal losses with age to decreased absorption, changes in the proportion of exchangeable bone, changes in vascularity and blood flow, hormonal changes and increased loss of Ca from body stores. Hansard et al. (1961) found the 5 mo old pig absorbed 53% of the ingested Ca, but only 48% of the ingested Ca was retained. The corresponding values in the 2 week old pig were 99% absorption and 98% retention. The rapid decreases in absorption and retention from 2 weeks to 5 mo of age were followed by slower decreases to full mature age. Addition of excess levels of dietary Ca did not compensate for the high fecal loss or change endogenous values significantly. Based on the results of slaughter studies, Nielsen (1972) found that pigs retained about 54% of the ingested Ca and 50% of the ingested P.

Moore and Tyler (1955) found absorption of Ca was most active from the proximal fourth of the small intestine, while absorption of P was most active in the proximal half. The solubility of Ca and P was greatest in the areas of the small intestine where absorption was greatest. Ca and P was shown to be secreted into the lumen in the upper small intestine, but reabsorption of these minerals occurred in

the lower segments of the small intestine. No secretion of

Ca and P into the large intestine was detected, and only

small amounts of endogenous Ca and P were found. It was

concluded that any fecal excretion of endogenous Ca and P

was due to incomplete reabsorption of the Ca and P secreted

into the small intestine. Partridge (1980) found that,

although the main site of absorption of Ca and P was the

small intestine, the relative importance of the regions

anterior and posterior to the mid-jejunum differed

depending on the type of diet fed.

Wasserman (1960) reported Ca is transported against a

concentration gradient by an energy-requiring active

transport system that can be inhibited by metabolic

inhibitors (NaCN, $NaNO_3$, NaF, $HgCl_2$, 2,4-dinitrophenol,

etc.), Mg or Co ions. Vitamin D is required for the active

transport of Ca, probably functioning through inducement of

Ca binding protein as well as Ca activated ATPase.

Additionally, Ca can also be absorbed by a passive ionic

diffusion, a process that may also require vitamin D, but

the active transport system largely accounts for most of

the uptake of Ca from the gut (Schuette and Linkswiler,

1984).

P absorption, like that of Ca, is largely via a

sodium-dependent active transport system, although passive

diffusion is possible. Vitamin D enhances the absorption

of P as well as Ca, and the effects of vitamin D on P
absorption are separate from its effects on Ca absorption.
Probably the largest single influence on P absorption in
the pig is the content of phytic-P in the diet, since the
utilization of phytic-P is limited in the pig; phytate can
form Ca-phytate salts which render Ca unavailable as well
(Harrison, 1984).

Five week old Large White pigs (n=14) were given an
isotonic solution of Ca chloride with ^{45}Ca (100 μCi·kg^{-1}
BW) intraperitoneally. They were killed after 30 min; 3, 6
or 24 h; or 2, 4 or 8 days. Although ^{45}Ca disappeared
rapidly from the blood, a small amount was still present at
8 days. There was more ^{45}Ca in the epiphysis of the femur
and humerus than in the diaphysis. Radioactivity was also
high in nasal bones (Mihai and Paun, 1982).

Comar et al. (1952) used autoradiographic studies to
show that the areas of periosteal origin in bone were
regions of slow deposition and resorption, while
endochondral bone rapidly exchanged Ca, P and Sr in
weanling pigs averaging 9 to 13 kg. In this rapidly
growing class of swine, they also found that the
assimilation by the bone of the radioactive Ca within 1
hour following carotid injection of the labeled Ca could be
due to exchange of the Ca with that previously in the bone,
formation and subsequent mineralization of new bone or a

combination of these two processes. Formation of 200 mg
new bone per hour would be required for bone formation to
account for all the entry of [45]Ca into bone, a reasonable
value for this age pig (Comar et al., 1952).

Hormonal Control of Ca and P Metabolism

Vitamin D. Vitamin D occurs in two forms in nature.
Vitamin D_2 (ergocalciferol) is of plant origin, and vitamin
D_3 (cholecalciferol) is produced by ultraviolet radiation
in the skin of mammals. Vitamin D_3 is used preferentially
by the feed industry as a dietary supplement, since all
species can utilize this form (Kornegay, 1986).

Whether absorbed from the gut or produced in the
animal itself, vitamin D must undergo two conversions to
yield the active form of the vitamin that functions in
metabolic regulation of Ca and P metabolism. DeLuca (1974)
described this activation process, whereby vitamin D is
first hydroxylated in the liver to 25-hydroxy-
cholecalciferol. This form is the major circulating
metabolite of vitamin D. Before the animal can use it for
metabolic reactions, the 25-hydroxy-cholecalciferol is
further hydroxylated in the kidney to 1,25-dihydroxy-
cholecalciferol. This renal hydroxylation is stimulated by
parathyroid hormone. Additionally, low serum P is also
thought to directly stimulate synthesis of 1,25-dihydroxy-
cholecalciferol, even in the absence of parathyroid hormone

(Hays, 1976; Jongbloed, 1987; Kornegay, 1986).

The principal target tissues of vitamin D are the small intestines, bone, and the kidney. The primary function of vitamin D appears to be enhancement of Ca absorption from the intestinal tract. Vitamin D is essential to the operation of both the Ca and P transport systems. Additionally, vitamin D also seems to enhance the availability of phytate P. Vitamin D increases renal tubular reabsorption of both Ca and P, resulting in increased retention of both minerals. Vitamin D does apparently exert a direct effect on the skeleton as well; in concert with parathyroid hormone, vitamin D stimulates resorption of Ca and P from previously formed bone (Harrison, 1984; Hays, 1976; Jongbloed, 1987; Kornegay, 1986; Pointillart, 1988; Schuette and Linkswiler, 1984; Wasserman, 1960).

Peo et al. (1986) reported that gain and efficiency will seldom be affected by lack of vitamin D, but percentage bone ash and bone strength are often reduced substantially, indicating that bone mineralization and integrity suffer. Pointillart (1988) observed that increases in bone bending moment accompanied supplementation of vitamin D in the diet. Nielsen et al. (1971) reported the rachitic effects of imbalanced Ca:P ratios were not detected when vitamin D sufficient diets

were fed. Levels of vitamin D in excess of 80,000 IU/kg
feed reduce performance, however, and can lead to
calcification of blood vessels, heart, kidney and muscle
(Peo et al., 1986). According to Stone and McIntosh
(1977), vitamin D had little effect on serum Ca and P
levels.

Pointillart et al. (1978a) found that insufficient
levels of vitamin D can affect calcitonin and parathyroid
hormone regulation of calcemia. Thomasset et al. (1979)
reported feeding low-Ca diets promoted intestinal Ca
binding protein synthesis due to the action of vitamin D in
the distal ileum, not normally considered a target tissue
for vitamin D. The increase in Ca binding protein probably
increases intestinal Ca absorption, as a mechanism of
adapting to the low Ca diets.

Taylor (1965) concluded that vitamin D probably
increases the utilization of phytate P in two ways: by
increasing the production of intestinal phytase and by
stimulation of Ca absorption, thus rendering the phytate
more soluble. In contrast, Pointillart (1988) found that
supplementation of vitamin D improves phytate P absorption,
but that the improvement was independent of an increase in
the level or activity of intestinal phytases or
phosphatases. The requirement for vitamin D increases as
the level of phytate P, as a percent of total P in the

diet, increases (Taylor, 1965). A P deficiency, classically associated with high phytate P feeding, is known to stimulate vitamin D metabolism (Pointillart, 1988).

Parathyroid Hormone. Parathyroid hormone is responsible to a large degree for the regulation of serum Ca levels, exerting its effects on the intestine, kidney and bone. Parathyroid hormone is thought to have immediate effects on the bone, but the effect on the intestines may be a delayed action. Parathyroid hormone release is stimulated by low serum Ca levels. Both the 25-hydroxy- and the 1,25-dihydroxy-cholecalciferol forms serve to suppress parathyroid hormone release. Parathyroid hormone directly and/or indirectly enhances absorption of Ca and P from the small intestine. The effect of parathyroid hormone on intestinal absorption may be in large part due to the stimulation of 1,25-dihydroxy-cholecalciferol production. In conjunction with vitamin D, parathyroid hormone increases resorption of Ca and P from bone, with a concomitant resorption of organic matrix; contrary to the effects of vitamin D, however, parathyroid hormone inhibits tubular reabsorption of P from the kidney (Harrison, 1984; Jongbloed, 1987; Kornegay, 1986; Schuette and Linkswiler, 1984).

Parathyroid hormone is thought to be involved in the

generation of the intestinal Ca and P concentration gradients. Parathyroid activity appeared to mirror changes in plasma Ca concentration (Stone and McIntosh, 1977). Plasma parathyroid hormone increases concomitantly as plasma calcitonin levels fall (Pointillart et al., 1978b). Calcitonin. Calcitonin is released in response to elevated serum Ca levels. Its known effects are to rapidly inhibit bone resorption, without affecting bone mineralization, decrease serum Ca and P, and to inhibit P reabsorption from the ascending loop of Henle and the distal convoluted tubule in the kidney. Compared to parathyroid hormone, calcitonin acts as a "fine tuner" in maintenance of Ca and P homeostasis due to its rapid release, action and elimination (Jongbloed, 1987; Kornegay, 1986; Schuette and Linkswiler, 1984).

Other Hormones. Thyroxine has been suggested to increase bone turnover rate, and a thyroidectomy seems to depress the rate of bone growth and differentiation. The effects of thyroxine on bone may be indirect, but through these effects, thyroxine would be affecting Ca and P. Growth hormone stimulates bone formation, but the effect appears to be dependent on thyroxine. Administration of thyroxine and growth hormone has been reported to increase phosphate reabsorption and the activity of the Na-dependent phosphate transport system (Jongbloed, 1987).

Factors Affecting the Calcium and Phosphorus Metabolism of the Pig

Many factors have been identified as affecting the metabolism or requirements for Ca or P. These factors include dietary factors, genetic factors, and physical factors in addition to the hormonal controls discussed earlier. A brief discussion of some of these effects is appropriate, since they could possibly affect the results of a Ca and P investigation. Although Ca and P levels in the diet and Ca:P ratio effects are treated as separate subjects in this review, it should be recognized that in practice, the effects of levels and ratios of Ca and P in the diet are inescapably linked.

Dietary Factors.

Level of Ca and P in the Diet. Vipperman et al. (1974) and Oksbjerg and Fernàndez (1987) found urinary Ca decreased and Ca retention increased as the dietary level of P increased, with no effect on Ca absorption. Increasing Ca content of the diet had the same effects on urinary P excretion and retention. Ca supplementation to 1.2% total dietary Ca resulted in decreased P balance and increased urinary P excretion (Forbes, 1960). To the contrary, Pointillart (1988) found that supplementation of 1.4% Ca in the diet had no effect on P utilization. The total amount of Ca excreted, absorbed and retained increased when the dietary Ca level increased to 1.4% Ca,

but the absorption and retention relative to the level of
dietary intake was higher for pigs fed .9% dietary Ca.
Mudd et al. (1969) reported that the total body Ca and P
and net retention of Ca and P was higher for pigs fed
.94/1.13 (1:1.20 Ca:P ratio) or 1.16/1.47% (1:1.27) than
for pigs fed .30/.38 (1:1.27) or .58/.68% (1:1.17) Ca/P.
Nielsen (1972) reported P excretion in the urine increases
with a decreasing Ca:P ratio or increasing P level, while
urinary Ca increased with an increasing Ca:P ratio or
decreasing P level. Morgan et al. (1969) found that
increasing Zn or Ca in the diet produced a fall in serum P
content. Increasing dietary Ca increased Ca and decreased
P retention, but had no effect on Zn retention. Zinc
supplementation had no effect on the utilization of P by
the growing pigs. Pigs retained about 50% of the Ca and P
ingested.

van Kempen et al. (1976) found that the linear growth
of bones, percentage ash of bones, and the Ca, P and Mg
content of the bones increased as the level of Ca and P in
the feed increased. The Ca:P ratio did not affect the Ca
and P content of the bone, but did affect the Mg content.
As P increased, the hydroxyproline percentage of dry fat-
free bone substance decreased, indicating decreased bone
formation; the decrease occurred at P levels above .5% of
the diet when the Ca:P ratio was 1.3:1, but was linear for

P levels from .40 to .70% of the diet when the Ca:P ratio was 1.9:1. Bone formation increased with increasing dietary Ca level, at constant P in weanling swine (Coalson et al., 1971). Kaantee (1983a,b) also reported the ash content of the third metacarpal bone decreased when Ca in the feed was low. Kornegay (1986) summarized early research results where it was established that a deficiency of P does not alter the P content of the soft tissue, with P needs for soft tissue development being met at the expense of bone mineralization. Nielsen et al. (1971) found the Ca:P ratio in bone ash remained constant even when dietary Ca and P levels were increased from .48 to 1.20% and .40 to 1.0%, for Ca and P, respectively. Similar results were reported by Okoński (1962) when requirement and 2.75 times requirement levels of Ca and P were fed.

Pointillart et al. (1978b) found that high P diets induced temporary modifications in hormonal Ca and P regulation, particularly that calcitonin levels were elevated, that may result in effects on plasma Ca levels. Adaptation appeared to have occurred by the third week, however, suggesting there is probably no long term effect. Pointillart et al. (1979) reported feeding low Ca diets to growing swine induced a simultaneous decrease in plasma Ca and calcitonin, and a marked rise in urinary hydroxyproline, which was indicative of increased bone

resorption. Dietary Ca deficiency over a long period of
time had no marked effect on bone Ca content or percentage
bone ash, as no differences were detected when compared to
controls. Response to low Ca diets and, in a previous
report, to high P diets demonstrate that adaptation to
unbalanced Ca:P ratios is possible, but additional
mechanisms (i.e. intestinal absorption changes) as well as
hormonal changes are probably responsible for the
adaptation response (Pointillart et al., 1978a, 1979).
Kayongo-Male et al. (1977) found that the poor performance
associated with low P in the diet (.35%) was more
pronounced than the effects of excess Ca or P or inverse
Ca:P ratios.

Dietary Ca:P Ratio. The Ca:P ratio has been shown to
effect the response of pigs to dietary Ca and P levels.
Chapman et al. (1962) found the Ca:P ratio to be most
critical when minimal levels of P were used in a diet,
while the P level had more effect on the response criteria
than did Ca level. Stober et al. (1979) reported that when
dietary P was deficient, wide Ca:P ratios severely
depressed performance, as well as bone strength and bone
ash. Reinhart and Mahan (1986) found decreased gain and
efficiency were associated with Ca:P ratios greater than
1.3:1 when the P levels were .05% below NRC-
recommendations. When the P level was increased to .10%

above NRC-recommendations, depression of performance was not seen until the Ca:P ratio exceeded 2.0:1. Bone bending moment and bone stress also decreased as the Ca:P ratio widened.

Bohstedt and coworkers (1942) found that a Ca:P ratio of 1.25:1 was most efficiently used. A Ca:P ratio of 1.25 to 1.5 is recommended for swine. Although the ratio of Ca:P in bone is relatively constant at a 2:1 ratio, the narrower ratio recommended for swine reflects the greater proportion of total body content of P found in soft tissues (about 20%), as compared to Ca (approximately 1%). Therefore, a greater proportion of the P absorbed is used for soft tissue needs than is the case for Ca, so the proportion of dietary P must be higher than would be needed simply for bone metabolism (Hays, 1976).

Cromwell (1979) reported that swine can tolerate a wide Ca:P ratio if the diet contains adequate P. He further stated that in cases where the level of P in the diet was deficient, a wide Ca:P ratio suppresses performance as well as bone mineralization. The growing pig can adjust to an adverse Ca:P ratio of .43:1 when dietary P level is .7%. The pig is not able to compensate for an adverse Ca:P ratio of .21:1 when dietary P level is 1.4%. However, performance markedly improved when the Ca:P ratio was changed to .42:1 at a dietary P level of 1.4%

(Veum et al., 1970a,b). Pond et al. (1978a) concluded that growing pigs could adapt to a wide range of dietary Ca and P levels with no effect on bone Ca, Mg, Cu, Mn or Zn, although Ca:P ratios less than .5:1 or greater than 2:1 produce abnormal bone formation.

Calvert et al. (1978) found that when the Ca:P ratio is wide, bone development is depressed. Doige et al. (1975) observed that dietary Ca and P effects were minimal when low levels of either mineral was fed in a Ca:P ratio of 1.25:1. However, when low Ca was accompanied by high dietary P, parathyroid enlargement, reduced bone mass, and increased numbers of osteoclasts and fibrous replacement of bone was observed. High Ca, low P diets resulted in hypophosphatemia, reduced bone ash and overgrowth of the epiphyseal plates. Nielsen (1972) found the Ca:P ratio had no effect on the percent of Ca or P found in the bones. Stone and McIntosh (1977) found plasma Ca and P concentrations were significantly influenced by dietary Ca:P ratio.

Dietary Na and K. Lindemann et al. (1984) and Kornegay et al. (1984) found no effects of Na on bone mineralization, bone strength, and the Ca and P content of bone ash, although feedlot performance, serum Na and Na content of bone ash was reduced at Na levels less than 1350 $mg \cdot d^{-1}$. Partridge (1978) reported reduced Na intake

enhances Ca absorption anterior to the terminal ileum. In contrast, Patience et al. (1987) found no changes in Ca absorption, retention or overall balance when 1.3 or 2.6% $NaHCO_3$ was fed, or when 3.0% $KHCO_3$ was included in the diet.

Dietary F. Forsyth and coworkers (1972a,b,c) found depressive effects of F on bone dimensions and an apparent interference with collagen metabolism in the bone. The mineral composition of the bone ash of pigs at birth was not affected by F incorporated in bone. They concluded that this indicated that F, therefore, does not exert a specific effect on retention of Ca in the bone. Forsyth and associates also found that high levels of dietary Ca/P reduced F accumulation in bone for animals fed additional F. High levels of F were found to depress growth rate and feed intake in weanling pigs. Rantanen et al. (1972) found thyroidectomy, removing the source of thyrocalcitonin, reduced or completely eliminated the adverse effects of feeding F on bone formation. Addition of Ca and P compensated for, but did not completely counteract, the effects of F on bone.

Dietary Mn. Kayongo-Male et al. (1977) investigated the interactions of Mn, Ca and P in the growing pig. They found added Mn decreased Ca and Mg in the first left rib and resulted in less production of compact bone in the metacarpal diaphysis, but had no effect on metacarpal breaking strength. These researchers also found that the poor performance associated with feeding .35% P in the diet was more pronounced than the effects of excess Ca and P or inverse Ca:P ratio, regardless of the level of supplemental Mn.

Dietary Protein Level. Schiefelbein et al. (1979) found no interaction between protein level and Ca/P levels above NRC-recommendations for performance of weanling pigs. Bone ash was higher for Ca/P levels of .8/1.0% at the 18% crude protein diet, but dietary Ca/P at .8/.8% produced highest bone ash when the dietary protein level was 21%. Kornegay et al. (1981a) found that feeding a 16/14% crude protein sequence, compared to a 18/16% sequence, had little or no effect on bone criteria. Bone length and ash did, however, tend to be lower (P<.10) for boars fed the higher sequence, with no effect on bone strength. Irlam et al. (1975) found that the Ca and P needs of the pig were not changed by feeding an increased dietary protein level. Conversely, Reinhard et al. (1976) suggested that the Ca and P requirement of the animal is increased by high

protein diets. Fammatre et al. (1977) found significant effects of higher dietary protein level on bone ash in the grower period, but the effects were not found in the finisher period. Similar differential effects of protein on bone mineralization with growth has been also been demonstrated with young and mature rats (Howe and Beecher, 1983).

Dietary Fat. In a Canadian trial, Atteh and Leeson (1983) investigated the effects of increasing dietary fat on Ca and P metabolism. They found that increasing dietary fat had no effect on the digestibility of Ca, P or Mg or on the bone or serum levels of these minerals. Newman et al. (1964a) found Ca digestibility was decreased 5% by feeding 10% tallow. Ca soaps in fecal samples were highest from pigs fed high-Ca, high-tallow diets. They concluded there was an inhibition of fat digestion by Ca while Ca digestibility was not materially affected by added fat. According to Newman et al. (1964b), tallow had no effect on femur ash or breaking strength, indicating the addition of 10% tallow did not increase the P requirement of swine. Jordan and Weatherup (1978) and Pakhomov (1979) found no detrimental effects on mineral metabolism of weanling pigs when 5 to 10% fat was added to the diet.

Dietary Lactose. Moser et al. (1978, 1980a) found the stress to strain ratios of pigs fed 30% dietary lactose were lower than the controls fed no lactose. Pigs fed low Ca diets had higher serum Ca levels when supplemented with 30% lactose, but lactose had no effect on serum Ca in pigs fed higher levels of dietary Ca. The lack of any effect of diet on the level of alkaline phosphatase activity indicated that normal bone calcification was occurring for all pigs at the time of slaughter.

Dietary Phytate. Interest in phytate in animal nutrition is more in relation to the availability of P, rather than of Ca, due to the expense of providing P in the diet relative to providing Ca (Taylor, 1965). Pierce et al. (1977) found pigs fed supplemental P provided by Ca phytate had substantially poorer performance than pigs fed the same levels of dietary P provided by dicalcium phosphate. Apparent digestibility of Ca decreased as the P level of the diet increased from .35% to .50%, but a further increase to .65% had no effect in pigs receiving dicalcium phosphate; pigs receiving Ca phytate exhibited a further reduction in apparent Ca digestibility in response to increasing the P level to .65%. The effects of dietary phytate level on performance, bone development and apparent digestibility of the nutrients indicated that at least .20% supplemental inorganic P was required in cereal based

diets.

Cromwell (1979) discussed the nutritional implications of the presence of phytates in animal feeds. One of the most notable effects, which can impact on the metabolism of Ca as well as reduce the availability of P, is the ability of the phytate molecule to strongly bind certain cations, including Ca, reducing their availability for absorption. Pedersen (1940) found 80% of the P in corn is bound by inositol as phytin P, and together with Ca, phytin forms Ca-phytate, which generally decreases the resorption of both Ca and P. According to Møllgaard (1945, 1947), the phytin P cannot be absorbed until after decomposition by the phytase enzyme. Qi et al. (1984) found the availability of total P in the diets for rats, chicks and pigs was significantly correlated with their phytate content. Phytic P was also found to affect the utilization of Ca, Zn, Cu and Fe.

According to Cromwell (1979), monogastrics have a very limited ability to hydrolyze the ortho-phosphate radicals of the phytate molecule, therefore utilization of phytate-P is minimal. Phytate P is partially digested by the pig under appropriate conditions. Presumably, dietary phytate is broken down by phytases in the feed, the digestive secretions, or by bacteria resident in the digestive tract. Since the optimum pH for cereal phytase is approximately

5.0, though the phytase retains some activity down to a pH of 3.0, it is unlikely that significant amounts of the enzyme survive the acid of the stomach to resume activity in the intestines. Appreciable breakdown of dietary phytate may occur in the stomach before gastric pH is reduced enough to inactivate the phytase. Research has demonstrated that substantial hydrolysis of phytate from a mixed cereal diet occurs in the stomach (Hill and Tyler, 1954; Taylor, 1965). Moore and Tyler (1955) found that when Ca phosphate was included in diet of the pig, hydrolysis of phytate P occurred in the stomach and in the large intestine.

Pointillart (1988) reported the results of comparisons of phytate P utilization in wheat or triticale diets with the utilization of the phytic P in corn diets. He found greater phytic P utilization in wheat and triticale diets, which contain higher levels of dietary phytase, than for the corn diets. Further, a rice-bran diet, when compared to a corn-soybean meal control diet, produced higher phytic P absorption and retention, and greater P digestibility for phytate-free dietary P. The high-phytase/low inorganic P diets had no effect on Ca absorption, but did result in improved Ca retention and higher bone mineral, bone density and breaking strength measurements. These effects were found to be independent of the effects of vitamin D,

although Pointillart concluded that vitamin D and dietary phytase activity may function synergistically to alter Ca and P metabolism.

Phytates can influence calcification in one or two ways. Phytates interfere with Ca absorption by formation of insoluble Ca-phytate. The P content of phytates is also not available to the animal as an equivalent amount of inorganic P. Thus, phytate can produce rickets when low Ca is fed due to a deficiency of Ca, and on high Ca diets due to a deficiency of P (Taylor, 1965). Phytate utilization can be further depressed by feeding diets marginal or deficient in vitamin D, with high levels of Ca or wide Ca:P ratios.

Dietary Fiber. Moser et al. (1980b,c) found fiber addition decreased serum Ca linearly, but increased serum P linearly. Peak force for third and fourth metatarsal bones increased linearly as the level of fiber was increased in the diet. Therefore, it appeared dietary fiber, provided as solkafloc, enhanced P utilization. Partridge (1978) reported that the apparent digestibility of Ca, P, Mg, K and Zn were reduced by inclusion of 3 or 9% solkafloc in the diet of growing pigs, due to reduced absorption posterior to the terminal ileum. Properties of cellulose other than its cation binding capacity must be involved in the reduction of mineral absorption, since reduced

absorption of P in this experiment could not be explained
on that basis. Possible explanations offered included the
high moisture content of the digesta on high cellulose
diets reducing trans-mucosal concentration and
electrochemical gradients in the large intestine, or simply
increased rate of passage through the large intestine
reducing the opportunity for mineral absorption.
Absorption of Na and K was not affected by amount of
cellulose in the diet but that of Ca, P and particularly Mg
decreased with increased cellulose in the diet (Martin et
al., 1980). To the contrary, Moore et al. (1986, 1987,
1988) reported no dietary effects on apparent Ca or P
absorption or retention when a corn-soybean meal basal diet
was compared to diets containing oat hulls, soybean hulls
or alfalfa meal. Davies (1978) found high dietary fiber
increases fecal excretion of Zn, Ca and P in humans, and is
associated with increased rate of passage through the
absorptive area of the small intestine.

<u>Genetic Factors</u>.

Effect of Breed. Rymarz et al. (1982) found no
significant differences between breeds (Norwegian Landrace,
Large White and Hampshire) for the ash and Ca or P contents
of growing-finishing gilts. Similarly, Nimmo et al.
(1980a) found no differences between breeds for bone
strength.

Effect of Sex. Crenshaw et al. (1981b) found that bone ash values were similar for boars, barrows and gilts. Nielsen (1972) found little indication of an effect of sex on total body retention of Ca and P in swine, and concluded little genetic difference exists for the requirement of pigs for Ca and P. Mudd et al. (1969) found there were no differences in total body Ca or P between sexes. Sex also had no effect on the calculated net retention of Ca, but barrows had higher P retention than gilts.

Effect of Frame Size. Irlam et al. (1975) found that the Ca and P needs of small frame and large frame animals were not different. Larger frame animals had longer metacarpal bones but the breaking strength of the bones did not differ from those of small frame animals.

Physical Factors.

Confinement Rearing. Some researchers postulated that the move to total confinement rearing of swine would result in increased needs for dietary Ca and P. Parker et al. (1974) found that pigs raised on soil had higher bone strength than pigs raised on concrete. Exercise or inclusion of 3% dirt in the diet did not compensate for the differences, suggesting that the increased bone strength and ash of pigs reared on the soil is due to factors other than forced exercise and consumption of dirt (Parker et al., 1975a). Although weaker bones and reduced ash content

were observed for pigs raised on concrete, compared to
those raised on dirt lots, feeding additional Ca and P to
these pigs did not improve bone mineralization, suggesting
the Ca and P requirements are similar for confinement and
pasture- or dirt lot-reared pigs.

Steam Pelleting the Diet or Ingredients. Steam
pelleting was shown to increase the P absorption in diets
containing no added P by Bayley and Thomson (1969). They
further found that steam pelleting of a diet containing
0.5% total P improved gain and feed efficiency over that
obtained with the meal diet, although it could not be
attributed to increased P availability or increased
digestibility of the energy or nitrogen components of the
diet. In balance studies of the effect of steam pelleting
and dietary P levels of .35 and .57% P on Ca and P
digestibility and retention, Harmon et al. (1970a) showed
that Ca and P absorption and retention were increased by
increasing P levels for both weanling and finishing pigs.
Young pigs were found to retain 39% of P from plant origin,
while finishing pigs retained only 32% of plant P. Harmon
(1972) concluded that the increases in feed efficiency
associated with pelleting corn-soybean meal diets must be
exerted through other means than simply improving P
availability since the increase in efficiency occurs on
both deficient and adequate levels of P. Pelleting of

individual diet ingredients has not been shown to enhance the P availability in corn, wheat bran or rice bran (Corley et al., 1980; Ross et al., 1983).

Feed Restriction. Brennan and Aherne (1986) found no effect of feed restriction on the mineralization of bone as measured by the metacarpal bending moment. Kornegay et al. (1983) and Lepine et al. (1985b) reported that bone length, weight, circumference and mechanical characteristics of ad-libitum fed pigs were greater than for feed-restricted animals, but that when the data was corrected for differences in body weight, the effects of energy were negated or reversed. Ad libitum versus restricted feeding was also found to have no effect on bone ash mineral contents. Kornegay (1986) and Lepine et al. (1985b) reported heavier, thicker bones were observed when developing boars were fed ad libitum, as compared to restricted-fed boars. Restricted-fed boars, however, were found to have larger bones than ad libitum-fed boars, on a equal body weight basis, suggesting skeletal growth rate was not reduced as much as soft tissue growth by restricted energy.

Effects of Calcium and Phosphorus on
Other Nutrients and Diseases

Ca and P have been shown to exert effects on the metabolism of other minerals, as well as having been implicated as a factor in the etiology of some disease conditions. Here, a brief review of some of these influences will be addressed.

Zinc Metabolism and Parakeratosis

In 1955, Tucker and Salmon showed that pigs fed diets high in Ca developed parakeratosis, a condition that could be prevented by supplementing the diets with Zn. Similarly, Hoekstra et al. (1956) demonstrated that excessive Ca in the diet aggravated the parakeratosis syndrome in pigs by feeding high levels of bone meal to growing pigs. They also established that the antagonism of parakeratosis by Ca was not due to changing the pH of the intestinal tract, and that addition of 2% bone meal to the diet did not alter Zn content of blood or tissue criteria.

In agreement with Hoekstra et al. (1956), Hoefer and coworkers (1960a,b) demonstrated that high levels of Ca exacerbate the symptoms of parakeratosis in growing pigs. The conditions were prevented by supplementing Zn to the diets, suggesting that Ca interferes with Zn metabolism.

Forbes (1960) concluded that the most probable interference of Zn function by Ca occurs at the cellular level. Taylor (1965) proposed that one possible

explanation for the interference in Zn metabolism by high
Ca levels is that the breakdown of phytate by phytase is
reduced at high levels of Ca, so that more Zn could be
precipitated as Zn-phytate, and thus rendered unavailable
for absorption.

Newland et al. (1958) found increased specific
activity of ^{65}Zn in the liver, a trend for increased
specific activity in the blood and kidney and a greater
proportion of fecal Zn from endogenous sources in pigs
receiving a high Ca, low Zn diet, suggesting an increased
rate of Zn metabolism in pigs receiving high Ca diets
without supplemental Zn. Morgan et al. (1969) reported Zn
content of the kidneys and liver were decreased by feeding
high Ca levels, but dietary Ca had no effect on Zn
retention. Pond et al. (1975, 1978b) could find no effect
of Ca and P on utilization or retention of Zn. For all of
these studies, only a small number of animals were used on
each diet, casting doubt on the reliability of results.

Batterham and Holder (1969) concluded that the effect
of high Ca on Zn, among other effects, depends on many
factors aside from the percentage Ca in the diet, including
the type of cereal, type of protein supplement, dietary
phytic acid level, feeding level and the level of other
minerals and vitamins.

Effect on Other Minerals

In general, increasing the level of Ca in the diet has been shown to increase the requirements for Zn, Mg and Mn, while the requirements for Fe and Cu are reduced in an indirect manner (Conrad & Beeson, 1957; Hoefer et al., 1960b; Lewis et al., 1956; Luecke et al., 1956; Stevenson & Earle, 1956; Whiting & Bezeau, 1958). In 1959, Carter et al. and Miller et al. reported increasing dietary Ca increased the concentration of liver Cu. In later research, Prince et al. (1984) found that high Ca and P supplementation increased liver Cu when pigs were supplemented with 250 ppm Cu, but had no effect on the liver Cu stores of pigs receiving no Cu supplement. Pond et al. (1978a) reported no effects of low (.2/.4%) Ca/P, high (1.2/1.0%) Ca/P or combinations of low and high P on Ca, Mg, Cu, Fe, Mn or Zn content of the liver or kidney.

van Kempen et al. (1976) reported increasing the P level in the diet from .4 to .7% of the diet resulted in a linear increase in the percentage of Ca, P and Mg in dry fat-free radius ash. Harmon et al. (1970b) reported bone Mg, Al and Na increased as dietary P level was increased from .34 to .50% of the diet, but a further increase to .75% dietary P did not increase these minerals further. Harmon and co-workers also reported dietary P level had no effect on bone K, Ba, Fe, Bo, Cu, Zn, Mn or Cr. Koch et

al. (1984) found no effect of dietary Ca:P ratio or of
increasing P level from .23 to .55% of the diet on bone
content of Ca, P, Mg, Na, K, Fe, Cu, Zn, Ba, Al or Ti in
weanling pigs. Similar results were reported by Koch and
Mahan (1985) in growing swine.

Mineral-vitamin level (100 vs. 150% NRC-
recommendations) had no effect on the P, Mg, Cu, Zn, Fe and
Mn content of the ash of the third or fourth metacarpals or
metatarsals. Ca content of the third metatarsal was higher
when 150% NRC Ca and P levels were fed. Similar,
nonsignificant trends were observed for third metacarpal
and fourth metatarsal (Lepine et al., 1985b).

Pond et al. (1975) found no differences in the
retention of Ca, Co, Cu, Mg, Mn or Zn in the bone when
normal (.5/.4%) or high (1.2/1.0%) Ca/P levels were fed
during the growing-finishing phase. High Ca/P diets did
result in significant reduction in bone Fe content, but
this was the only indication of any detrimental effect of
the high level of Ca/P used in this study. Radius/ulna Fe
content was reduced by feeding high dietary Ca/P (1.2/1.0%)
compared to low Ca/P (.2/.4%), or combinations of low and
high Ca and P, in a study by Pond et al. (1978a). There
were no effects of the diet on bone Mg, Cu, Mn or Zn.
Also, Fe concentrations in the bone ash were found to be
higher in pigs fed a normal Ca diet than pigs fed high Ca

levels (Pond and Yen, 1980).

Effect on Other Nutrients

Effects of Ca and P on Lipid Metabolism. Newman and Hecker (1969) investigated the effects of dietary Ca and P level on the lipid metabolism of swine. They found total lipid in feces and serum was lowest ($P < .05$) in pigs fed the low mineral level, whereas there were essentially no differences in total lipids of liver and hearts due to diet. Lipid P in feces, liver, heart and serum was unaffected by dietary level, or source of mineral or sex. Combs et al., (1962) demonstrated that high levels of Ca significantly depressed digestibility of ether extract.

Effects of Ca on Vitamin K. Hall et al. (1985) reported an incidence of a hemorrhagic syndrome in growing pigs, which these researchers attributed to the effects of feeding 1.8 or 2.7% dietary Ca with .9% dietary P. The condition was prevented by supplementing vitamin K to the diet. They suggested that the high Ca may inhibit the synthesis or absorption of vitamin K, or partially destroy vitamin K within the gut.

Hall (1988) investigated the effects of addition of vitamin K to the diet on Ca homeostasis in growing pigs. He found that net absorption of Ca from the gastrointestinal tract, and Ca excretion, appeared to be regulated by vitamin K dependent Ca-binding proteins in the

body. A vitamin K-dependent enzyme, gamma-glutamyl carboxylase has been characterized in a number of body tissues. This enzyme was shown to catalyze the post-translational carboxylation of specific glutamate residues, increasing the Ca-binding affinity of the protein.

Osteochondrosis and Structural Soundness

The Ca and P status of the pig has been implicated by some researchers as a factor contributing to the incidence of osteochondrosis and other unsoundness problems in swine. Brennan and Aherne (1986) investigated this relationship in 100 and 130 kg boars and gilts. Three diets, based on the NRC (1979) recommended Ca/P, ARC (1983) recommended Ca/P and 130% of ARC recommended Ca/P were fed, and half of the pigs were slaughtered at 100 kg while the rest were continued to slaughter at 130 kg. Bone strength parameters increased with increasing dietary Ca and P, but performance, incidence of osteochondrosis and soundness were not affected by the Ca and P level of the diet. Grøndalen (1974) reported variations in mineral supplementation had no influence on the incidence of leg weakness. Calabotta et al. (1982), Kornegay et al. (1981a, 1983) and Lepine et al. (1985a,b) also reported no effect of Ca and P on soundness. Brennan and Aherne (1986) concluded that young animals with high growth rates are predisposed to mechanical injury to cartilage, which may

lead to subsequent failure of ossification, but there was
no evidence dietary Ca and P were involved.

Atrophic Rhinitis in Swine

Atrophic rhinitis (AR) is a disease of swine
characterized by shrinkage, distortion or absence of the
nasal turbinate, and is sometimes accompanied by a twisted
snout, sneezing and nosebleeding. Symptoms identical to
these have been produced by some researchers in growing
swine by feeding diets deficient in Ca or with imbalanced
Ca:P ratios. These researchers believe AR to be a part of
a generalized osteitis fibrosis resulting from nutritional
secondary hyperthyroidism. The secondary hyperthyroidism
is thought to be the direct result of underfeeding Ca
and/or overfeeding P. Others attribute the etiology of AR
to infection by one or more of a variety of infectious
agents.

Brown et al. (1966) and Pond (1967) implicated dietary
Ca and P levels in the etiology of AR. They reported that
turbinate atrophy was evident in pigs fed low Ca or P
levels, while high levels of Ca and P prevented the
condition. Although the involvement of Ca and P status in
the incidence of AR has since been discounted, this report
did result in a flurry of research on Ca and P.

Peo et al. (1969) investigated the effects of low,
normal and high dietary Ca levels, fed with low, normal or

high P, on the incidence of AR in pigs. Diets had no
effect on the incidence of AR. Horváth and Papp (1972), in
response to reports that AR in pigs could be experimentally
induced by feeding low Ca levels in the diet, conducted a
study of the predisposing role of Ca depletion in the
etiology of AR. The concluded that dietary Ca depletion
plays no fundamental role in the etiology of AR, and at
most is a secondary factor in the development of the
disease.

Logomarsino et al. (1974a) studied the effects of
dietary Ca, low Ca:P ratios and instillations of a 4%
acetic acid solution on the incidence of AR. They found no
effects of dietary Ca or Ca:P ratio on the incidence of AR,
but were able to successfully induce turbinate deformity in
pigs instilled with the acetic acid. Histological
evaluation of the turbinate bones revealed there was a
generalized osteopenia produced by low dietary Ca, but
there was no effect of diet on turbinate deformity or
rhinitis, and high Ca did not prevent deformity or rhinitis
in acetic acid-instilled pigs. These researchers concluded
that two separate mechanisms were affecting the pathology
of the turbinates: 1) a generalized osteopenia of the
turbinates and other bones caused by low dietary Ca; and 2)
a localized irritation resulting in turbinate deformity and
rhinitis.

Hsu et al. (1976) fed low, optimum or high Ca/P levels with and without nasal washings obtained from pigs showing clinical AR signs to weanling pigs. They found Ca and P level in the diet had no effect on the incidence of AR, while AR could be readily produced experimentally by instillation of nasal washings from infected pigs.

Logomarsino et al. (1974b) investigated the effects of inoculation with <u>Bordatella bronchiseptica</u> and feeding high or low Ca on turbinate morphology. Turbinate osteopenia was found in all pigs on low Ca diets, but deformity in only 4 of 8 pigs. Inoculation with <u>Bordatella bronchiseptica</u>, on the other hand, resulted in turbinate deformity in 7 of 8 pigs, compared to 1 of 8 pigs that were not inoculated. The effects of the inoculation were found to be localized in nature, and high Ca was ineffective as a preventative agent.

In further investigation of AR in swine, Horváth et al. (1972) found that in swine inflicted with AR the tail vertebra bone weight was higher, but the Ca content of the bone was lower than that for healthy, unexposed swine. Peo (1970) summarized a number of research reports on the involvement of Ca and P in the incidence of AR in swine. He concluded that, although bone formation undoubtably was influenced by dietary Ca and P levels and the turbinates would react quickly to these effects, any possible

relationship between Ca and P level and AR in swine is coincidental, and secondary to the effects of the causative agent.

Availability of Calcium and Phosphorus in Feedstuffs

Many different techniques have been used to determine the availability of P and other minerals in feedstuffs and inorganic mineral supplements for swine. There are advantages and disadvantages to each technique and corresponding differences in the absolute values and the precision of the estimates obtained, due in part to the criterion chosen as well as the technique differences. Usually, within a given experiment, the relative differences between 2 or more P sources tested are usually consistent among most comparisons of those same sources.

The availability of P is of particular concern in swine nutrition, since a large proportion of the P in commonly-fed dietary ingredients for swine is organically bound in phytate, which cannot be readily utilized by monogastrics (Cromwell, 1979). Additionally, since Ca levels in most cereals and plant protein sources is low, most of the Ca in the diet comes from inorganic sources. The P content of these same feedstuffs is higher, and may account for 50 to 65% of the total P in the diet; the P in many feedstuffs is known to be poorly utilized by the pig (Kornegay, 1986).

Many of the estimates of P availability in feedstuffs are based on the determination of apparent digestibility of P. This procedure can be useful for comparing the relative availability of P, but tends to underestimate true digestibility of P unless the data are corrected for the endogenous P in the feces. Endogenous P can comprise a relatively large proportion of the total fecal P, particularly under conditions of low dietary P as are encountered when a diet of predominately natural ingredients, with no supplemental P, is used in availability determinations (Cromwell, 1979). Conversely, Oksbjerg and Fernàndez (1987) concluded that P retention, measured in balance experiments, could be used as a response criterion for evaluation of P utilization in inorganic feed phosphates, if dietary P levels used were between 2.8 and 4.8 g/d for 25 kg pigs.

Radioisotopes have been successfully used in swine mineral research to aid in quantifying the portion of excreted mineral that is of endogenous origin. Whittemore and Thompson (1969) proposed a carcass ratio be used to assess Ca and P availability in animals. The ratio was obtained by comparing the radionuclide recovery from pairs of animals, one dosed orally and the other intramuscularly with identical amounts of [45]Ca and/or [32]P. This method was found to compare favorably with results obtained by the

traditionally used comparative balance.

Recently, many researchers are making relative comparisons of the availability of Ca or P in organic or inorganic feedstuffs to that in a standard Ca or P source known to be well utilized by the pig. This often means the slope of the response of a bone criterion (bone ash, bone strength) to the Ca or P in a test ingredient is compared to the slope obtained for that bone criterion when the Ca or P is provided by the standard source.

Koch and Mahan (1985) evaluated a number of criteria for use in assessing P status of growing swine. They found bone bending moment was influenced by both the dietary P level and the Ca:P ratio. Ash weight, organic matrix weight, percentage bone ash, and organic component weights all responded linearly to increases in dietary P, but were unaffected by Ca:P ratio. Serum levels of Ca, P and Mg and growth performance were found to be less sensitive to dietary P levels. Thus, Koch and Mahan (1985) concluded that bone component weights, net ash accretion and percentage bone ash offered a more sensitive criteria for evaluating growing swine's response to dietary P than other factors considered. Koch et al. (1984) came to the same conclusions when these biological characteristics were evaluated in weanling swine. A critical review of the use of bone strength criterion for mineral nutrition research

by Crenshaw et al. (1981a) is discussed more fully under
the section on the effects of Ca and P on bone.

Availability of Ca from Plant or Inorganic Sources

Few researchers have reported estimates of
availability of Ca in feedstuffs or inorganic supplements.
Ross et al. (1984) estimated the availability of Ca in
calcitic limestone, oyster shell flour, gypsum, marble dust
and aragonite ranged from 93 to 102% as available as a Ca
carbonate precipitate standard, while the availability of
the Ca in dolomitic limestone was substantial less (51 to
78% as available). Pond et al. (1982b) found no
differences in the availability of limestone due to
particle size or the reactivity of the material, based on
performance and bone criteria.

Newton and Hale (1979) reported cement kiln dust
(28.6% Ca) offered promise as a dietary additive for swine,
perhaps in increasing the efficiency of gain. Subsequent
evaluation of cement kiln dust by Pond et al. (1982a)
indicated, however, that 3% cement kiln dust in the diet
depressed growth and caused osteonecrotic lesions of the
humerus, much like those encountered due to nutritional
secondary hyperthyroidism or rickets. Therefore, it
appears cement kiln dust, although containing a substantial
concentration of Ca, contains substances detrimental to the
pig. The only report of an estimate of Ca availability in

feedstuff came from Cromwell et al. (1983), who reported
the Ca in dehydrated alfalfa meal was 20% as available as
the Ca carbonate standard.

Availability of P from Inorganic Sources

Based on numerous research reports, it would appear
that the P in most inorganic P supplements is highly
available to the pig. The P in monosodium phosphate,
trisodium phosphate, potassium phosphate, phosphoric acid,
dicalcium phosphate and monocalcium phosphate has been
reported to be equally available. The P in defluorinated
phosphate, superphosphate and ammonium polyphosphate, if
not equally available to those sources, has been found to
be very nearly as available. The P in sodium
pyrophosphate, sodium hydrophosphate and Ca hydrophosphate
also appears to be as available as dicalcium phosphate.
Although not strictly an inorganic P source, it appears
that the P in meat and bone meal is equally as available as
dicalcium phosphate, as well. Only soft phosphate and
colloidal clay are generally classified as poor sources of
inorganic P, and the P in Curaco Island phosphate is
considered less available to the growing pig than dicalcium
phosphate (Ammerman et al., 1963; Bohstedt et al., 1942;
Chapman et al., 1955; Clawson and Armstrong, 1981; Cromwell
et al., 1976; Cupàk et al., 1972; Harmon et al., 1967,
1970b, 1974; Huang and Allee, 1981; King, 1980; Kornegay,

1972; Newman and Elliot, 1974; Noland et al., 1964, 1971;

Partridge, 1981; Plumlee et al., 1958; Procházka et al.,

1972; Tunmire et al., 1983; Wahlstrom et al., 1975).

Availability of P in Feedstuffs

A fair amount of research regarding the availability

of P in grain and protein feeds has been reported in the

last 15 years. A number of estimates of bioavailability,

relative to a monosodium phosphate standard have been made

for commonly used feeds. The other standard used in a few

of the relative bioavailability estimates summarized here

was potassium phosphate, but there appear to be no

inconsistencies due to the choice of standard in these

studies so the availability of the two standards will be

assumed to be equal. Reports on apparent P digestibility

by other workers, although not reported here, appear to

support the relative ranking of these feedstuffs in regard

to the extent to which the P in them can be used by the

pig.

The availability of P in dry corn appears to average

about 15% as available as the P in monosodium phosphate,

while that of high moisture corn is 40 to 45% as available.

Reconstituting dry corn increases the availability somewhat

(25% available), while dry pelleting the corn does not seem

to offer any advantages (12% available) (Abrams et al.,

1975; Boyd et al., 1983; Cromwell, 1979; Cromwell et al.,

1974; Huang and Allee, 1981; Miracle et al., 1977; Ross et al., 1983). The availability of sorghum (19%) appears to be slightly higher than that of corn; like corn, high moisture storage of sorghum results in increased P availability (42%) (Tonroy et al., 1970; Trotter and Allee, 1979).

The P in wheat appears to be about 50% as available as the P in monosodium phosphate (Cromwell et al., 1974, 1985; Huang and Allee, 1981; Miracle et al., 1977). The P in wheat bran and wheat middlings has a relative bioavailability of 30 to 35% (Corley et al., 1980; Stober et al., 1980b). Oat and barley P appears to be 25 to 35% as available as the P in monosodium phosphate (Besecker et al., 1976; Cromwell, 1979; Cromwell et al., 1974; Huang and Allee, 1981; Stober et al., 1980b), and an 18% relative P bioavailability for rice bran has been reported (Corley et al., 1980).

Among protein feeds used in swine diets, it appears that the availability of P in regular (44% crude protein) soybean meal is greater than that in dehulled (49% crude protein) soybean meal (28 to 37 vs. 20% available for regular and dehulled soybean meals, respectively) (Burnell et al., 1988; Huang and Allee, 1981; Miracle et al., 1977; Ross et al., 1982; Tonroy et al., 1973). In one study, Stober et al. (1980a) found cottonseed meal P to be totally

unavailable to the pig and Burnell et al. (1988) found the
availability was 3%, while other researchers (Huang and
Allee, 1981) reported the P in cottonseed meal was 42% as
available as that in monosodium phosphate. Canola meal
(21% available) and sunflower seed meal (3% available) and
cottonseed meal (2% available) were found to be poor
sources of organic P when compared to monosodium phosphate.
Cromwell et al. (1983) reported the P in dehydrated alfalfa
meal was 100% as available as P from monosodium phosphate.

Availability of P in Mixed Diets

Estimates of the apparent digestibility of P in
unsupplemented corn-soybean meal diets range from 15.4% to
36% (Bayley and Thomson, 1969; Bayley et al., 1975b; Pierce
et al., 1977; Vipperman et al., 1974). When the corn-
soybean meal diet was supplemented with an organic P
source, the apparent digestibility of P decreased to less
than half of that for the unsupplemented diet (Vipperman et
al., 1974). Bellaver et al. (1983), using isotope dilution
techniques, estimated the "true availability" to be 48% and
46% for the unsupplemented and supplemented diets,
respectively. Cornelius and Harmon (1974) found the
apparent digestibility of the P in a high-moisture corn-
soybean meal diet was higher than for a dry-corn-soybean
meal diet; supplementation of P to the high-moisture corn-
soybean meal diet increased the apparent digestibility of

the P. The apparent digestibility of P in a wheat-soybean meal diet was found to be 15.4% (Pierce et al., 1977). Using a radioisotope-based carcass ratio, Whittemore et al. (1971) reported the true availability of P in a standard (British) starter diet was 40%.

According to Cromwell (1979), if the non-phytate P of feeds is assumed to be available, then the bioavailability of P in most feedstuffs of plant origin would only be 20 to 40%. The greater availability of P in wheat and barley may be attributable to the presence of naturally occurring phytases in these grains, since the phytate P proportion is similar for corn, wheat, sorghum and barley (Nelson et al., 1968). McCance and Widdowson (1944) reported that wheat and rye contain high levels of phytase, barley contains moderate levels and corn and oats contain very little phytase.

Some researchers have suggested that possible explanations of the increased availability of P in high-moisture corn included hydrolysis of phytic acid in the high-moisture corn while in storage or hydrolysis in the gut of the pig. Frape et al. (1979) indicated that intestinal flora likely contribute the bulk of the phytase activity detected in the pig. Further, they reasoned that since the majority of these flora are located in the hind gut of the the pig, the significance of this phytase

activity was of questionable value in providing for
increased P utilization in the pig. Therefore, it would
seem that the most logical explanation of the increased
utilization of P in high-moisture corn would lie in
hydrolysis of the phytate molecule during the storage of
the high-moisture corn.

Effect of Calcium and Phosphorus and Time on Growth

Effect on Gain

In weanling pigs, the effects of Ca and P on growth
appear to be affected more by the ratio of Ca and P than
the level of Ca or P per se. As was indicated earlier,
when the Ca:P ratio remains between .9:1 and and 2:1, Ca
levels as low as .25 and P levels as low as .44% were
reported to have no effect on gain (Combs et al., 1962;
Combs and Wallace, 1962; Mahan, 1982). Within these Ca:P
ranges (.9 to 2.0:1), it does appear that the pig can
respond to increasing dietary P levels, although there is
no strong evidence that the currently recommended levels
are not adequate (Koch et al., 1984; Mahan, 1982; Mahan et
al., 1980). When the Ca:P ratio is greater than 2:1, gain
is depressed even when dietary Ca and P levels are in
excess of the recommendations (Reinhart and Mahan, 1985).
Conversely, when dietary Ca and P levels are lower than
recommended, it appears that a narrower Ca:P ratio provides
more desirable performance (Combs and Wallace, 1962).

During the growing phase, the Ca:P ratio remains very
critical. Both wide Ca:P ratios (>2:1) and imbalanced
(<.9:1) ratios have been shown to depress performance,
regardless of the Ca and P levels, relative to the
requirement (Hall et al., 1985; Nielsen et al., 1971;
Reinhart and Mahan, 1985). Within a range of Ca:P ratios
of 1:1 to 2:1, some researchers report the pigs may respond
to higher than NRC (1979) recommendations (Fammatre et al.,
1977; Hickman et al., 1983). Others have clearly
demonstrated that gain is depressed when less than NRC-
recommended levels are fed (King, 1980; Mahan et al.,
1976a). Conversely, Koch and Mahan (1985), feeding P
levels below the requirement in Ca:P ratios of 1:1 to 3:1,
and Thomas and Kornegay (1981), feeding 75 to 125% of NRC-
recommended P levels with 100 or 125% NRC Ca levels,
reported no significant effects of Ca:P ratio or Ca and P
level, respectively, on gain in growing swine. Reinhart
and Mahan (1985) reported that increasing the Ca:P ratio
from 1.3:1 to 4:1 depressed performance when dietary P
levels of .4% were fed.

For finishing swine, researchers report few effects of
Ca and P level in the diet on gain (Fammatre et al., 1977;
Mahan et al., 1976b; Reinhart and Mahan, 1985; Thomas and
Kornegay, 1981). As was the case for growing pigs,
Reinhart and Mahan (1985) did, however, demonstrate that

Ca:P ratios greater than 2:1 depresses performance when requirement levels of P are fed, and increasing the Ca:P ratio from 1.3:1 to 4:1 depressed performance when less than requirement levels were used.

When the overall grower-finisher period is considered, most researchers agree that there is no effect of Ca and P on gain when the P requirement is at or near the NRC (1979) suggested value (Combs et al., 1962; Crenshaw et al., 1981b; Cromwell et al., 1972, 1979; Fammatre et al., 1977; Hickman et al., 1983; Kornegay et al., 1981a; Lepine et al., 1985a; Libal et al., 1969; Miller et al., 1981; Nimmo et al., 1980b, 1981; Pond et al., 1978a; Schroeder et al., 1974). When Ca was maintained at .35% of the diet, Libal et al. (1969) reported gain increased as P in the diet increased from .30 to .70%., while Kesel et al. (1983) reported improved rate of gain when boars were fed 150% of NRC-recommended Ca and P levels, as compared with animals fed recommended levels. Thomas and Kornegay (1981) demonstrated that pigs fed 75% of the NRC-recommended (1979) P level, with the recommended Ca level had poorer gains than pigs fed at or above the NRC-recommended P level. In general, a number of researchers have concluded that extreme Ca and P levels will affect gain, as well as efficiency, and the effects of extreme levels of dietary P are more pronounced than for extremes in Ca (Kornegay,

1986).

Crenshaw et al. (1981b) and Kesel et al. (1983), investigating the effects of Ca/P levels and time on swine performance, reported gains that increased in quadratic manner over time from weaning to market. Crenshaw (1986), in developing best fit prediction equations for performance and bone criteria in growing-finishing pigs, found that average daily gain could be best described as decreasing with dietary P level, increasing with time, decreasing with time x time, and increasing with P level by time interaction, and decreasing nonsignificantly with Ca level.

Kornegay (1986), reviewing Ca and P research, combined the daily gain data of 11 studies, conducted since 1969, in which the Ca/P levels fed during the growing phase were reduced during the finishing phase (reduced data). A second data set was also compiled, wherein daily gain data of eight studies, in which the level of Ca/P fed during the grower were continued in the finishing phase, were combined (continuous data). All Ca/P levels fed were expressed as a percentage of NRC (1973). Asymptotic models, relating daily gain to the percentage of NRC Ca/P fed, were fit within each combined data set, and indicated that daily gain increased rapidly then plateaued in both data sets. In the reduced data, 97% of maximum daily gain was reached when NRC-recommended levels of Ca and P were fed, whereas

in the continuous data set, 97% of maximum daily gain was obtained when 81% of NRC-recommended Ca/P levels were used.

It would appear from the studies summarized here that gain would likely increase with increasing Ca and P level (using a constant Ca:P ratio) to the levels approximated by the NRC-recommendations (1979) and then plateau. Levels in excess of NRC-recommendations would not be expected to offer any advantage in improved gain, while levels substantially below these recommendations would be expected to depress performance. Gain would also be expected to respond quadratically to time, from weaning to market.

Effect on Feed Efficiency

Researchers, using weanling pigs, have reported decreased efficiency of feed utilization (EFU = gain:feed ratios) with increasing Ca level in the diet (Combs and Wallace, 1962; Mahan, 1982), and increased EFU with increasing P level (Koch et al., 1984; Mahan, 1982; Reinhart and Mahan, 1985). The Ca:P ratios in these studies were also allowed to vary, though, and in a number of reports, decreased EFU has also been associated with increasing Ca:P ratios (Combs et al., 1962; Combs and Wallace, 1962; Koch and Mahan, 1985; Mahan, 1982; Reinhart and Mahan, 1985). Mahan (1982) found that when dietary P was increased from .50 to .90% with Ca held constant at .70% of the diet, there was no effect on EFU, but when the

Ca was held constant at .90% of the diet, EFU increased with increasing P level; when averaged across the two Ca levels, there was a quadratic increase in EFU with P level. When the levels of Ca and P were increased in a constant Ca:P ratio, there was no effect of increasing minerals on EFU (Hickman et al., 1983; Mahan et al., 1980).

In growing swine, increasing Ca:P ratios were also associated with decreased EFU, particularly when P levels were below NRC-recommendations (Koch and Mahan, 1985; Mahan et al., 1976b; Reinhart and Mahan, 1985; Thomas and Kornegay, 1981). EFU was depressed by feeding P levels below NRC-recommendations (Mahan et al., 1976b; Thomas and Kornegay, 1981), although these changes were also confounded with changes in the Ca:P level. Koch and Mahan (1985) reported EFU increased with increasing P level (.12% to .50%), but at P levels increasing from .50% to .90%, Miller et al. (1981) found no effect of P level on EFU. Although Kornegay et al. (1981a) reported EFU was improved by feeding 125% of the NRC-recommended Ca and P levels in a constant Ca:P ratio, other reports indicate there is no advantage in EFU to feeding higher than NRC-recommended levels (Fammatre et al., 1977; Hickman et al., 1983; Lepine et al., 1985a; Nimmo et al., 1980b; Thomas and Kornegay, 1981).

During the finisher phase, Thomas and Kornegay (1981)

showed that feeding P levels below NRC-recommendations
depressed EFU. Although the treatments used by Thomas and
Kornegay resulted in changes in Ca:P ratio being confounded
with changes in dietary P, Reinhart and Mahan (1985) found
that EFU also decreases with increasing Ca:P ratio in the
finishing pig, the effect being more pronounced when
dietary P levels were below NRC-recommendations. In
contrast, Miller et al. (1981) reported no effect of
dietary treatments on EFU, even though the Ca:P ratios
decreased from 2:1 to 1:1. Fammatre et al. (1977) reported
EFU improved when Ca and P levels were increased in a
constant Ca:P ratio above the NRC-recommendations.
Conversely, other researchers report no advantage in EFU by
finisher pigs for feeding Ca and P levels above NRC
(Hickman et al., 1983; Kornegay et al., 1981a; Thomas and
Kornegay, 1981).

For the combined grower-finisher phase, Thomas and
Kornegay (1981) reported overall EFU was reduced when pigs
were fed 75% of the NRC-recommended P level. Combs et al.
(1962) reported a linear EFU response to P level from .40
to .48% of the diet. Nielsen et al. (1971) reported Ca:P
ratios of .1:1, .2:1 or 3:1 reduced EFU of growing-
finishing pigs. Other researchers report no significant
effects of Ca and/or P, at or above NRC-recommended levels,
or of the Ca:P ratio (Combs et al., 1962; Crenshaw et al.,

1981b; Cromwell et al., 1972; Fammatre et al., 1977; Kesel et al., 1983; King, 1980; Kornegay et al., 1981a; Lepine et al., 1985a; Libal et al., 1969; Mahan et al., 1976b; Nielsen et al., 1971; Nimmo et al., 1980b, 1981; Pond et al., 1978a; Thomas and Kornegay, 1981). The combined-data summary of research by Kornegay (1986) indicated that 97% of maximum feed efficiency (feed:gain ratios) were obtained with 93% and 81% of NRC-recommended Ca/P levels for reduced and continuous Ca/P levels, respectively.

Data from Kesel et al. (1983) indicate EFU declines in linear fashion with time, whereas data from Crenshaw et al. (1981b) implies the response may be quadratic, increasing to about 4 mo of age, then decreasing. Crenshaw (1986) found the best fit prediction equation for EFU indicated EFU decreased nonsignificantly with Ca level, increased with P level, decreased with squared P level, and decreased with time. Considered overall, one would expect little difference in EFU due to diet, and that EFU would decline over time.

Effect of Calcium and Phosphorus Levels and Time on Bone Characteristics

Bone strength and mineralization criterion are the most often used measures of Ca and P status of the pig reported in the literature. However, comparison of the results of one study to those of another study is complicated. This is because of the lack of

standardization of the instruments used and/or the testing procedures, differences in how the bones are prepared for testing, and inadequate reporting of the details of the testing procedure to allow the reviewer to compensate for these differences. This is particularly the case with research using bone strength criterion to evaluate the Ca and P status of the pig (Crenshaw et al., 1981a).

Dried bones were found to demonstrate more variability in breaking strength than wet bones (Crenshaw et al., 1981a; Kornegay et al., 1981b; Maxon and Mahan, 1983). It has been shown by Crenshaw et al. (1981a) and Kornegay et al. (1981b) that changes in moisture content of the bone can alter the mechanical properties of the bone, including the amount of force that the bone will withstand before integrity of the bone is compromised. Crenshaw et al. (1981a) reported that as little as 10 min exposure to air could result in changes in the mechanical properties of wet bones. Sedlin (1965) showed, however, that freezing of wet bone did not alter the bone mechanical properties as long as dessication of the bone during the freezing period was prevented.

Crenshaw et al. (1981b) found that different responses to dietary Ca and P levels were obtained with the femur and metatarsal bones in pigs less than 4 mo of age. Schroeder et al. (1974) found the third metacarpal bone was more

sensitive to dietary Ca and P levels than the the femur.
Parker et al. (1974) reported the correlation between
humerus and second metatarsal bending strength was very
small (r=.16). Metatarsals were found to be heavier and
longer than metacarpals, and outside (fourth) metatarsals
and metacarpals were heavier and longer than inside bones
(third); differences were greater between metacarpals and
metatarsals than between inside and outside bones (Kesel et
al., 1983). Lepine et al. (1985b) reported that metatarsal
bones were more sensitive to dietary Ca and P level than
metacarpal bones; whereas inside (third) metacarpals and
metatarsals were more sensitive than outside (fourth)
metacarpals and metatarsals.

The ideal bone for assessing mineralization in swine
may vary with age, sex and other biological and economic
considerations. Metacarpals and metatarsals are widely
used since they come from the front and hind feet,
respectively, and are easily obtained. Additionally,
recovery of the metacarpals and metatarsals from the feet
is economically more appealing than reducing the retail
value of the ham or shoulder in order to recover the femur
or humerus (Crenshaw et al., 1981b).

Bone strength is measured by a number of criteria,
including force (or load), maximal bending moment, bending
stress, and modulus of elasticity (measures the resistance

of the bone to bending). For purposes of this review, only three of these strength criteria will be considered; those three are force, bending moment and stress. In this section the effects of Ca and P level and time on a number of bone criteria will be reviewed. These criteria will include the physical measurements of the bone, the Ca and P content of the bone, the ash content of the bone, and measures of bone strength. Additionally, a brief summary of differences between force, bending moment and stress will be included.

Effect of Ca and P Level and Time on Physical Measurements of Bone

Lepine and coworkers (1985b) found no effect of feeding 150% NRC-recommended (1979) mineral and vitamin levels on metacarpal and metatarsal weight and length, and little effect on other physical measurements of bone. Fresh weight, length, shaft diameters and wall thickness, however increased significantly with age. Kesel et al. (1983) reported increases in metacarpal weight, metacarpal length and dimensions of the bone for boars with age, with the rate of change decreasing as the boars aged. Metacarpal weight and bone wall thickness was greater for pigs fed 150% Ca and P levels, although the magnitude of difference was not as great when the values were adjusted for the body weight of the pig.

Kornegay (1986) concluded that, in general, weight, length and outside diameters of bones were not very responsive to dietary Ca and P levels. Thickness of the bone wall was found to be responsive to Ca and P levels, particularly when the levels were below the NRC-recommendations (Crenshaw et al., 1981a; Kornegay, 1986). It also appears that most of the physical measurements of the bone increase with time, concomitant with the increase in body size.

<div align="center">

Effect of Ca and P Level and Time on
Bone Ca and P Content

</div>

Kornegay and Thomas (1981) found little effect of feeding Ca and P levels, in the range of 75 to 125% of NRC-recommended (1979) Ca and P levels, on bone Ca and P. The magnitude of increases in bone mineralization with supplementation of minerals above NRC-recommendation was inconsistent, and when observed, the magnitude of increases was small. Maximum bone mineralization in response to dietary Ca and P occurred when the Ca:P ratio was approximately 1.3:1. Koch and Mahan (1985) found that increasing Ca:P ratios (1:1, 1.5:1 and 3:1) produced increasing Ca and P percentage of the femur ash. Increasing dietary P levels from .12 to .50% had no effect on femur Ca, P or Mg, while bone Na, K, Fe and Zn declined.

The Ca and P content of bone, expressed as a percent of bone ash, is relatively constant over a wide range of

dietary Ca and P intakes and ages. This makes bone Ca and P content a very dietary Ca and P insensitive response criteria. However, when expressed as percent of dried fat-free bone, the Ca and P content of bone is as sensitive as bone ash (Kornegay, 1986).

Effect of Ca and P Level and Time on Bone Ash

Increasing the Ca level in the diet, while holding the P constant at a level at or above the P requirement appears to produce a quadratic increase in percent bone ash (Coalson et al., 1974; Cromwell et al., 1972; Mahan, 1982). Increasing dietary P, with a constant Ca level, also results in increased bone ash percentage. Both linear and quadratic components of the response have been reported to be significant by most researchers (Cline, 1981; Combs et al., 1962; Cromwell et al., 1970, 1974, 1979; King, 1980; Koch and Mahan, 1985; Kornegay and Thomas, 1981; Mahan, 1982; Miller et al., 1981; Parker et al., 1974, 1975b; Reinhart and Mahan, 1985).

When Ca and P levels are concomitantly increased in a Ca:P ratio between 1:1 and 2:1, bone ash increases. The response of bone ash to Ca/P level is linear and/or quadratic, both above and below the NRC-recommendations. Even in studies where 3 or more Ca/P levels are fed and only linear response is reported, there appears to be a trend toward quadratic response (Blair and Benzie, 1964;

Brennan and Aherne, 1986; Crenshaw et al., 1981b; Fammatre et al., 1977; Hickman et al., 1983; Kornegay and Thomas, 1981; Lepine et al., 1985b; Mahan et al., 1980; Maxson and Mahan, 1983; Nimmo et al., 1980a,b; Parker et al., 1975b).

Kornegay (1986) compiled bone ash and bone strength data from various Ca/P research studies conducted since 1969; one set of data included studies where the Ca/P levels were reduced during the finishing phase, and another set of data combining studies where continuous levels of Ca/P were fed through both the grower and the finisher phases. All Ca/P levels were expressed as a percentage of NRC (1973)-recommended Ca/P levels. Asymptotic analysis indicated that the response of bone ash to Ca/P level plateaued above 100% NRC-recommendations for the "reduced" group, and at approximately 75% of NRC-recommendations for the "continuous" group.

Crenshaw (1986) found that bone ash could not be reliably predicted by the Ca and P level in the diet. He found that only the Ca by time interaction was significant, indicating that bone ash changed over time, with the slope of the curves influenced by the Ca level in the diet. Crenshaw also found that bone ash maximized at .7 to .8% dietary P (a dietary range of .4 - 1.2% P was used in the study), but no maximum was reached with the Ca level used (within a range of .4 - 1.2% dietary Ca), indicating bone

ash was affected more by Ca than by P. Time effects on bone ash were far greater than Ca effects in this study. Other researchers have also reported no effect of Ca and P level in the diet on bone ash percentage (Harmon et al., 1974; Hsu et al., 1976; Liptrap et al., 1970; Peo et al., 1969; Schroeder et al., 1974). Bone ash appears to increase in a quadratic manner from weaning to market weight (Crenshaw, 1986; Hickman et al., 1983; Lepine, 1985b; Maxson and Mahan, 1983).

Effect of Ca and P Level and Time on Bone Strength

A number of researchers have reported linear trends in the force required to cause three-point bending for different bones in response to increasing dietary P level (Bayley et al., 1975a; Libal et al., 1969; Parker et al., 1974, 1975b; Schroeder et al., 1974). Quadratic effects of dietary P level on force have also been reported (Bayley et al., 1975a; Cline, 1981; Cromwell et al., 1979; Hall et al., 1985; Kornegay and Thomas, 1981; Libal et al., 1969; Parker et al., 1974). Both linear and quadratic increases in bending moment in response to increasing dietary P level have been shown as well (Crenshaw, 1986; Koch and Mahan, 1985; Koch et al., 1984; van Kempen et al., 1976). When the effect of dietary P level on stress is considered, only linear effects of diet have been reported (Miller et al., 1981; Schroeder et al., 1974).

Increases in force, bending moment and stress have been reported by a number of workers in response to Ca supplementation, although only two levels of Ca were used in the diets (Crenshaw, 1986; Libal et al., 1969; van Kempen et al., 1976), while Schroeder et al. (1974) found no effects of Ca or P level on stress in the femur or third metacarpal. Linear effects of Ca on force (Cromwell et al., 1972; Schroeder et al., 1974), bending moment (Koch et al., 1984) and stress (Crenshaw, 1986) have been reported. Hall et al. (1985) reported nearly quadratic effects of Ca on force, whereas Crenshaw (1986) and van Kempen et al. (1976) found quadratic effects of Ca on bending moment.

A number of researchers have compared force, bending moment or stress at only 2 levels of Ca/P, both levels in a constant ratio. All three measures of bone strength were reported to be higher at the higher Ca/P level (Crenshaw et al., 1981b; Kesel et al., 1983; Lepine et al., 1985b; Nimmo et al., 1980a, 1981; Parker et al., 1975b). Where sufficient levels were used to describe the response curve, Kornegay et al. (1981a) found force responded in linear and/or quadratic manner to increasing Ca/P level, whereas Nimmo et al. (1980b) reported linear effects of Ca/P on force. Kornegay and Thomas (1981) reported Ca/P levels less than NRC-recommendations (1979) reduced force, while there were no consistent trends in response to levels above

NRC-recommendations. Maxson and Mahan (1983) found bending moment of the metacarpal responded linearly to increasing Ca/P level, where as the metatarsal bending moment responded linearly to dietary Ca/P in the early portion of the growing period, and quadratically later in the grower period. Brennan and Aherne (1986) reported quadratic effects of Ca/P level on bending moment in the grower-finisher period. Only Nimmo et al. (1980b) reported effects of dietary Ca/P level on stress, finding the response was linear as Ca/P level increased. In the "reduced" vs. "continuous" data compiled by Kornegay (1986), the response of bone strength to Ca/P level plateaued above 128% NRC-recommendations for the "reduced" group, and at approximately 91% of NRC-recommendations for the "continuous" group.

When the effects of time on bone strength are considered, few researchers reported data from which time effects could be estimated. Crenshaw (1986) found stress responded linearly over time, while bending moment increased linearly over time, with the slope of the response dependent on the Ca level. Crenshaw et al. (1981b) found bending moment of the femur, humerus, third and fourth metacarpals and metatarsals, and the rib responded linearly over time; quadratic effects of time on humerus, femur and rib stress were reported as well, while

metacarpal and metatarsal stress decreased from 1 to three mo, then increased again through 7 mo of age. Both Kesel et al. (1983) and Lepine et al. (1985b) reported linear effects of time on metacarpal bending force, while Lepine et al. found metatarsal bending force increased quadratically over time.

Considering time and diet information together, it appears one could expect the third and fourth metacarpal and metatarsal force and stress to respond primarily in linear fashion as dietary Ca/P level increased, while the effects of time on the metacarpals would likely be linear, and on the metatarsals linear and quadratic for force and linear for stress. Crenshaw (1986) reported best fit prediction equations for a number of bone strength measures. Force was best described as increasing with linear P level, decreasing with quadratic P level, and increasing with time, with the slope of the response to time increasing with Ca level. Stress, on the other hand, increased with Ca level and time, with little effect of P level, although the overall fit of the equation was very poor ($R^2=.41$).

Comparison of Force, Bending Moment and Stress as Measures of Bone Strength

Crenshaw et al. (1981b) defined force as a measure of the amount of load a bone can withstand until breakage. Therefore, a bone with a larger size and/or a higher degree of mineralization would be expected to withstand a greater force. Bending moment adjusts force for the distance between the two supports in the three point bending test. Calculation of bending moment allows comparison of force required to cause bending of bones of differing lengths, because the spacing of the support fulcra between tests can affect the recorded force (Crenshaw et al., 1981a; Wilson et al., 1984).

Force, an externally applied load on the bone, does not account for differences in the size or shape of the bone, therefore, alterations in the size or shape of the bone due to dietary Ca or P levels might result in a misinterpretation of the adequacy of the dietary levels tested (Crenshaw, 1986). Stress is a mechanical property of bone, and allows an evaluation of changes in the degree of bone mineralization because it is a calculation of the amount of force a bone will withstand per unit area of the bone. Additionally, the calculation of stress is based on the area moment of inertia, which accounts for differences in the geometry of the area over which the force is applied, i.e. for differences in size as well as shape.

Bones are irregular in shape, presenting problems for
determination of the moment of inertia (Crenshaw, 1986;
Crenshaw et al., 1981b).

Stress and force or bending moment respond differently
to dietary Ca and P levels (Crenshaw et al., 1981b).
Maximization of stress indicates that nutrient levels are
adequate for mineralization of bone. Force or bending
moment, on the other hand, increases in response to
increases in the total amount of bone. Measuring force or
bending moment may be desirable for the determination of
recommended levels, but stress would be more appropriate
for establishing minimum requirements. For example,
desired mineralization of the bone matrix would occur at
maximum stress, but more total bone (i.e. at maximum force)
might be needed to maintain structural integrity in an
animal entering the breeding herd (Crenshaw et al., 1981a).
Because of changes in the response obtained relative to
time and the engineering principles that define stress,
Crenshaw (1986) concluded that an assessment of the amount
of stress withstood by bone was critical to the
interpretation of responses to dietary Ca and P levels over
a range of .4 to 1.2% and .4 to 1.0% of diet, for Ca and P,
respectively.

Use of Shear as an Alternative to Three-Point Bending for Measuring Bone Strength

Wilson et al. (1984) reports that shear deflection can also affect the amount of force measured in a three-point bending test. In particular, when the ratio of the space between the fulcra (L) to the outside diameter of the bone (D) is less than 10, then shear deflection becomes a part of the measured force deflection for the bending test. When L/D is approximately 5, shear accounts for 50% of the total deflection, whereas, when L/D is > 10, shear deflection is approximately zero. Since few researchers report what value of L was used in their studies, it is impossible to determine if there is any confounding of shear deflection in the reported bending test results. To overcome the effects of shear deflection on bending test results, Wilson et al. (1984) recommend that when the L/D ratio is less than 10, a pure shear test should be used to evaluate bone strength.

Summary of Ca and P Effects on Bone Criteria

Kornegay et al. (1981b) reported the correlations between a number of bone criteria; correlations between criteria were small to modest at best. Metacarpal weight was positively correlated to breaking strength in four trials and to ash in 2 trials (r=.30 and r=.21 for breaking strength and ash, respectively). Breaking strength was correlated to ash in only one trial, with a small

coefficient (r=.19).

Kornegay (1984) summarized the results of a number of experiments where the response of bone criteria to Ca and P were measured. A positive relationship was evident between dietary Ca and P level and bone ash and bone bending strength in a number of studies, with a plateau in response between 100 and 150% NRC-recommended Ca and P level. The magnitude of response to Ca and P decreased with maturity, with complete compensation in bone mineralization noted by the time animals reached market weight. Correlations between bone characteristics and soundness scores were nonsignificant and approached zero.

Crenshaw et al. (1981b) tested the homogeneity of slopes between the percentage of ash and a number of bone strength parameters. They reported these tests were nonsignificant, indicating that there is little relationship between percentage of ash and bone strength characteristics.

Crenshaw et al. (1981a) reported that in rachitic bones with incomplete calcification, the bone strength measures of force and stress, and bone ash, all increase as calcification becomes more complete. In completely calcified bone, force increases as the amount of total bone tissue increases, while stress and bone ash do not.

Crenshaw et al. (1981a) found that stress was a more

sensitive indicator of mineralization than percentage of ash, based on responses of stress and percentage of ash across sexes. Bones recovered from boars had significantly lower stress values than did bones from gilts or barrows, while percentage ash showed nonsignificant numerical trends in this regard, indicating boars had bones that were less mineralized than bones from barrows or gilts.

Nimmo et al. (1981) found bone ash values are relatively accurate indices of skeletal development in the young, rapidly growing pig, but one cannot derive any measure of bone strength or indication of bone density per unit area from this criterion. Based on the results of a study of the reliability of dietary Ca and P levels as predictors of bone mechanical properties, Crenshaw (1986) concluded that, for studies with the objective of evaluation of requirements relative to the role of minerals in the structural support of the animal, the criteria should involve an assessment of strength. He found that bone ash could not be used as a reliable predictor of bone strength; in an attempt by the animal to maintain structural integrity, compensation in bone strength could be detected by mechanical test before changes in ash content were detectable.

Alternatives to Slaughter for Collection of Bone Data

There are few studies reported in the literature that utilize non-slaughter techniques for collection of bone data in swine. Blair and Benzie (1964) reported radiographs were taken weekly to monitor bone development in pigs from 3.6 to 11.4 kg, but reported few results of this analysis, and did not offer any evaluation of the technique.

Sorenson and Cameron (1967) proposed a photonbeam-transmission technique as a reliable in vivo means of determining bone mineral content in humans. Crenshaw (1986) used a similar procedure to determine bone mineral content of femur and third metatarsal bones in the pig, but performed the determination on bone cross-sections of freed bone, rather than in vivo.

There have been no reports of a bone biopsy procedure used in nutrition studies with the pig. This technique calls for removal of a sample of bone from the live animal, under local or general anesthesia, with the wound closed and sutured as needed. This would allow subsequent sampling of the same animal following a dietary treatment, or at various times along the treatment period.

Little (1972) described a biopsy procedure for use in cattle and sheep for recovery of bone tissue to analyze for P status in these species. In cattle, the area to be

sampled was clipped and sterilized, and a local anesthetic
was infiltrated along the line of rib sufficient to allow a
skin incision of 9 to 10 cm in length. Skin, fascia and
muscle were incised down to the rib and the edges were then
retracted. A 4 to 5 cm incision was then made through the
periosteum and two incisions were made perpendicular to and
across the periosteum incision, so the periosteal tissue
could be retracted. A 1.5 cm diameter trephine was then
used to remove a 700 to 1000 mg fresh weight sample. The
wound was then cleaned to remove all small particles of
bone left by the trephine, and the periosteum flaps were
replaced loosely without suturing. Muscle and skin were
then sutured back separately. A similar procedure was
followed for sheep, except that an entire section of the
rib was removed using bone-cutting forceps.

Little (1972) found bone composition (ash, Ca or P
content of bone) per unit of volume was a reliable measure
of changes in bone composition of cattle during periods of
nutritional depletion and repletion in these species. With
yearling cattle, Little (1972) found significant
differences in ash content, and in Ca and P contents of the
bone when the data was expressed per unit of volume or
weight of fresh bone, but bone Ca and P differences were
not found when expressed per unit of dry fat-free bone.
Following a depletion period, P was supplemented in small

and increasing amounts, and changes in bone composition were monitored with serial biopsy. Although nonsignificant, increases in bone composition characteristics in response to the supplementation were observed, indicating the effects were referable to the inadequacy of P in the depleted state. During the depletion period, Little (1972) found the changes were due to failure of the normal remodeling process, with poor calcification of new osteoid tissue, and resultant increases in the percentage of water. Thus, when analyzed on the dry fat-free basis, differences were suppressed. When "before and after" serial biopsy samples were analyzed as independent observations, differences in Ca and P contents were no longer significant. This was found to indicate that greater sensitivity was associated with serial sampling, therefore, serial biopsy technique was preferable.

Little and McMeniman (1973) applied the biopsy procedures described by Little (1972) to the study of bone composition in grazing sheep. As was reported with cattle data in the earlier study, expression of bone composition on the basis of unit of volume or wet weight provided a more sensitive index of P status than when composition was expressed on a dry fat-free basis. Little and McMeniman (1973) found the technique was sufficient to demonstrate

differences in skeletal reserves due to the effects of lactation in grazing ewes.

In a third study by Little and co-workers (1978), bone biopsy of Merino ewes was used to study the effects of varying P intake and stage of production on skeletal mineralization. Biopsy samples taken at the beginning and end of treatment showed significant loss of bone mineral due to lactation; the loss of bone mineral in ewes suckling twins could be differentiated from the loss sustained by ewes suckling a single lamb. Feeding supplemental P prevented significant losses of bone mineral in lactating ewes. Little et al. (1978) concluded that detection of differences in bone mineral in the absence of effects on production was indicative of the sensitivity of the biopsy technique for measuring variations in P storage.

Summary and Conclusions

The Ca and P nutrition of the pig is, to say the least, a very complicated set of interactions between these two minerals and between these minerals and other nutrients. The availability of these minerals in inorganic supplements and in feedstuffs normally fed to swine, particularly that of P, is critical to the determination of levels required to produce adequate performance and/or bone strength.

Bone growth increases earlier than muscle growth in
the pig, as has been reported for other species.
Performance has been shown to maximize at levels of Ca and
P in the diet lower than those required to maximize bone
strength and mineralization. Bone criteria are more
sensitive to dietary Ca and P levels than are performance
criteria. Perhaps the best way to illustrate the
differences one might expect between basing conclusions on
a performance criteria or on a bone criteria, and the
interactions that must be involved, is to relate the
results of one more Ca/P study.

Kornegay et al. (1981b) reported the correlations
between a number of performance and bone criteria;
correlations between criteria were small to modest at best.
Daily gain was positively correlated to dried metacarpal
weight in 3 trials, negatively in one, and was unrelated in
a third (r=.30). The same pattern held true for the
correlation of daily gain to bone breaking strength
(r=.23). Daily feed intake of barrows was positively
correlated to breaking strength in one study and negatively
correlated in the second study (r=.52); for boars, the
correlation was r=-.28. Efficiency was not significantly
correlated to bone criteria in any trials. These
correlations clearly illustrate two points: 1) a
researcher should not base his conclusions solely on either

performance or bone criteria alone, but must look at the
overall performance and bone status of the pig in making
recommendations about the Ca and P needs of the animal; and
2) there is great variability inherent in the response of
the pig to various Ca/P treatments, not only within a
study, but also from study to study.

Describing the performance and bone responses to Ca/P
level and time in a data set large enough to overcome a
large portion of the inherent variability in the
performance and bone measures, so that prediction of the
responses can be reliably made, seems to be a lofty goal.

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CHAPTER 3

Running Head: Ca/P Response Curves for Performance

CALCIUM AND PHOSPHORUS REQUIREMENT OF SWINE FROM
WEANING TO MARKET: DEVELOPMENT OF RESPONSE CURVES
FOR PERFORMANCE[1,2]

ABSTRACT

Three trials involving a total of 300 pigs were
conducted to establish response surfaces for the effects of
Ca/P level and time on postweaning pig performance to
market weight.   Five dietary Ca/P levels (70, 85, 100, 115
and 130% of the NRC recommended (1979) dietary Ca and P
levels) were fed from weaning to market.   Nine replicates
of 5 blocks each (5 pigs/block) were used across the 3
trials, and 1 block of pigs per replicate was slaughtered
every 4 wk following the start of the trials.   Efficiency
of feed utilization (EFU) was found to be insensitive to
diet within the range of Ca/P levels used, whereas BW, ADG
and average daily feed intake (ADFI) were linearly ($P<.01$)
and quadratically ($P<.01$) related to both diet and weigh-
period.   Asymptotic models relating continuous effects of
total Ca + P intake relative to the NRC recommended (1979)
Ca + P intake (CAP) and days on test at the midpoint of
each weigh-period to observed performance were fitted to
the data and used to derive response surfaces describing

the effects of CAP and days on test on BW, ADG, and ADFI. Ninety, 95 and 98% of maximum ADG, ADFI and EFU were also determined; 98% of maximum ADG was reached with a CAP level of 99.8% of NRC recommendations, whereas CAP levels of 95.3 and 83.5% of NRC recommendations were required to produce 98% of maximum ADFI and EFU, respectively.

(Key Words: Growth rate, Feed Intake, Calcium, Phosphorus, Pigs.)

Introduction

Formulation of diets to meet Ca and P needs of postweaning swine depend on accurate requirement data over the entire period from weaning to market. Genetic improvement of swine over the last 20 yr has markedly increased leaness in market hogs. This change in body composition has been accompanied by improved efficiency of feed utilization, but average daily gains have not changed greatly during the past few years (Kornegay, 1986).

Few studies have determined effects of different Ca and P levels on performance over the entire growth period. Small increases in ADG and efficiency of feed utilization of growing-finishing swine have been reported in response to levels of Ca/P slightly above NRC (1979) recommendations (Fammatre et al., 1977; Parker et al., 1975; Thomas and Kornegay, 1981). In much of the subsequent work, the range of Ca and P levels used was often restricted to only levels in excess of that suspected to produce desired performance.

Therefore, the first objective of this study was to monitor performance of postweaning swine in response to differing levels of dietary Ca and P from weaning to market. A second objective was to establish response surfaces predicting effects of Ca and P intake on performance of swine over a wide range of dietary Ca and P levels during the period from weaning to market.

Experimental Procedures

Three trials were conducted, using a total of 300 crossbred pigs (avg initial wt, 10 kg), to determine performance from weaning to market of pigs fed a wide range of dietary Ca/P levels, and to establish response surfaces for the effects of time and Ca/P on performance. Five dietary treatments were used, representing 70, 85, 100, 115 and 130% of NRC recommended Ca/P levels (NRC, 1979). Proportions of calcium carbonate and dicalcium phosphate in the diet were varied to obtain desired Ca/P levels. The same relative Ca and P levels for each dietary treatment were maintained across four phases of growth as defined by the NRC recommended nutrient density changes. Pigs were changed to the next phase diet when the average weight of all pigs reached reached the next higher weight classification. Table 1 gives composition of the control (100% NRC) diet for each phase.

Pigs were blocked by sex and sire; each block contained five pigs and there were five blocks per replicate. One pig from each block within a replicate was randomly assigned to each of the five dietary treatments, resulting in five pigs per treatment in each replicate. At the time of allotment, one block per replicate was randomly assigned to each of five slaughter times (approximately every 4 wk). Ten pigs in trials 1 and 2 and six pigs in trial 3 were slaughtered at the beginning of the trial. One additional replicate in each trial (25 pigs per trial) was randomly selected for periodic biopsy (Combs et al., 1989). Pigs in the biopsy replicate contributed gain data throughout the trials and were all slaughtered at the end of the trial.

Pigs were initially housed in temperature-controlled nurseries (.22 $m^2 \cdot pig^{-1}$ of floor space) which were maintained at 27 C for the first 2 wk of the trial, with a 2 to 3 C decrease each week for the remaining 2 wk in the nurseries. Each pen contained the pigs for a diet-replicate combination. Pigs were moved to growing-finishing facilities at the end of 4 wk on test (9 to 10 wk of age and 20 to 25 kg body weight). Initially, at least .45 $m^2 \cdot pig^{-1}$ was provided in these pens. This space allowance was increased to approximately 1.85 $m^2 \cdot pig^{-1}$ at market weight. Feed and water were provided ad libitum.

Pigs were weighed bi-weekly and feed intake per pen during the preceding 2 wk was recorded. There were 11 periods each in trials 1 and 3, and 10 periods in trial 2. The lengths of trials 1, 2 and 3 were 138, 140 and 131 d, respectively. At the end of wk 4, and approximately every 4 wk subsequently, one block of pigs per replicate was slaughtered, for a total of five slaughter periods, in addition to the initial slaughter at the beginning of the trial.

Because of some variation in calculated and analyzed dietary Ca and P levels, analyzed values were used for statistical analysis. The Ca plus P ratio term (CAP) used to characterize different dietary Ca/P levels was defined as the ratio of the analyzed average daily intake of Ca plus P across the entire length of each trial divided by the calculated NRC average daily intake of Ca plus P recommended during the same time. The recommended average daily Ca plus P was calculated using table values for ADG, and the expected efficiency and average daily feed intake (ADFI) corresponding to the NRC (1979)-recommended Ca and P levels in each of four dietary phases used in the study. Averaged over trials, mean analyzed values for CAP were 80.8, 88.3, 102.3, 118.8 and 132.2% of the NRC (1979)-recommended Ca/P levels for diets 1 through 5, respectively. Therefore CAP and time on test corresponding

to the midpoint of each weigh-period were unique to each trial, but generally similar.

For analysis of weight, ADG, ADFI and EFU (gain:feed ratios), a series of ANOVA models were fit to evaluate either discrete effects of trial, diet, pen within trial and diet, period and their interactions, or continuous effects of CAP, time on test (in days) and their interactions. Orthogonal polynomial contrasts for the effects of Ca/P level and weigh-period on performance were determined for the fixed effects model. Based on the consistency of results across trials, the data from the three trials were pooled for final analysis.

Effects of the biopsy procedure on performance criteria were evaluated by ANOVA using a model including diet and biopsy effects within each trial and weight period. Effects of sex on performance criteria were evaluated in similar fashion. Effects of biopsy and sex within trial and period were generally nonsignificant (P>.10) for performance criteria, therefore these effects were excluded from the final model for performance criteria.

Since the response of performance criteria to CAP and time was expected to be asymptotic, nonlinear asymptotic models were considered as response surface models for performance data. As a first step, the asymptotic model

$Y = A[1 - Be^{(-k \times SCAP)}]$ was fit within each trial and
period (Model 1) to the trial x diet x period least squares
means for performance (generated from the model shown in
Table 2). In this model, SCAP is the scaled CAP ratio,
where SCAP = (CAP - minimum CAP) for each trial, and the
asymptotic parameters A, B and k are defined such that:

A = maximum performance

B = percentage reduction in Y between the maximum (A)
and the lowest CAP level.

k = the rate at which maximum performance is
approached; i.e., k is the proportion by which the
distance to maximum is reduced for each unit
increase in CAP.

Estimates of A, B and k for each period were plotted
by time for each trial in order to determine if any
discernible patterns for these parameters over time
emerged. Total error sums of squares for Model 1 were
calculated by summing the error sums of squares from the
nonlinear models across periods and trials. The mean
square error from this model was used as the estimate of
residual mean square error in subsequent lack of fit tests.

Using the estimates of A from each trial and period
from model 1, the observed BW, ADG, ADFI and EFU were
divided by the trial-period maximum to determine the
percentage of maximum (Y/A) for each datum. Linear and
quadratic regressions of estimates of A for each period on
time were likewise performed for each trial to describe

predicted maximum performance (A) as a function of time. Then, as a second step (Model 2), the asymptotic model $Y/\tilde{A} = [1 - (B_0 + B_1t + B_2t^2)e^{(-k \times SCAP)}]$ was fit within each trial where $\tilde{A}$ was a linear and quadratic function of time (i.e., $\tilde{A} = \alpha_0 + \alpha_1t + \alpha_2t^2$ and where t is day on test). The percentage reduction in Y between the highest and lowest Ca/P level was fitted as a linear and quadratic function of time as well (i.e., $\tilde{B} = \beta_0 + \beta_1t + \beta_2t^2$). The purpose of Model 2 was to allow performance within each trial to be described as a continuous function of time and Ca/P level.

The model $Y/\tilde{A} = [1 - \tilde{B}e^{(-k \times SCAP)}]$ was also fit across trials (Model 3) for SCAP = (CAP - lowest CAP in any trial). Values for $\tilde{A}$ were derived from second degree polynomials on time within each trial (Model 3) or across the 3 trials (Model 4). Values for $\tilde{A}$ were identical for Models 2 and 3 but were calculated ignoring trial effects for Model 4. $\tilde{B}$ was fit as a linear and quadratic function of time in both models. Models 3 and 4 allowed data to be pooled across trials. Model 3 used within-trial estimates of $\tilde{A}$ under the hypothesis that differences among trials were primarily attributable to differences in A (maximum). Model 4 used common estimates of $\tilde{A}$ across trials.

Error sums of squares for all $Y/\tilde{A}$ models were calculated by multiplying predicted values $Y/\tilde{A}$ by $\tilde{A}$ to

derive an estimate of Y, squaring the deviation of Y from the actual values and summing these deviations. Total sums of squares used to calculate R^2 for the models was obtained from Model 1.

Mean squares for lack of fit on each model were calculated by dividing the difference in error sums of squares between the model in question and Model 1 by the difference in error degrees of freedom. Tests of lack of fit were the obtained by dividing this mean square by the residual mean square error from Model 1. Although not an exact test (due to non-independence of LS means), it gives an estimate of the reduction in fit observed with each sequential simplification of the model. R^2 values for each model were calculated by dividing the difference between total sum of squares and error sum of squares of each model by the total sum of squares.

Overall ADG, ADFI and EFU ratios were also determined for each trial and diet. These values were fit to the asymptotic model $Y = A[1 - Be^{(-k \times SCAP)}]$ (ignoring trials) and the predicted CAP level associated with 90, 95, and 98% of maximum overall performance was determined.

Results and Discussion

<u>Effects on Sex on Performance Criteria.</u> The effects of sex were never significant in two successive periods for any performance criterion, and sex effects were significant in

only three periods (P<.05) for ADG, which had the highest
number of significant cases. Crenshaw et al. (1981)
reported no differences in gain or feed efficiency of gilts
and barrows in response to dietary Ca/P levels of .4/.4 or
.8/.8% Ca/P, fed from 1 to 3 mo of age, although barrows
gained faster at 5 and 7 mo of age. Likewise, Thomas and
Kornegay (1981) reported barrows gained faster with equal
efficiency, when compared with gilts during the finishing
phase. To the contrary, Nielsen (1972) concluded little
genetic difference due to sex exists for the requirement of
pigs for Ca and P.

Evaluation of Slaughter carcasses. Slaughter carcasses
were visually appraised for gross skeletal abnormalities,
particularly for beading of the ribs as indications of
dietary Ca and P insufficiency. At the time of slaughter,
beading of carcass ribs was scored using a scale of 1
through 5 corresponding to severe, moderate to severe,
moderate, mild, very mild, or no beading. The overall
incidence of beaded ribs (score of 2 or higher) was 18%.
The majority of the observed cases of beaded ribs were
associated with the lowest CAP level (58% of the total
cases); 30, 8, 0 and 4% of the total cases were observed on
diets 2, 3, 4 and 5, respectively. Overall, 52% of the
pigs fed the lowest diet exhibited beaded ribs at
slaughter. The incidence of rib beading decreased at a

decreasing rate (P<.01) with increasing CAP level, and increased linearly (P<.01) with time on test. The response to the diets also differed over time (P<.01); very few cases of beaded ribs were observed on diets 4 and 5 at any time, whereas the incidence of beaded ribs on diets 1 and 2 increased steadily with time. Rib beading has been shown to accompany deficiency of both Ca and P (Miller et al., 1962, 1964). The high incidence of beaded ribs in pigs fed the 70% NRC recommended CAP diet, and to a lesser extent, in pigs fed the 85% NRC recommended CAP diet, indicates that the lower levels of Ca/P used in this study were deficient with respect to the needs of the pig.

<u>Diet Effects on Performance.</u> For the discrete effects model (Table 2), BW, ADG and ADFI increased at a decreasing rate with increasing dietary Ca/P (P<.01), whereas EFU only tended to increase linearly (P<.10) as Ca/P increased (Tables 3, 4, 5 and 6). Similarly, Maxson and Mahan (1983) reported that ADG and ADFI increased linearly and quadratically in response to increasing dietary Ca and P levels in a constant Ca:P ratio, but that there were no significant effects of dietary Ca/P level on EFU. Reinhard et al. (1976), Fammatre et al. (1977), Calabotta et al. (1982) and Kesel et al. (1983) have also reported improved performance in response to increased Ca and P levels with a constant Ca:P ratio. Contrary to the results obtained in

the current study, Pond et al. (1975), Hines et al. (1979) and Nimmo et al. (1980) have reported no effect on ADG, ADFI or EFU due to increasing the Ca/P level in the diet; however, the levels used in those studies were from 100 to 230% of NRC recommendations.

Time Effects on Performance. The effect of period in the discrete effects model (Table 2) was significant for all performance criteria. Orthogonal contrasts of period means indicated that BW increased at a decreasing rate (P<.05) from weaning to market (Table 3). ADG and ADFI increased at a decreasing rate (P<.01) with time during the growing-finishing period (Tables 4 and 5), while EFU declined at a decreasing rate (P<.01) over the same period (Table 6). Few researchers have quantified changes in performance criteria with time over the entire weanling, growing and finishing period. Previously, Crenshaw et al (1981) and Kesel et al. (1983) reported that daily gain increased at a decreasing rate with time from weaning to market. Kesel (1983) reported a linear decline in feed efficiency, whereas Crenshaw (1981) found that efficiency increased until the pigs were about 4 mo of age and then declined.

Interaction Effects on Performance. Diet by period effects were significant for BW (P<.01), ADG (P<.05) and ADFI (P<.01); in all three cases, the interaction was due to smaller increases in performance over time for pigs on the

lowest Ca/P level (Tables 3, 4 and 5). The diet by period interaction was also significant for EFU, although differences between the diets over time were due to differences in slope alone (diet by linear time effect; $P<.05$; Table 6). Differential effects of Ca or P level on performance criteria over time have been reported previously. Crenshaw (1986) found that gain increased with P level and that the magnitude of response differed over time. Kesel et al. (1983) reported that BW was higher for boars fed 150% NRC recommended Ca/P than for boars fed 100% Ca/P, and that the difference increased over time.

Interactions of trial with diet or period were due mainly to slight differences in CAP level and period length between trials, and no trial by diet by period interactions were significant. Therefore, pooling the data across trials for derivation of response surfaces was considered proper. Although the time trends for EFU were strong, the effects of CAP on this variable were not consistent; therefore, we concluded that a response surface could not be derived for EFU.

Asymptotic Performance Models. The asymptotic model fit within trial and period (Model 1) had R^2 = 99.9, 90.8 and 98.0, for BW, ADG and ADFI least squares means, respectively. The degrees of freedom, mean square error and R^2 for this model are shown in Table 7. Estimates of A

increased in a nearly linear manner with time for BW and ADFI, but was clearly quadratic for ADG for each trial (Figure 1). Estimates of B for BW, ADG and ADFI were quadratic across period for each trial (Figure 2). There was no clear pattern discernible for k, although k did vary significantly among periods. Values of A were very consistent across trials, while estimates of B and k were less consistent.

Pooling across periods (Model 2) was deemed absolutely necessary for interpretation of the relationships of the response criteria to diets over time. There was no significant lack of fit for ADFI (P<.10), but significant lack of fit was encountered for BW and especially for ADG due primarily to differences in the behavior of the B and k parameters. The lack of fit for BW and ADG was viewed as an unavoidable consequence of the stated objectives. The R^2 for Model 2 were 99.7, 66.2 and 94.1%, for BW, ADG and ADFI, respectively (Table 7). The interpretation of diet and time trends were consistent across all trials for the three response criteria (Table 8), suggesting that the lack of fit detected did not affect the general conclusions that could be drawn from the data and that pooling across trials would be appropriate.

In Model 3, the maximum (A) was determined within each trial and B and k were determined across trials. Lack of

fit was significant for the across-trials model (Model 3) for all three performance characteristics (P<.01 for BW, ADFI and ADG). This result was likely a carry-over from differences in the behavior of B and k cited earlier. R^2 values for the across-trials models were 98.5, 61.1 and 90.6 for BW, ADG and ADFI, respectively (Table 7).

The results obtained when all parameters were estimated ignoring trials (Model 4) were consistent with the previous model. R^2 values were 97.6, 57.0 and 82.9% for BW, ADG and ADFI, respectively (Table 7). The estimates of A and B, as functions of time, and k for this model are presented in Table 8 and the resultant response surfaces for BW, ADFI and ADG, respectively, are shown in Figures 3, 4 and 5.

Using the estimates of A expressed as function of time across trials, the lack of fit was significant for all three performance characteristics (P<.01 for BW, ADFI and ADG). The predicted values, generated from this model, mirror the mean data rather well, however, suggesting that the actual lack of fit detected was small in magnitude.

The asymptotic analysis of BW indicated that the R^2 remained very high for all models, even when pooling the data across periods within trials, and subsequently, across trials and periods. For ADFI, lack of fit was not significant when the data was pooled across periods; the

incremental decreases in R^2 were similar when the data were pooled across trials and by-trial estimates of A were used. For ADG, the greatest increase in error was associated with pooling the data across periods (see Figure 2), with smaller decreases in R^2 for subsequent pooling. These data suggested that the significant lack of fit seen for BW, ADFI and ADG as further pooling was employed was due to trial differences. The variation in trials probably is unavoidable, however.

The asymptotic response surface predicted for BW (Figure 3) shows a very linear response to time from weaning to market. This response to time is very similar in shape to that shown by Kesel et al. (1983) although the response is of greater magnitude. The predicted response of ADFI to time (Figure 4) from weaning to market appeared to be mostly linear, although it tended to increase at a decreasing rate during the finishing phase. ADG (Figure 5) was predicted to increase quadratically with time, and decrease after 110 d on test. This agrees with the reports of Crenshaw et al. (1981) and Kesel et al. (1983).

The predicted BW and ADFI responses to CAP level were nearly linear initially and became increasingly curvilinear over time; there appeared to be little difference among the four highest CAP levels at any time. ADG responded quadratically to CAP from the beginning, with little

difference among the four highest CAP levels; the magnitude
of difference between pigs on the lowest CAP level and
those on the upper four CAP levels increased with time. In
a summary of Ca and P research, Kornegay (1986) fit
asymptotic models to combined data from a number of
research trials conducted since 1969. He showed rather
conclusively that ADG increased until 80 to 85% of the NRC
(1973) recommended Ca/P level was reached and that the
response then plateaued, as was seen in the current study.
This pattern indicates that there is no advantage in daily
gain when higher than NRC recommended levels are fed.

Good agreement of the results of our study with the
published literature suggests that the response surface
equations reported for BW, ADG and ADFI can be used to
predict responses to dietary Ca/P within the range used in
this study at any time from weaning to market weight.
Overall Performance Maximums. The CAP levels associated
with 90, 95 and 98% of the overall maximum ADG, ADFI and
EFU are reported in Table 9. Although the overall diet
effects on EFU were only nearly significant, EFU was
predicted to approach maximum at the lowest CAP levels (60,
70 and 84% of NRC recommended Ca/P for 90, 95 and 98%
maximum overall EFU). This was a reflection of the
insensitivity of EFU to the range of dietary CAP levels
used in this study. The CAP level associated with maximum

EFU was midway between that obtained in the combined data summary of Kornegay (1986) for pigs fed lower finishing levels of Ca/P and those fed the same level in the growing and finishing periods. The range in Ca/P levels and in the response was smaller in the current study than in the combined data of Kornegay (1986) and may have contributed to the differing maximums obtained.

ADG and ADFI required higher CAP levels to achieve 90, 95 and 98% of maximum performance. The 98% maximum ADG in this study was obtained at approximately 100% of the NRC recommended level, whereas Kornegay (1986) reported that 98% of maximum ADG was obtained with 81% of the NRC recommended Ca/P level. The percentage of maximum associated with each CAP level for the current study is shown graphically in Figure 6. Based on these predicted maximums, current NRC recommendations for Ca and P appear to be adequate to produce at least 98% of maximum ADG in growing-finishing pigs, whereas these dietary Ca/P levels are far in excess of that required to maximize the efficiency of gain.

Implications

The results of this research indicate that the current

NRC recommended levels of Ca/P are adequate with respect to

the growth performance of the pig from weaning to market

weight. The response curves derived for body weight, daily

gain and daily feed intake can be used to predict the

response of Ca/P levels from 70 to 130% of the NRC

recommendation for any period of time between weaning and

market. This will aid in interpretation of Ca/P effects

obtained for smaller time periods in other studies.

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TABLE 1.   PERCENTAGE COMPOSITION OF CONTROL DIET (100% NRC)

Ingredient	Phase[a]			
	1	2	3	4
Ground corn	72.07	77.67	77.97	83.15
Soybean meal, 49% CP	24.89	19.86	19.81	14.81
Dicalcium phosphate[b]	1.15	.98	.70	.53
Ground limestone[b]	.89	.89	.92	.91
Vitamin-Se premix[c]	.35	.20	.20	.20
TM premix[d]	.05	.05	.05	.05
Salt	.35	.35	.35	.35
Antibacterial[e]	.25	-	-	-

[a]Diets were changed according to phase as follows: Phase 1 - 10 to 20 kg; phase 2 - 20 to 35 kg; phase 3 - 35 to 60 kg; phase 4 - 60 to 100 kg.

[b]Proportions were altered to obtain 70, 85, 115 and 130% of basal diet levels for diets 1, 2, 4 and 5, respectively, with some adjustments in corn and soybean meal.

[c]Supplied (per kg diet): 4400 IU vitamin A, 440 IU vitamin D_3, 11 IU vitamin E, 1.1 mg vitamin K, 4.4 mg riboflavin, 22 mg pantothenic acid, 22 mg niacin, 22 ug vitamin B_{12}, 440 mg choline, 440 ug biotin, .3 mg Se during phase 1; during subsequent phases, provides: 3520 IU vitamin A, 352 IU vitamin D_3, 8.8 IU vitamin E, .88 mg vitamin K, 3.52 mg riboflavin, 17.6 mg pantothenic acid, 17.6 mg niacin, 17.6 ug vitamin B_{12}, 352 mg choline, 352 ug biotin, and .1 mg Se.

[d]Supplied (per kg diet): 100 mg zinc, 50 mg iron, 27.5 mg manganese, 5.5 mg copper and .75 mg iodine.

[e]Supplied (per kg diet): 110 ppm chlortetracycline, 55 ppm procaine penicillin and 110 ppm sulfamethazine.

TABLE 2. MEAN SQUARES AND SIGNIFICANCE OF EFFECTS
OF TRIAL, DIET, PEN, PERIOD AND INTERACTIONS ON
BW, ADG, ADFI AND EFU[a]

Source	df[b]	BW	ADG	ADFI	EFU
Trial	2	492.8**	.034+	3.581**	.098**
Diet[c]	4	2269.4**	.442**	4.015**	.003+
Trial x Diet	8	32.4**	.009	.257**	.000
Pen(trial x diet)	25	77.9**	.017	.224**	.005**
Period[d]	10	40668.3**	.637**	23.730**	.444**
Trial x Period	19	237.5**	.088**	.676**	.025**
Diet x Period	40	119.5**	.019*	.164**	.004**
Trial x Diet x Period	76	8.2ns	.014	.083	.002
Error	245	9.5	.013	.070	.002
R^2		99.5	79.8	94.7	93.3

[a]The significance is denoted as follows: **P<.01, *P<.05, +P<.10.
[b]Degrees of freedom for ADG, ADFI and EFU; for BW, there were 11 df for period, 21 df for trial x period, 84 df for trial x diet x period and 270 df for error.
[c]Linear (P<.01) and quadratic (P<.01) effects of diet on BW, ADG and ADFI; linear (P<.10) effect of diet on EFU.
[d]Linear (P<.05) and quadratic effect of time on BW; quadratic (P<.01) effect of time on ADG, ADFI and EFU.

TABLE 3. LEAST SQUARES MEAN BODY WEIGHT (KG), BY DIET AND
PERIOD, FOR PIGS FED FIVE DIETARY CA/P LEVELS FROM
WEANING TO MARKET WEIGHT

Period[c]	Ca/P level (% of NRC)[ab]					SEM
	70	85	100	115	130	
0	10.0	10.0	9.9	10.0	9.9	.1
1	14.9	15.6	15.7	15.9	15.8	.2
2	20.5	22.2	22.8	22.8	22.6	.3
3	26.6	29.3	30.0	29.8	29.3	.4
4	34.1	39.2	40.6	39.9	40.2	.5
5	41.9	49.7	51.8	51.1	51.7	.6
6	48.4	59.7	61.8	61.0	62.1	.7
7	54.8	67.4	70.8	70.3	70.5	.8
8	63.4	80.7	83.3	83.3	83.8	1.0
9	73.9	94.2	95.9	97.5	97.1	1.2
10	80.7	101.8	102.7	105.2	102.9	1.2
11	87.7	110.4	107.0	110.5	109.4	1.6

[a]Linear (P<.01) and quadratic (P<.01) increase in response
to increasing dietary CAP level.
[b]Significant diet by period interaction (P<.01) due
primarily to poorer performance over time for pigs fed
lowest CAP level.
[c]Linear (P<.05) and quadratic (P<.01) increase in response
to increasing time on test.

TABLE 4. LEAST SQUARES MEAN AVERAGE DAILY GAIN (G), BY DIET AND PERIOD, FOR PIGS FED FIVE LEVELS OF DIETARY CA/P FROM WEANING TO MARKET WEIGHT

	Ca/P level (% of NRC)[a][b]					
Period[c]	70	85	100	115	130	SEM
1	355	413	428	430	428	24
2	500	579	620	608	595	33
3	534	618	622	631	600	34
4	520	706	748	724	761	40
5	513	717	776	775	802	41
6	528	789	783	767	834	42
7	647	768	904	879	803	46
8	621	907	891	885	920	48
9	677	887	801	927	845	47
10	612	646	708	740	717	39
11	791	846	742	843	819	54
Avg.	551	703	728	737	731	14

[a]Linear (P<.01) and quadratic (P<.01) increase in response to increasing dietary CAP level.
[b]Significant diet by period interaction (P<.05) due primarily to poorer performance over time for pigs fed lowest CAP level.
[c]Quadratic (P<.01) increase in response to increasing time on test.

TABLE 5.   LEAST SQUARES MEAN AVERAGE DAILY FEED INTAKE (G),
BY DIET AND PERIOD, FOR PIGS FED FIVE LEVELS OF CA/P FROM
WEANING TO MARKET WEIGHT

Period[c]	Ca/P level (% of NRC)[ab]					
	70	85	100	115	130	SEM
1	674	733	711	690	725	28
2	1,000	1,148	1,173	1,140	1,153	45
3	1,201	1,351	1,414	1,371	1,366	54
4	1,471	1,793	1,836	1,801	1,897	70
5	1,479	1,911	1,959	2,004	1,974	75
6	1,704	2,249	2,299	2,356	2,457	89
7	1,844	2,655	2,808	2,577	2,657	100
8	2,158	2,871	2,996	2,960	2,875	111
9	2,249	3,132	3,040	3,189	3,073	118
10	2,501	2,848	3,063	3,117	2,729	114
11	2,523	3,324	2,995	3,217	3,278	122
Avg.	1,628	2,069	2,130	2,120	2,091	22

[a]Linear (P<.01) and quadratic (P<.01) increase in response
to increasing dietary CAP level.
[b]Significant diet by period interaction (P<.01) due
primarily to poorer performance over time for pigs fed
lowest CAP level.
[c]Quadratic (P<.01) increase in response to increasing time
on test.

TABLE 6.   LEAST SQUARES MEAN EFFICIENCY OF FEED UTILIZATION
(GAIN:FEED), BY DIET AND PERIOD, FOR PIGS FED FIVE LEVELS
OF CA/P FROM WEANING TO MARKET WEIGHT

	Ca/P level (% of NRC)[ab]					
Period[c]	70	85	100	115	130	SEM
1	.531	.566	.608	.632	.599	.027
2	.515	.516	.546	.546	.529	.025
3	.462	.467	.450	.470	.446	.021
4	.353	.396	.408	.404	.403	.018
5	.347	.375	.392	.385	.406	.018
6	.315	.359	.345	.331	.340	.016
7	.363	.293	.327	.339	.304	.015
8	.290	.320	.298	.299	.319	.014
9	.300	.286	.272	.290	.277	.013
10	.241	.246	.232	.250	.287	.012
11	.314	.252	.250	.265	.253	.014
Avg.	.372	.383	.388	.395	.391	.006

[a]Linear (P<.10) increase in response to increasing dietary
CAP level.
[b]Significant diet by period interaction (P<.01).
[c]Quadratic (P<.01) increase in response to increasing time
on test.

TABLE 7. DEGREES OF FREEDOM, MEAN SQUARES AND R^2 VALUES FOR THE ASYMPTOTIC MODELS FIT TO LEAST SQUARES MEAN BW, ADG AND ADFI DATA

Criteria	Model	df^a Regr	df^b Error	Mean Square[c] Error	R^2
Weight	Model 1[d]	105	70	1.7008**	99.9
.	Model 2[e]	18	157	3.7953**	99.7
	Model 3[f]	12	163	16.0311**	98.5
	Model 4[g]	6	169	24.6571**	97.6
ADG	Model 1	96	64	.00655**	90.8
	Model 2	21	139	.01110**	66.2
	Model 3	13	147	.01210**	61.1
	Model 4	7	153	.01284**	57.0
ADFI	Model 1	96	64	.03569+	98.0
	Model 2	18	142	.04660+**	94.1
	Model 3	12	148	.07135**	90.6
	Model 4	6	154	.12445**	82.9

[a,b] Degrees of freedom for regression (regr) and error, respectively.

[c] Significant increases in mean square error between sequential models indicating significantly greater lack of fit: **$P<.01$, +$P<.10$.

[d] Model 1: $Y = A\{1-Be^{(-k \times SCAP)}\}$, fit within trial and period. Three weight parameters (A, B and k) estimated in each of 12 periods for Trials 1 and 3 and 11 periods for Trial 2. For ADG and ADFI, the three parameters were estimated in each of 11 periods for Trials 1 and 3, and 10 periods for Trial 2.

[e] Model 2: $Y/\tilde{A} = \{1-\tilde{B}e^{(-k \times SCAP)}\}$, fit within trial. For ADG, $\tilde{A}$ was derived as $(\kappa_0 + \kappa_1 t + \alpha_2 t^2)$ where t is days on test and $\tilde{B}$ was fit as $(\beta_0 + \beta_1 t + \beta_2 t^2)$; for weight and ADFI, $\beta_0 = 0$ since no initial differences would be expected.

[f] Model 3: $Y/\tilde{A} = \{1-\tilde{B}e^{(-k \times SCAP)}\}$, fit across trials. $\tilde{B}$ was fit as linear and quadratic function of days on test. $\tilde{B}$ and k were estimated across trials, while $\tilde{A}$ was obtained within each trial.

[g] Model 4: $Y/\tilde{A} = \{1-\tilde{B}e^{(-k \times SCAP)}\}$, with all parameters fit across trial. $\tilde{A}$ was calculated as a linear and quadratic function of days on test ignoring trials.

TABLE 8. PARAMETER ESTIMATES FOR BY-TRIAL AND ACROSS-TRIAL ASYMPTOTIC BW, ADG AND ADFI MODELS[a]

	Coefficients of A as a function of time			Coefficients of B as a function of time			
Trial	A^0	A^1	A^2	B^0	B^1	B^2	k

------------------ By Trials (Model 2) ------------------

------------------------ BW, kg ------------------------

	A^0	A^1	A^2	B^0	B^1	B^2	k
1	10.029	.7052	.00020	–	.0052	-.000031	.2516
2	9.613	.5842	.00143	–	.0033	-.000013	.4040
3	9.043	.7957	.00045	–	.0061	-.000033	.2078

------------------------ ADG, kg ------------------------

1	.416	.0086	-.00004	.2211	-.0006	.000015	.1228
2	.329	.0107	-.00006	.0328	.0067	-.000044	.3900
3	.389	.0128	-.00008	.0698	.0081	-.000055	.1564

------------------------ ADFI, kg ------------------------

1	.697	.0302	-.00010	–	.0066	-.000044	.2262
2	.136	.0410	-.00015	–	.0077	-.000053	.3217
3	.446	.0381	-.00013	–	.0078	-.000049	.0939

------------------ Across Trials (Model 4)[b] ------------------

BW	9.652	.6950	.00069	–	.0058	-.000033	.0927
ADG	.378	.0107	-.00006	.1846	.0023	-.000006	.0791
ADFI	.446	.0381	-.00013	–	.0078	-.000049	.0939

[a]Refer to Table 7 for definition of models.
[b]Model 3 used with coefficients of A as a function of time ignoring trials and with the across-trial estimates for B, as a function of time, and k.

TABLE 9. PREDICTED CA/P LEVEL, EXPRESSED AS A PERCENTAGE
OF NRC-RECOMMENDED LEVELS, WHICH PRODUCE 90, 95 AND 98
PERCENT OF MAXIMUM AVERAGE DAILY GAIN, AVERAGE DAILY FEED
INTAKE AND EFFICIENCY OF FEED UTILIZATION (GAIN:FEED) FOR
GROWING-FINISHING PIGS

| | Percent of maximum | | |
	90	95	98
Overall ADG	81.5	89.4	99.8
Overall ADFI	79.0	86.0	95.3
Overall EFU	59.7	70.0	83.5

[a]Derived by solving the equation $X = \{1 - Be^{(-k \times SCAP)}\}$, where X = percentage of maximum.
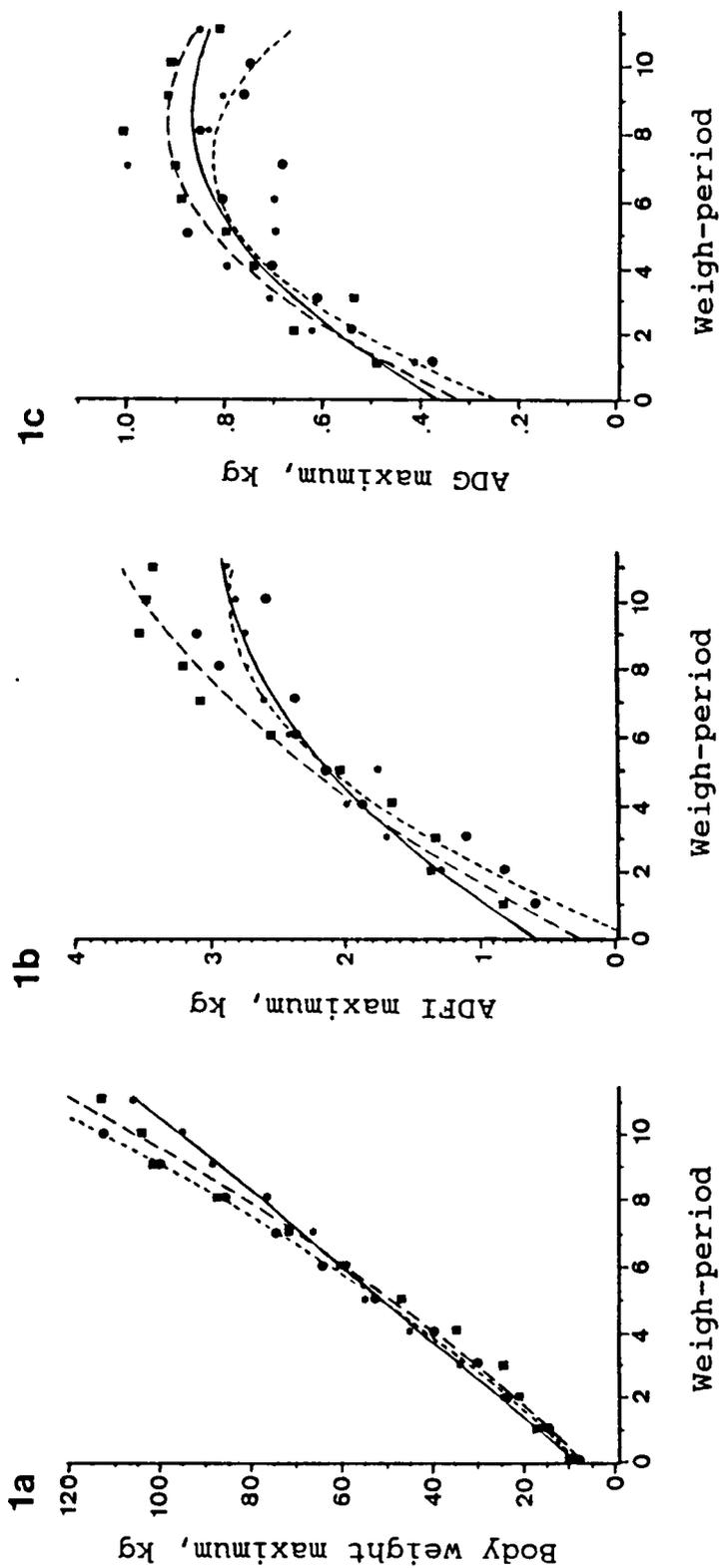
Figure 1. Asymptotic estimates of maximum body weight (1a), ADFI (1b) and ADG (1c), by period for trials 1 (———), 2 (-·•·-) and 3 (—■—), for pigs fed five levels of Ca/P from weaning to market weight.
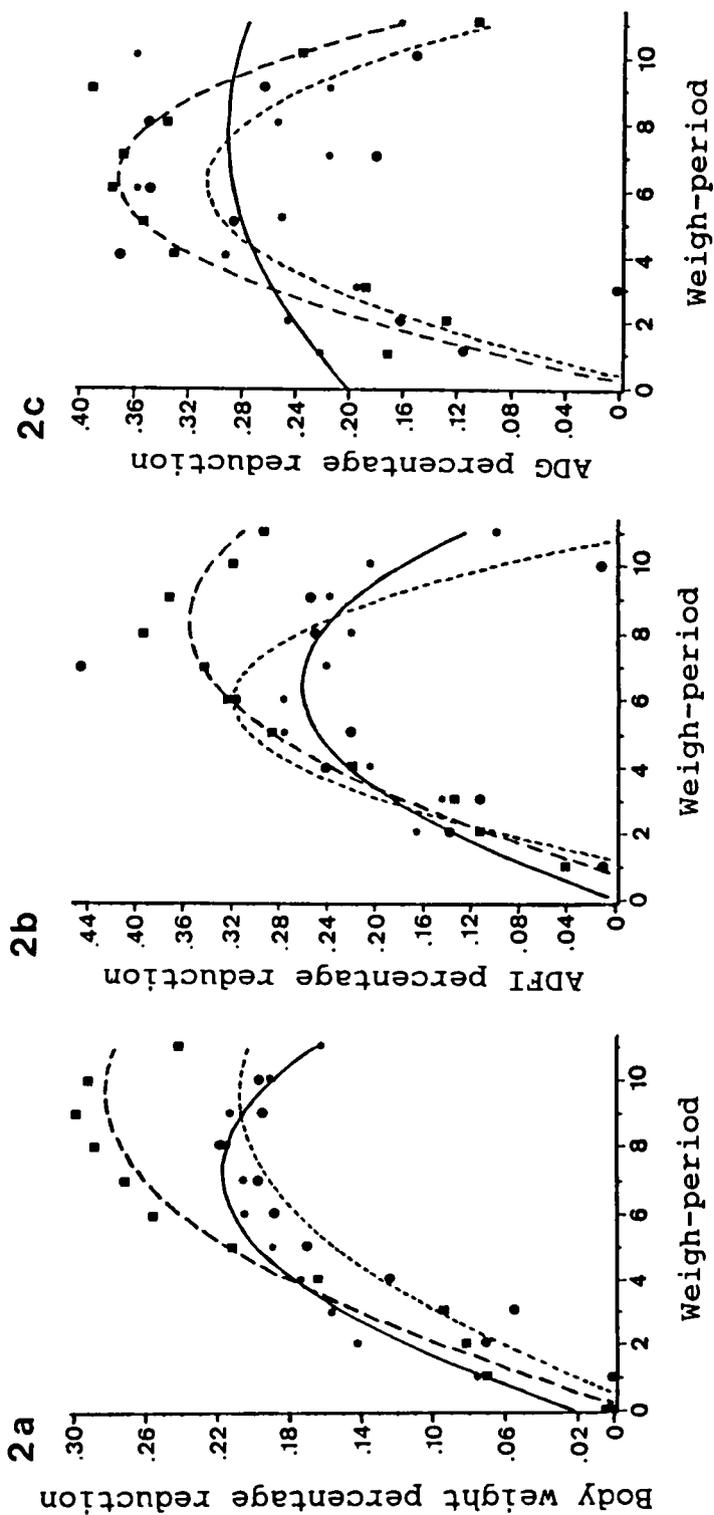
Figure 2. Asymptotic estimates of the percentage reduction in body weight (2a), ADFI (2b) and ADG (2c) associated with the lowest CAP level (i.e. asymptotic B), by period for trials 1 (———), 2 (-·•-) and 3 (-■-), for pigs fed five levels of Ca/P from weaning to market weight.
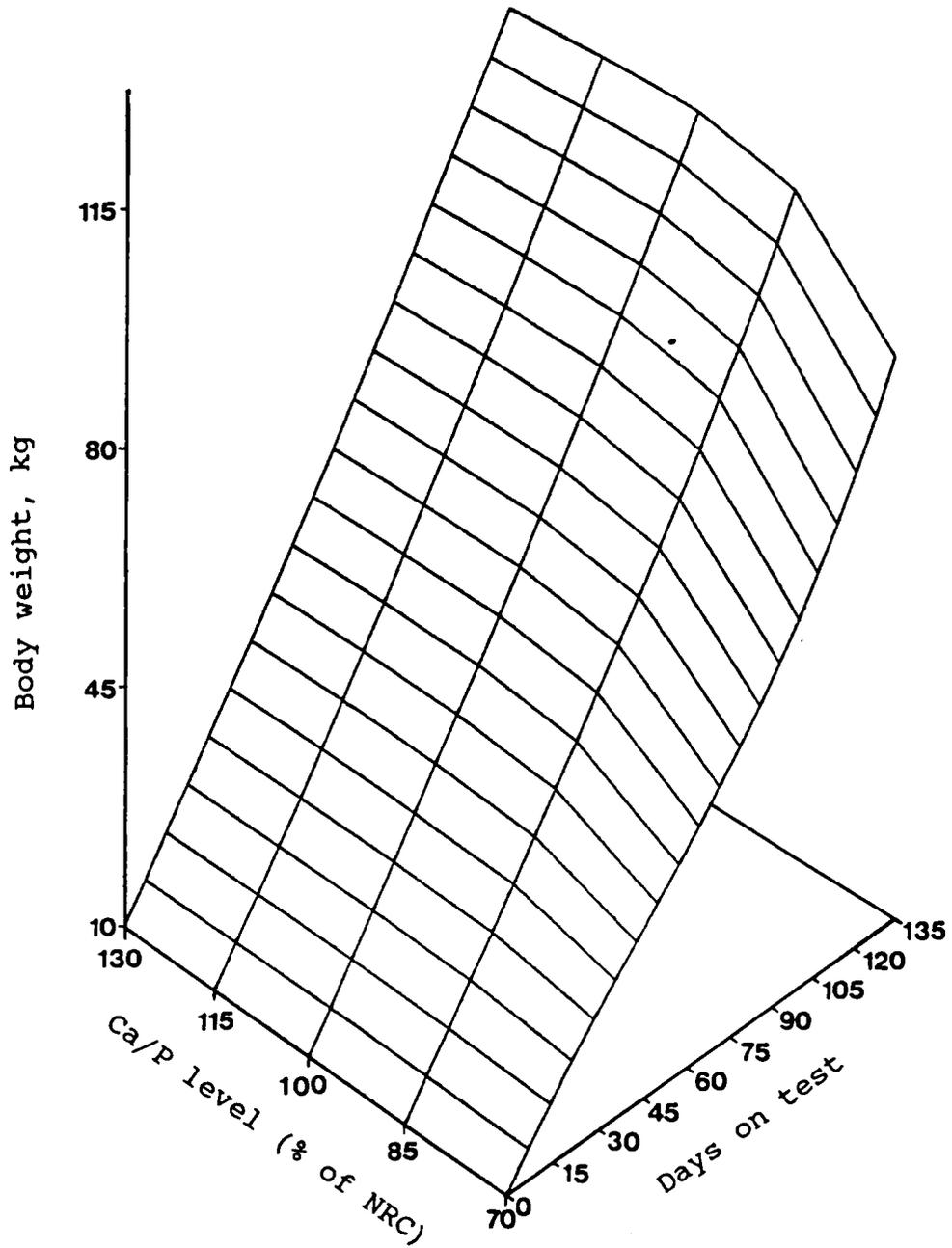
Figure 3. Asymptotic response surface relating the effects of CAP level and time on test to the observed cumulative body weight of pigs fed five levels of dietary Ca/P from weaning to market weight.
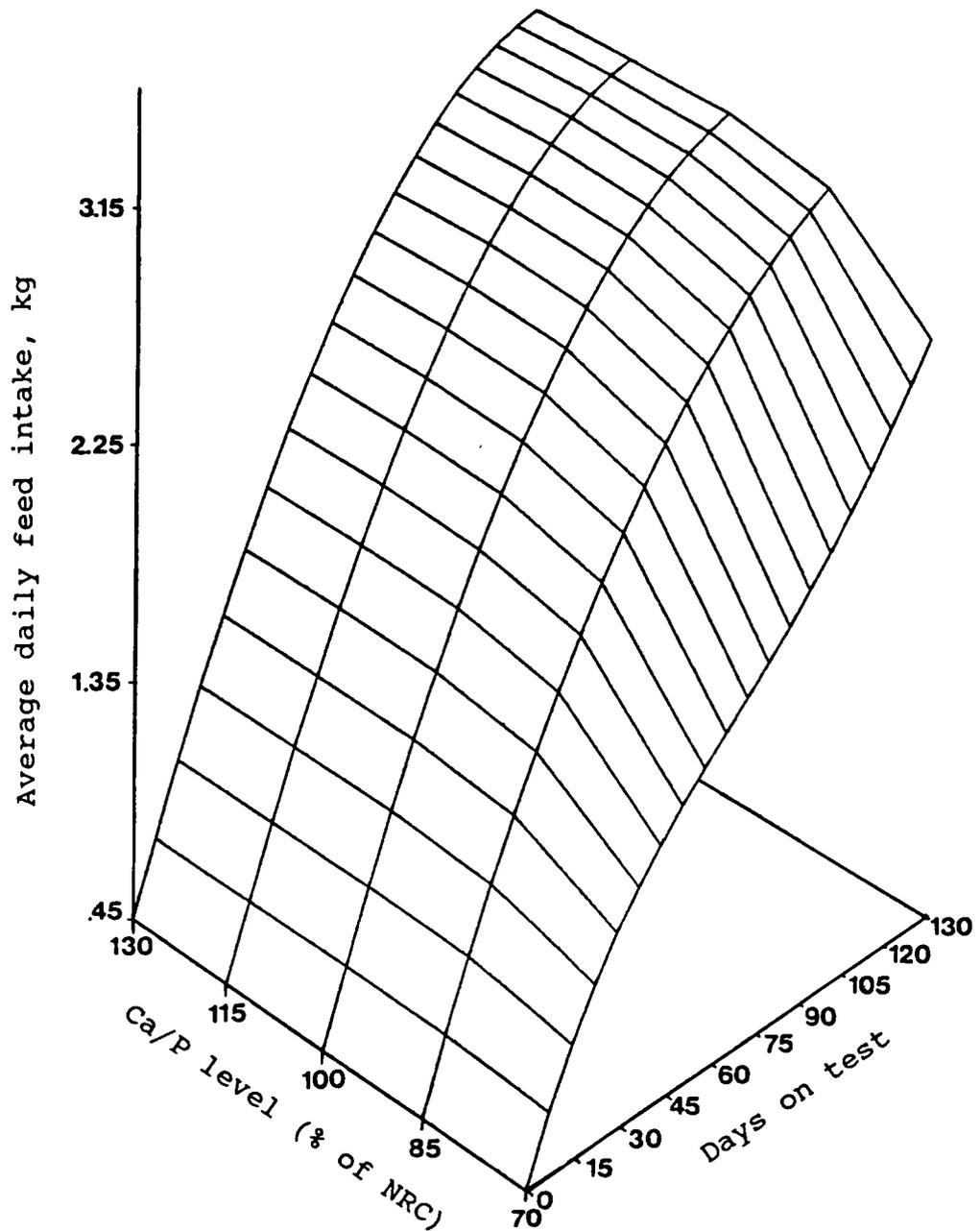
Figure 4. Asymptotic response surface relating the effects
of CAP level and time on test to the observed average
daily feed intake of pigs fed five levels of dietary Ca/P
from weaning to market weight.
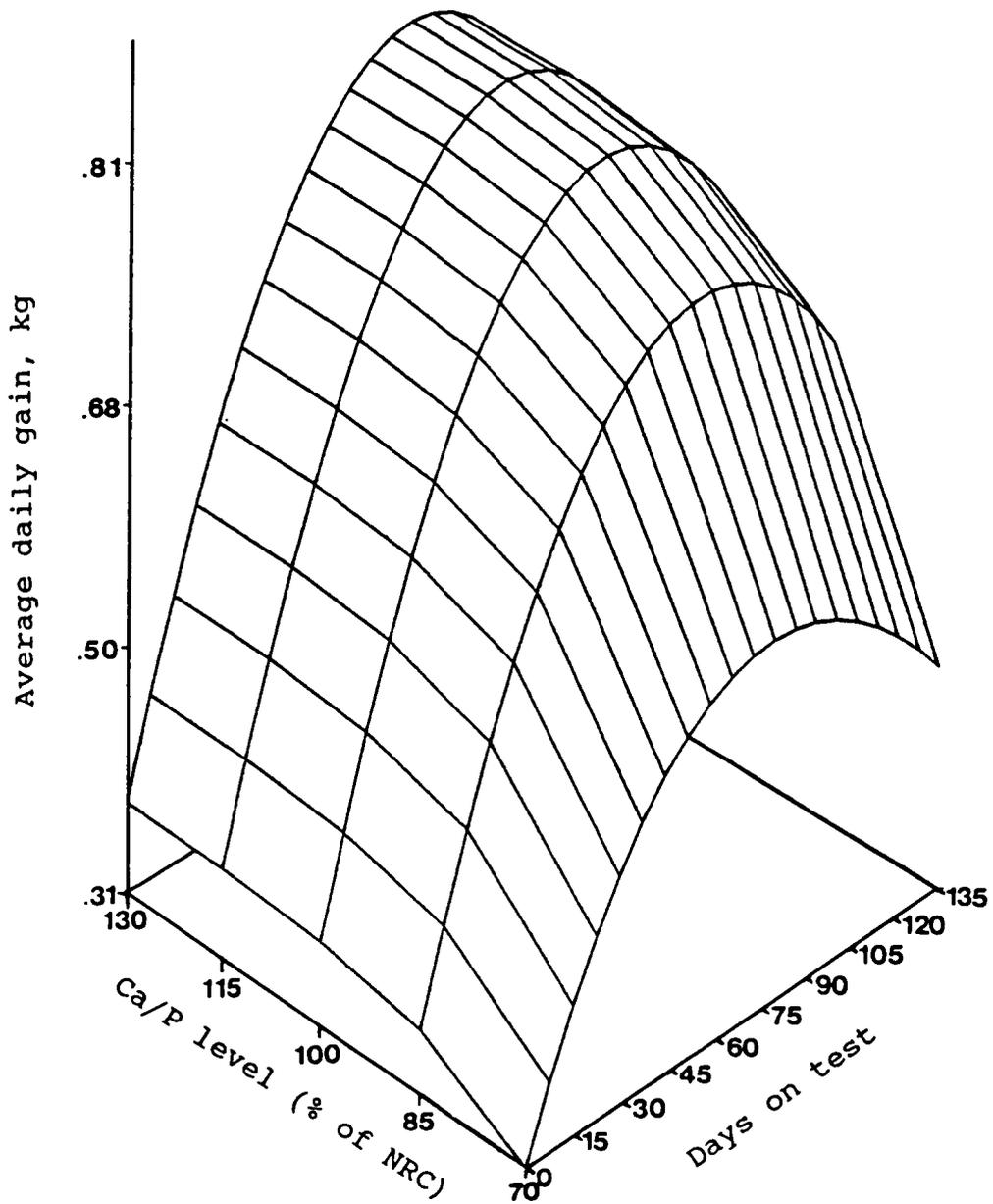
Figure 5. Asymptotic response surface relating the effects of CAP level and time on test to the observed average daily gain of pigs five level of dietary Ca/P from weaning to market.
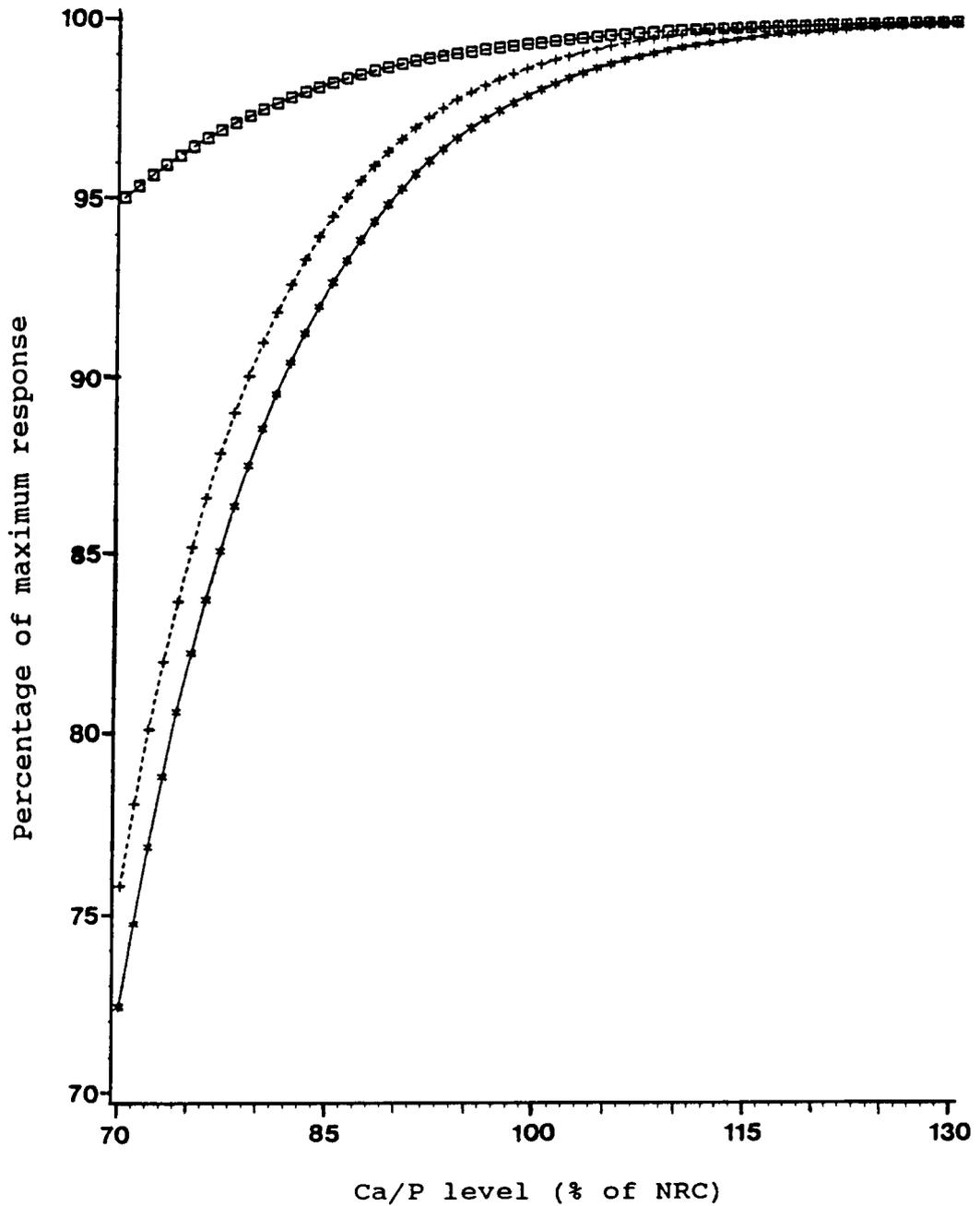
Figure 6. Percentage of maximum average daily gain (*),
average daily feed intake (+) and efficiency of feed
utilization (gain:feed, □) associated with each CAP
level for growing-finishing pigs.

CHAPTER 4

COMPARISON OF BONE BENDING AND SHEAR TESTS FOR
DETERMINING CALCIUM AND PHOSPHORUS STATUS OF SWINE FED
FIVE CALCIUM-PHOSPHORUS LEVELS FROM WEANING TO MARKET[1,2,3]

ABSTRACT

Three trials using 251 pigs were conducted to evaluate

bone bending and shear stress for determining the Ca/P

status of pigs fed five dietary Ca/P levels from weaning to

market [70, 85, 100, 115 and 130% of NRC (1979) recommended

Ca/P]. Shear stress was determined for fourth metacarpal

and metatarsal bones, and compared with stress determined

by three-point bending tests on third metacarpal and

metatarsal bones.   Effects of dietary CAP intake were

linear for bending stress (P<.01), and linear and quadratic

for shear stress (P<.05), with lower SEM for shear stress.

Time effects were linear for both bending and shear stress

(P<.01), with lower SEM for shear stress. Shear stress had

higher R^2 values and lower CV than did bending stress.

Residual correlations between bending and shear stress were

modest, but significant, indicating that the two tests

produced consistent results, although bending stress was

more repeatable across bones from the same animal than was
shear stress. Shear force was less susceptible to effects
of bone length and orientation in the testing apparatus
than was bending force, and calculation of other measures
of bone integrity were simpler for shear stress.
Therefore, shear testing is suggested as a more desirable
method of determining effects of dietary CAP and time on
bone stress.

(Key Words: Bending stress, Shear stress, Calcium,
Phosphorus, Pigs.)

Introduction

The maximum force required to cause three-point
bending failure in bones has long been used as a primary
response variable in Ca and P nutrition studies. Maximum
force is responsive to dietary Ca/P over a wide range of
intake levels, although there is a large amount of
variation in maximum force required to produce failure
(Crenshaw et al., 1981a). Bending force, however, can be
influenced by the spacing of the three points, and may be
confounded with other forces, including shear (Crenshaw et
al., 1981a; Wilson et al., 1984). When the ratio of the
space between the fulcra supporting the bone in a three-
point bending test (L) to the outside diameter of the bone
(D) is less than 10, shear deflection becomes a part of the
measured force deflection for the bending test (Wilson et

al., 1984). Specifically, when the L/D ratio is approximately 5, shear accounts for 50% of the total deflection, whereas, when the L/D ratio is greater than 10, the shear deflection is approximately zero.

Bones that differ in shape also differ in the shape of the area over which force is applied in bending tests, thus affecting the measured force deflection (Crenshaw et al., 1981a). Bending stress adjusts maximum force for the distance over which the force is applied, the orientation of the bone and the area of the bone at the midpoint where force is applied. Bending stress is, therefore, considered a purer expression of bone strength than is simple bending force (Crenshaw et al., 1981a,b; Crenshaw, 1986).

Use of a shear test as an alternative to bending tests has been proposed, since the maximum shear force is unconfounded with other forces that could result in bone failure. Additionally, the shear test is independent of the length of the bone that is tested, and is much less sensitive to the orientation of the bone than is the bending test (Crenshaw et al., 1981a; Wilson et al., 1984).

Therefore, the objective of this study was to compare bone bending and shear stress of third and fourth metacarpals and metatarsals of pigs fed five Ca/P levels from weaning to market for use in evaluating Ca and P status of growing-finishing swine.

Experimental Procedures

Third and fourth metacarpal and metatarsal bones were
recovered from growing-finishing swine fed five Ca/P levels
[70, 85, 100, 115 and 130% of NRC (1979) recommended Ca/P]
and slaughtered at monthly intervals from weaning to market
in order to compare bone bending and shear stress. Pigs
came from a larger Ca/P study designed to examine the
performance and bone characteristics of swine fed widely
varying Ca/P levels. A more detailed description of
experimental procedures was reported by Combs et al.
(1989).

Three trials, involving a total of nine replicates
with five blocks of five pigs in each (25 pigs per
replicate), were conducted. Pigs were blocked by sex and
sire when the average weight of all pigs reached 10 kg.
One pig per block was randomly assigned to each of five
Ca/P treatments. Ten, ten and six pigs were slaughtered at
the beginning of Trials 1, 2 and 3, respectively, to
provide baseline data for initial Ca and P status. Of the
remaining pigs, one block per replicate was randomly
assigned to each of five slaughter times (approximately 4
wk intervals) with final slaughter at approximately 140 d
on test (110 $\pm$ 1.2 kg average body weight).

Immediately after slaughter, metacarpals and
metatarsals were removed and frozen at -20 C in air-tight

plastic bags. Bones were later thawed and extraneous
tissue was removed. Then, bones were weighed and the
overall length of each bone plus the width of the bone
shaft at the narrow and wide dimensions of the bone shaft
were measured (Figure 1). Bones were then refrozen in
moisture-proof bags. Bending strength of the third
metacarpal and metatarsal and the shear strength of the
fourth metacarpal and metatarsal of each slaughter pig was
subsequently determined on an Ingstrom Universal Testing
Machine[4] using the bending and shear fixtures shown in
Figure 2.

Bones were thawed in the air-tight plastic bags
immediately before testing to prevent dessication, and
maintained under refrigeration until testing was completed,
at which time the bones were promptly refrozen for
subsequent measurement of wall thickness and analysis of
ash content. Wet bones have been found to demonstrate less
variability in breaking strength than dry bones (Crenshaw
et al., 1981a; Kornegay et al., 1981; Maxson and Mahan,
1983). Crenshaw et al. (1981a) and Kornegay et al. (1981)
demonstrated that changes in moisture content of the bone
can alter the mechanical properties of the bone, including
the amount of force that the bone will withstand before

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[4]Model 1123, Ingstrom Corp., Canton, MA.

integrity of the bone is compromised. Crenshaw et al. (1981a) reported that as little as 10 min exposure to air could result in changes in the mechanical properties of wet bones. Sedlin (1965) showed, however, that freezing of wet bone did not alter the bone mechanical properties as long as dessication of the bone during the freezing period was prevented.

Force was applied to the bones at a speed of 10 mm · min^{-1} and resulting force curves were plotted at a chart speed of 50 mm·min^{-1}. Maximum force required to cause bending or shear failure was electronically measured and recorded in kg of force.

After bending or shear testing, the bones were cut in half at the midpoint, using a band-saw[5]. Wall thickness at the midpoint of the narrow, wide and perpendicular dimensions was measured using a dial caliper[6] and averaged to determine a value for the wall thickness at the midpoint of the overall bone length (Figure 1). The area of the bone tissue perpendicular to the overall bone length was assumed to be that of a quarter circle. The radius was assumed to be the average of the narrow and wide bone shaft dimensions.

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[5]1.27 cm metal cutting blade, 5.5 teeth per cm, estimated 1.6 mm saw kerf.
[6].001 in. scale, Mitutoyo Corp., Tokyo, Japan.

In order to compare the force required to produce bending failure to that required to cause shear failure, the common unit of stress was calculated for both bending and shear forces. Shear stress ($Stress_s$) was calculated according to the formula (Harner and Wilson, 1985):

$$Stress_s = \text{Maximum force} / (2 * \text{Area}).$$

Bending stress ($Stress_b$) was calculated according to the formula (adapted from Crenshaw et al., 1981a):

$$Stress_b = \frac{0.106 * \text{Maximum force} * \text{Length}}{[(0.44 * r^2 * t) - (1.32 * r * t^2)]}$$

Where: r = radius, t = wall thickness, and Length refers to the spread between the lower 2 points of the 3-point bending fixture (held constant at 3.175 cm in this study).

Effects of trial, slaughter time, diet and their interactions, as well as linear, quadratic and cubic orthogonal effects of dietary Ca/P and slaughter time on bending or shear stress were determined by ANOVA (Model 1[7]). Additionally, linear and quadratic effects of body weight (WT) and interactions of these effects with trial, slaughter time and diet were included as covariates in a second ANOVA model. Effects of sex on stress were evaluated by ANOVA. There were no differences in stress between barrows and gilts (P>.10) in any trial or slaughter time. Therefore, effects of sex were not included in the

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[7]See Table 3.

final model.

ANOVA indicated that trial interactions with effects of diet, slaughter time and slaughter weight were not significant for the third metacarpal (3C), third metatarsal (3T) and fourth metatarsal (4T) (P>.10), but several trial by main effect interactions were significant for the fourth metacarpal (4C) (P<.05). However, it was concluded that pooling data across trials for this analysis was acceptable and necessary, although some interactions involving trial might exist for 4C shear stress.

Comparisons of bending and shear tests were made on the basis of 1) the significance of diet effects, 2) the R^2 values for each test in each model, 3) the residual coefficients of variation for each model, and 4) the correlations between the two bending measures (3C, 3T) and the two shear measures (4C, 4T), and between each bending measure with each shear measure.

Results and Discussion

<u>Effects of Size and Orientation of the Bone.</u> Comparison of bending and shear testing on the basis of stress was necessitated by the possible confounding of other forces with bending force in the measured force deflection obtained in the bending test. In order to avoid confounding changes in the spacing of the fulcra with changes in force obtained with larger bones, the spacing of

the support fulcra was kept constant for all bones at 3.175 cm, which was the widest spacing that would accommodate the smallest bones. This decision could, however, result in confounding of crushing and shear-type failures with the bone failure associated with bending forces due to the dependence of the bending stress obtained on the spacing of the lower 2 points of the apparatus relative to the overall length of the bone.

Another factor that contributed to the need for comparing bending and shear tests on the basis of stress is the dependence of the bending force obtained on the orientation of the bone in the bending apparatus. Since the bone shaft is irregularly shaped, the bending force obtained would differ depending on how the bone was placed in the bending apparatus. We attempted to minimize this effect by consistently laying the bone on its medial surface (see Figure 1).

Effects of Ca/P and Time. Overall effects of both Ca/P level and slaughter time on bending (3C, 3T) and shear (4C, 4T) stress were significant (P<.01). Ca/P effects were linear and quadratic (P<.01) for shear stress (Table 1). The Ca/P effect was linear (P<.01) for bending stress, but the quadratic component of the response only approached significance (P<.10 and .12 for 3C and 3T, respectively). Nimmo et al. (1980) also reported that stress responded

linearly to increasing dietary Ca/P level. Others, using
only two levels of Ca and P ranging from 70 to 150% of the
NRC-recommended levels, have reported increasing bone
strength with increasing Ca/P level (Crenshaw et al.,
1981b; Kesel et al., 1983; Lepine et al., 1985; Nimmo et
al., 1981; Parker et al., 1975).

Both bending and shear stress were linearly (P<.01)
related to time (Table 2). Crenshaw (1986) found that
stress increased linearly with time, whereas Crenshaw et
al. (1981b) reported inconsistent effects of time on
metacarpal and metatarsal stress.

Time by diet interactions were significant for shear
stress measures, but not for bending stress (Figure 3,
P<.01). Based on the work reported by Crenshaw (1986),
time by diet interactions would have been expected for both
bending and shear stress. When the weight of the pigs at
slaughter was included in the model as a covariate, diet,
time and diet by time interactions became nonsignificant
for all bones (P>.10).

Time by diet interactions and orthogonal contrasts for
overall diet effects indicated that diet and time effects
differed for bending and shear stress measures.
Differential effects of diets at different times were
detectable for shear stress, whereas there appeared to be
no differences in diet effects over time with bending

stress, even though such differences would be expected. Additionally, the shape of the diet curves differed between bending and shear stress. Standard errors associated with the two shear measures were smaller relative to the mean and to the range of values than those associated with the two bending measures (Tables 1 and 2). These data also suggest that much of the effects attributed to diet reflected effects of diet on body weight. Kesel et al. (1983) and Lepine et al. (1985) demonstrated that the magnitude of differences in mechanical bone characteristics between boars fed 100 or 150% of NRC-recommended Ca/P level was reduced when the values were corrected for body weight differences.

In both models, the smallest R^2 was obtained for 3C, followed by 4C, 3T and 4T, in that order (Table 3). When the average R^2 of the two bending bones was compared with the average of the two shear bones, the average R^2 of the two shear bones was consistently higher than that of the two bending bones. Crenshaw (1986) reported poor overall fit (R^2=41%) for a prediction model relating third metatarsal bending stress to time and Ca and P level, in a similar study. The R^2 in this study averaged 75% for the bending stress equations and 82% for the shear stress equations, indicating that more of the variation in shear stress can be explained by the model than was the case for

bending stress.

The residual CV was largest for 3T bending stress, and smallest for 4C shear stress, in both models (Table 3). When the CV were averaged across bending and shear bones, the shear stress measures had lower average CV than the bending stress measures for both models, indicating that the residual variation was smaller for shear stress, relative to the mean, than for bending stress.

Although most correlations among and within bending or shear stress measures were significant, the correlations were modest, but consistent for both models (Table 4). Within bones used for bending or shear tests, values for the two bending bones (3C, 3T) were more highly correlated than were values for the two shear bones (4C, 4T) in both models (r=.52 for bending bones, r=.10 for shear bones). This indicated that the bending results were more repeatable within the same animal than were the shear results. The metacarpal (3C, 4C) bones were correlated at r=.35. Correlations between metatarsal bones (3T, 4T) were slightly lower, averaging .30. Correlation between 3C bending stress and 4T shear stress averaged .14, whereas 3T bending stress and 4C shear stress were correlated at r=.31. The significance of the correlations among and within bending and shear stress measures indicate that there is consistency between the results of the bending and

shear tests, although the bending results may be more repeatable.

Based on the results of this study, shear testing appears to be a more desirable method of determining bone strength, compared with bending testing, because: 1) it is a "purer" expression of bone strength, less subject to confounding by other effects than bending; 2) simpler calculations are required than for bending tests; 3) dietary Ca/P and time differences were detected more readily, due to reduced variation; 4) higher R^2 were achieved for models with and without weight-correction; 5) lower CV were achieved with and without weight correction, indicating less variation, relative to the size of the mean; 6) significant correlations with bending stress measures and between the two shear stress measures, indicate that results are consistent between measures, although results are less repeatable than between the bending measures.

Implications

The results of this study suggest that when one is interested in determining bone strength, particularly of the third or fourth metatarsal or metacarpal, shear determinations on the bone offer a test that is conducted in the same manner as bending tests, is as sensitive or more sensitive than the bending test to dietary Ca/P, and

offers simpler calculation of various measures of bone
strength than the bending test.

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TABLE 1.   LEAST SQUARES MEAN STRESS (KG·CM^{-2}) FOR THIRD
METACARPAL (3C) AND METATARSAL (3T) BENDING TEST
AND FOURTH METACARPAL (4C) AND METATARSAL (4T)
SHEAR TEST AS INFLUENCED BY DIETARY CA/P LEVEL

	Ca/P level (% of NRC)						
Bone	70	85	100	115	130	SEM	Range/ SEM
3C	248.9	296.7	353.1	366.4	396.6	10.7^a	13.8
3T	176.6	202.7	256.3	271.0	293.2	8.0^b	14.6
4C	72.7	95.3	110.4	126.6	127.4	2.8^c	19.5
4T	71.7	92.8	108.2	106.7	114.3	2.8^c	15.2

^{a}Linear (P<.01) and quadratic (P<.10) effects of Ca/P.
^{b}Linear (P<.01) and quadratic (P<.12) effects of Ca/P.
^{c}Linear (P<.01) and quadratic (P<.01) effects of Ca/P.

TABLE 2.   LEAST SQUARES MEAN STRESS (KG·CM^{-2}) FOR THIRD
METACARPAL (3C) AND METATARSAL (3T) BENDING TEST AND FOURTH
METACARPAL (4C) AND METATARSAL (4T) SHEAR TEST FOR
SLAUGHTER PERIODS FROM WEANING TO MARKET

	Average day on test						
Bone	27	55	83	112	138	SEM	Range/ SEM
3C	229.2	294.2	354.0	393.1	391.1	10.5^a	15.6
3T	178.0	201.3	244.8	276.5	299.1	7.9^a	15.3
4C	83.5	94.3	100.9	128.5	125.4	2.8^a	16.1
4T	71.5	81.0	95.0	114.8	131.4	2.7^a	22.2

^{a}Linear effect of time (P<.01).

TABLE 3. R^2 VALUES AND CV OBTAINED FROM FIXED EFFECTS
MODELS FOR THIRD METACARPAL AND METATARSAL (3C, 3T)
BENDING STRESS AND FOURTH METACARPAL AND METATARSAL
(4C, 4T) SHEAR STRESS

	Bending Stress			Shear Stress		
Model	3C	3T	Avg.	4C	4T	Avg.
-----	-----	-----	----- R^2, %	-----	-----	-----
1[a]	68	71	70	77	80	79
2[b]	79	81	80	85	85	85
Avg.	74	76	75	81	83	82
-----	-----	-----	----- CV, %	-----	-----	-----
1	20.9	21.5	21.2	17.2	17.6	17.4
2	20.9	21.6	21.3	17.5	18.6	18.1
Avg.	20.9	21.6	21.3	17.4	18.1	17.8

[a]Model 1: Stress = Trial + Diet + Trial x Diet + Time +
    Trial x Time + Diet x Time + Trial x Diet x Time.
[b]Model 2: Stress = Trial + Diet + Trial x Diet + Time +
    Trial x Time + Weight + Weight^2 + Diet x Time +
    Weight x Diet + Weight x Time + Trial x Diet x Time +
    Weight x Diet x Time + Weight x Trial x Diet x Time.

TABLE 4. ALL POSSIBLE CORRELATIONS OF THIRD METACARPAL
(3C) AND METATARSAL (3T) BENDING STRESS AND FOURTH
METACARPAL (4C) AND METATARSAL (4T) SHEAR STRESS
FOR BOTH MODELS OF STRESS

Model[a]	Correlation					
	3C-3T	3C-4C	3C-4T	3T-4C	3T-4T	4C-4T
1	$.55^b$	$.34^b$	$.17^d$	$.30^b$	$.29^b$	$.12^d$
2	$.49^b$	$.35^b$	$.11$	$.32^b$	$.31^c$	$.08$
Avg.	.52	.35	.14	.31	.30	.10

[a]Refer to Table 3 for definition of effects in each model.
[b,c,d]Correlation is different from zero (P<.001, P<.01 and
P<.05, respectively).

Overall length

Midpoint - location where radius and wall
thickness measurements were made.
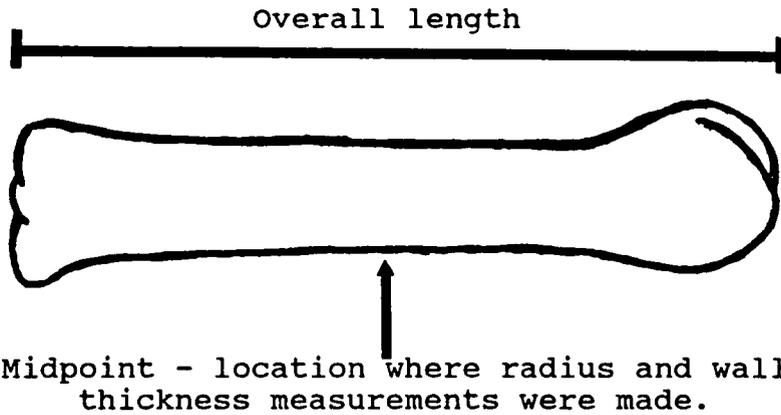
Direction of force application
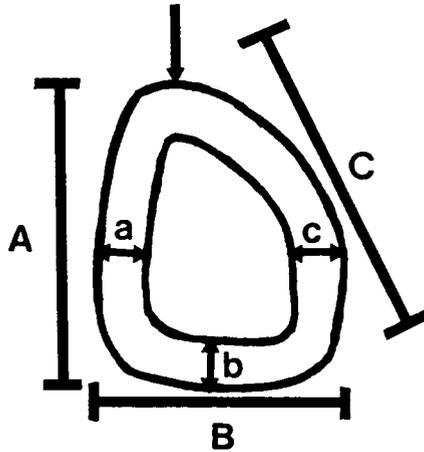
A = Wide dimension   a, b, c = locations of wall
B = Narrow dimension   thickness measurements
C = Perpendicular dimension

$$\text{Radius} = \frac{A + B}{2} = r \qquad \text{Wall Thickness} = \frac{a + b + c}{3} = t$$

$$\text{Area} = \left\{ \frac{\pi[r^2 - (r - t)^2]}{4} + (2rt - 3t^2) \right\}$$

Figure 1. Determination of bone dimensions used in
calculation of bending stress of the third metacarpals
and metatarsals and shear stress of the fourth
metacarpals and metatarsals.

2b

Shear Fixture
End-on View

.5 mm gap

.75 cm

.75 cm

.5 mm gap

.75 cm

.75 cm

2a

Bending Fixture
End-on View

1 cm

3.175 cm

1 cm

1 cm

1 cm

1 cm
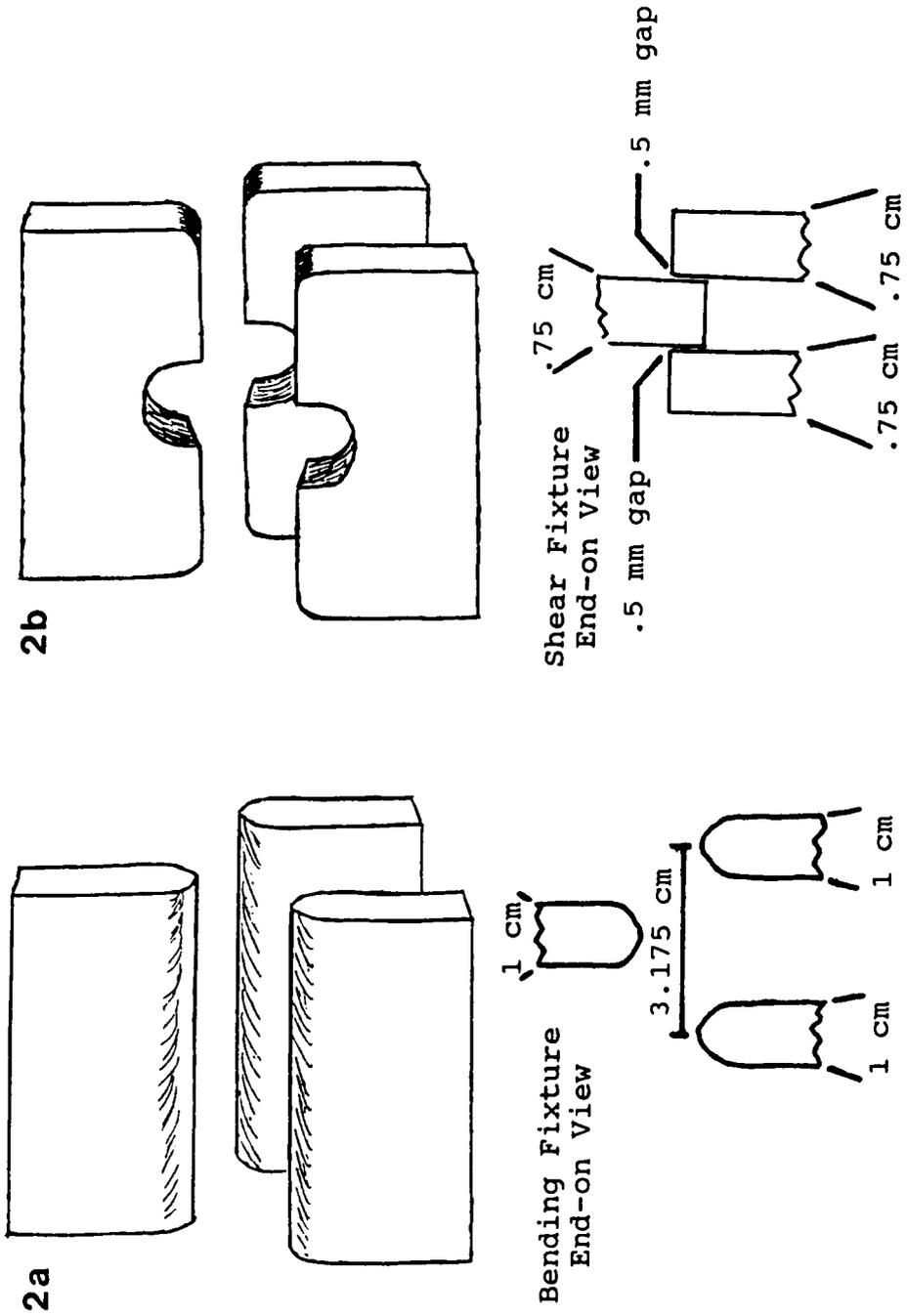
Figure 2. Diagram of bending (2a) and shear (2b) fixtures used on Ingstrom Model
1123 Testing Machine for bone strength determinations.
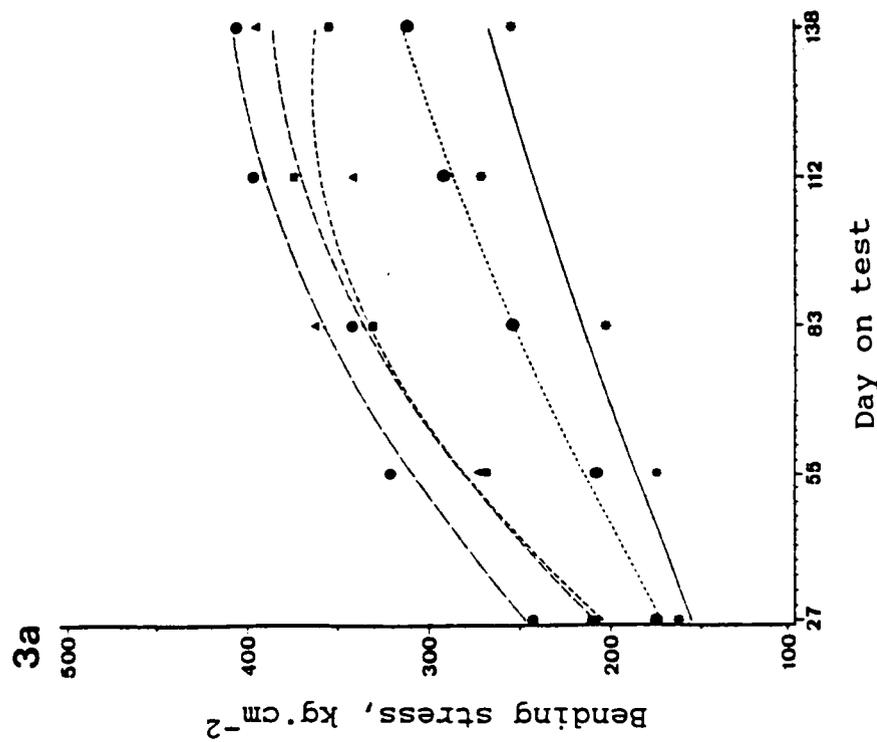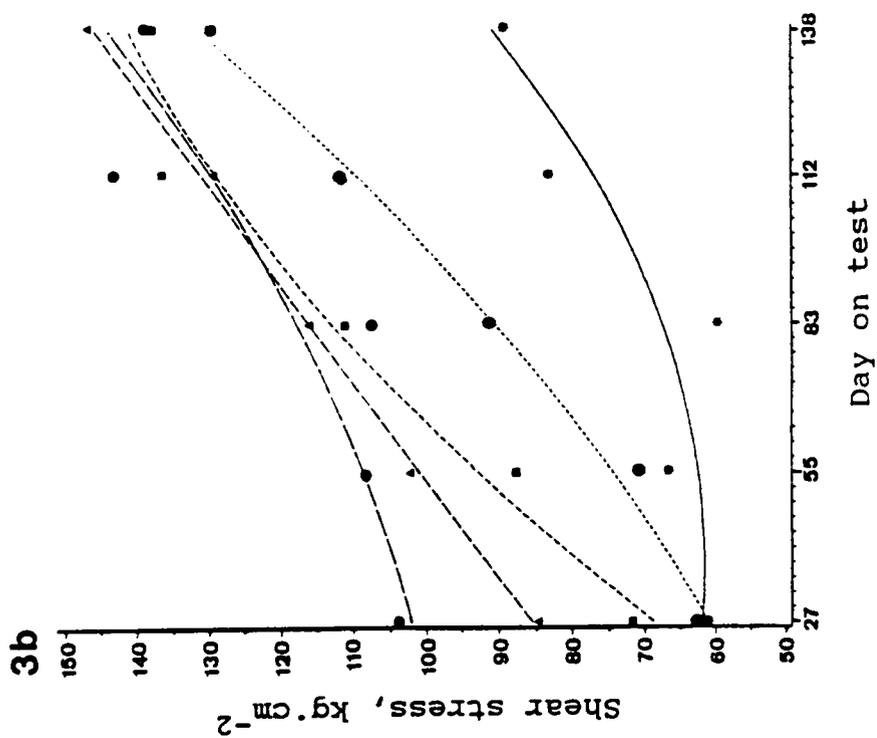
Figure 3. Bending stress (3a, avg third metacarpal and metatarsal) and shear stress (3b, avg fourth metacarpal and metatarsal), by day on test, for growing-finishing pigs fed five dietary Ca/P levels [70 (——), 85 (-•-), 100 (-●-), 115 (-▲-) and 130% (—●—) NRC-recommended levels] from weaning to market weight.

CHAPTER 5

CALCIUM AND PHOSPHORUS REQUIREMENT OF SWINE FROM WEANING TO
MARKET: DEVELOPMENT OF RESPONSE CURVES FOR BONE CRITERIA[1,2,3]

ABSTRACT

Three trials involving crossbred 251 pigs were
conducted to establish response surfaces for the effects of
Ca/P level [70, 85, 100, 115 and 130% of NRC (1979)
recommendations] and time (weaning to market) on bone
criteria. Nine replicates of 5 blocks each (5 pigs/block)
were used over the three trials. One block of pigs per
replicate was slaughtered every 4 wk following start of the
trials. Twenty-six additional pigs were slaughtered at the
beginning of testing to provide baseline data on initial Ca
and P status. Third metacarpals and metatarsals (3M) and
fourth metacarpals and metatarsals (4M) were collected at
slaughter, and the bone length, wet weight, wall thickness,
cross-sectional area, bending or shear force, bending or
shear stress, extracted weight, dry fat-free ash weight and
dry fat-free ash percentage were determined. Most bone
criteria responded linearly (P<.01) and quadratically
(P<.05) at a decreasing rate to increasing dietary Ca/P
intake, and linearly (P<.01) to time on test, although bone

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length, wall thickness and stress also increased quadratically (P<.05) at a decreasing rate in responses to time. Asymptotic models relating continuous effects of total Ca + P intake ratio (CAP; expressed as a percentage of NRC) and number of days on test before slaughter were fit to least squares slaughter time by diet means. These models adequately described the response of bone criteria to CAP and time. The CAP level associated with 95 and 98% of maximum for each bone criteria at 27, 55, 83, 112 and 136 d on test was also determined and compared to that required to produce maximum BW, ADG and avg daily feed intake. Bone length, weight, wall thickness and dry fat-free ash percentage reached 98% maximum at or near 100% NRC recommended CAP level, as did BW, ADG and avg daily feed intake; however, 98% of maximum bone force and stress required higher CAP levels.

(Key Words: Bones, Calcium, Phosphorus, Pigs, Growth models)

Introduction

Some researchers have recommended that the calcium and phosphorus levels suggested by NRC (1979) are inadequate to maximize bone development in some classes of swine (Guéguen and Perez, 1981; Maxson and Mahan, 1983; Nimmo et al., 1981; Reinhard et al., 1976; van Kempen et al., 1976). To date, however, there has been no conclusive evidence that maximization of bone development as defined by breaking

strength is necessary, particularly in pigs fed for slaughter (Guèguen and Perez, 1981). Maximization of growth and efficiency of feed utilization (EFU) can be shown to occur at dietary Ca and P levels lower than those required to maximize bone criteria (Crenshaw, 1986; Kornegay, 1986).

Few studies have determined effects of different Ca and P levels on performance and bone criteria over the entire growth period. Additionally, the range of Ca and P levels used is often restricted to only levels in excess of that suspected to produce desired performance.

This study was a part of a larger Ca/P study described by Combs et al. (1989a). The objectives were to evaluate bone characteristics of pigs fed diets containing various levels of Ca and P, and to derive response surfaces relating these bone criteria to dietary Ca plus P intake and time on test. Additionally, the Ca and P intake required to produce 95 and 98% of maximum for various bone criteria were compared with the Ca and P intake required to produce 95 or 98% of maximum BW, ADG and average daily feed intake.

Experimental Procedures

Two hundred fifty one pigs (nine replicates of five blocks each with 5 pigs per block plus 26 initial slaughter pigs) were used. Five dietary Ca/P treatments [70, 85, 100, 115 and 130% of the NRC recommended (1979) dietary Ca + P levels] were fed from weaning to market (avg body wt of $10 \pm .1$ kg to avg of 110 ± 2.5 kg). Total dietary Ca + P intake was determined from the analyzed Ca and P content of the diet and was expressed as a percentage of NRC recommended Ca + P intake (CAP) for derivation of response surfaces. Diet levels averaged 80.8, 88.3, 102.3, 118.8 and 132.2% of the NRC recommended Ca/P level for diets 1 through 5, respectively. Other details concerning diets, analysis of the feed and the management of animals can be found in Combs et al. (1989a).

One block of pigs per replicate was slaughtered every 4 wk (approximately) giving a total of five slaughter periods, in addition to the initial slaughter group. At slaughter, the third and fourth metacarpals and metatarsals from one side of the pig were removed. The right or left side was alternately sampled from one slaughter time to the next, so as to coincide with the side that was being biopsied in live pigs (Combs et al., 1989b) at that sampling time. Bones were frozen immediately after slaughter in air-tight plastic bags, were later thawed and

freed of extraneous tissue for dimension measurements, and were then refrozen in air-tight plastic bags to prevent dessication pending bending and shear testing.

For bending and shear tests, force was applied to the bones at a speed of 10 mm·min^{-1} and resulting force curves were plotted at a chart speed of 50 mm·min^{-1}. Maximum force required to cause bending or shear failure was electronically measured and recorded. Afterwards, proximal halves of the bones were dried and crushed. Fat was extracted by refluxing a 75%:25% chloroform: methanol mixture over crushed and dried samples in a 4 l soxhlet apparatus. Extracted samples were redried for 24 h and ashed at 600°C until a consistent white ash was obtained. A detailed discussion of the procedures used to prepare, measure and process the bone samples can be found in Combs et al. (1989c).

Bone criteria evaluated included length, wet weight, wall thickness, cross-sectional area at the midpoint, force required to produce three-point bending or shear, bending or shear stress, dry fat-free weight of the proximal half, dry fat-free weight of the ash of the proximal half, and dry fat-free ash percentage of the proximal half. Data were averaged across the two bones used for bending tests (third metacarpal and metatarsal bones, 3M), and across the two bones used for shear tests (fourth metacarpal and

metatarsal bones, 4M) before statistical analysis.

For statistical analysis, the fixed effects ANOVA models included effects of trial, diet, slaughter time and all two-way interactions. Days on test before slaughter was used as the continuous time effect in derivation of response surface curves, and was unique to each trial but generally was similar (averaging 27, 55, 83, 112 and 136 days on test for slaughter periods 1 through 5, respectively). Since diet and time trends were fairly consistent in all trials, data from all trials were pooled for analysis. Least squares means for diet-time subclasses adjusted for effects of trial, were used to fit double quadratic and asymptotic response surfaces using average values for CAP and day across trials as the independent variables. Response surface models included continuous effects of CAP and day on test.

Effects of sex on bone criteria were evaluated by ANOVA using a model including effects of sex and diet within each trial and time period. Effects of sex were never significant in two successive periods for any of the criteria; therefore, the effect of sex was excluded from the final model.

Nonlinear asymptotic response surfaces were derived for all 3M and 4M bone criteria. Asymptotic models relating bone criteria (Y) to dietary CAP level were fit to

diet least squares means at each time (Model 1: Y = A{1 − Be$^{[-k \times SCAP]}$}, where SCAP is the average CAP value for each diet minus the lowest CAP value). Using derived estimates of the asymptotic maximum value (A) for each criterion from these models, each datum (Y) was divided by the predicted maximum appropriate for that trait at that time, and asymptotic models were refit to the Y/A data. To define the final response surface model, across both time and dietary CAP, the asymptotic maximum for each bone criterion (A), and the percentage reduction in maximum on the lowest CAP level (B), were both fit as secondary polynomial functions of day on test (Model 2). Procedures for calculation of residual error sum of squares, lack of fit and R^2, described by Combs et al. (1989a) were followed. Response surface models were then solved for the CAP level associated with 95 and 98% of maximum for each bone criteria at 27, 55, 83, 112 and 136 d on test. For purposes of comparison, the CAP level associated with 95 and 98% of maximum BW, ADG and average daily feed intake (ADFI) at these same days on test were calculated from asymptotic performance models (Combs et al., 1989a) and compared to CAP levels associated with comparable maximums for bone data.

Results and Discussion

The effects of both time and diet on all bone criteria were significant (Table 1). The least squares diet by time means for both 3M and 4M length, wet weight, wall thickness, cross-sectional area, extracted weight and ash weight demonstrated similar patterns of response to diet and time. Therefore, only 3M least squares means are shown in Table 2. Least squares diet by time mean force, stress and dry fat-free ash are presented in Table 3 for both 3M and 4M bones.

The wet weight, wall thickness, cross-sectional area, dry fat-free extracted weight and dry fat-free ash weight of 3M and 4M bones, and the 3M bending force and stress all increased linearly with time (P<.01) from weaning to market (Tables 2 and 3). Bone length, radius and dry fat-free ash percentage of both 3M and 4M bones, and 4M shear force increased at a decreasing rate (P<.01) over time from weaning to market; 4M shear stress also increased at a decreasing rate (P<.05) with time. In agreement with the present study, increases in dimension measurements, bone strength and ash content, concomitant with increasing body size over time, have been widely documented and reported (Crenshaw, 1986; Crenshaw et al., 1981b; Kesel et al., 1983; Kornegay, 1986).

Bone length, wet weight, radius, wall thickness,

cross-sectional area, bending and shear force, shear stress, extracted weight, dry fat-free ash weight and dry fat-free ash percentage increased linearly ($P<.01$) and quadratically ($P<.01$) with increasing dietary Ca/P level (Tables 2 and 3); bending stress only increased linearly as dietary Ca/P increased.

In contrast to our findings, weight, length and outside diameters (radius) of bones were reported to be unresponsive to dietary Ca and P levels. However, in agreement with the results of the present study, wall thickness is responsive to dietary Ca/P, particularly at levels below NRC recommendations (Crenshaw et al, 1981a; Kornegay, 1986).

When Ca/P is increased in an equal ratio, both above and below NRC recommended levels, bone ash has been shown to increase (Crenshaw et al., 1981b; Lepine et al., 1985; Maxson and Mahan, 1983), as was found in this study. Nimmo et al. (1980) and Kornegay et al. (1981) reported linear and quadratic effects of Ca/P level on force. Nimmo et al. (1980) also found that stress increased linearly with increasing Ca/P level.

The linear and quadratic increases with increasing Ca/P level noted for the bone criteria in our study were due to large increases in the bone criteria associated with increasing dietary CAP from 70 to 100%, whereas there was

little difference noted in these criteria between the 100
and 130% CAP level (Tables 2 and 3).

Diet by time interactions were significant for 3M and
4M wet weight, force, dry fat-free extracted weight, and
dry fat-free ash weight, for 3M dry fat-free ash percent,
and for 4M stress (Table 1). Crenshaw (1986) previously
reported that the effects of time on third metacarpal
bending force and percentage ash could be modified by
dietary Ca level, whereas no interaction of Ca or P with
time was found for ash weight or stress. The trial by diet
interaction of 3M length and 4M stress was significant
(P<.01 and P<.05, respectively), indicating that the slope
of the response to diets differed for the three trials.
The slope of the response to time also differed in the
three trials for at least one bone pair of each criteria
measured, although the only significant trial by diet by
time interaction was for 4M stress.

The mean square errors, R^2 and lack of fit ratios for
the predicted asymptotic models are summarized in Table 4.
Using the estimated increase in error sums of squares as an
estimate of lack of fit, no differences in fit between
Models 1 and 2 were found for wet weight; whereas lack of
fit in Model 2, compared with Model 1, was significant for
at least one bone pair for all other bone criteria.
Asymptotic models fit within each slaughter time (Model 1)

had R^2 greater than 97% for all bone criteria. The R^2 when an asymptotic model was fit across all times (Model 2) were also high for all bone criteria, except for 4M stress, suggesting there was very little loss of predictive power associated with simplification of the model, contrary to what one might have expected from the lack of fit ratios. The reduced R^2 and the inflated lack of fit ratio for 4M stress was likely due to the significant interaction of trials, diets and time for this criteria (Table 1), suggesting that the predicted response surface for this criteria may not be as reliable as the response surfaces for other criteria. Therefore, with the exception of 4M stress, asymptotic models appear to reflect the responses of bone criteria very well.

The coefficients for asymptotic response surfaces for bone length, wet weight, wall thickness, cross-sectional area, bending or shear force, bending or shear stress, extracted weight, ash weight or dry fat-free ash percent are reported in Table 5. The 3M and 4M response surfaces for most bone criteria were very similar, with the exception of 3M and 4M dry fat-free ash percentage.

Dry fat-free ash percentage of the 3M bone pair (Figure 1a) was predicted to respond quadratically to both diet and time, plateauing near the 85% NRC recommended Ca/P level across the entire time period. Dry fat-free ash

percentage of the 4M bone pair (Figure 1b), on the other hand, was predicted to respond quadratically to diet and in linear manner to time; 4M dry fat-free ash percentage appeared to plateau near the 100% NRC recommended Ca/P level initially, and at a lower level later on, contrary to what would be expected from literature reports. Kornegay (1986), in summarizing Ca/P research conducted from 1969 to 1985, predicted that 99% of maximum bone ash would be obtained with 100% of NRC recommended Ca/P levels when the Ca/P level fed during the growing phase was reduced during the finishing phase, as was the case in this study. The shape of the response of bone ash to Ca/P, expressed as a percentage of NRC recommendations, in Kornegay (1986) was similar to the response obtained in this study with the 3M bones. In the current study, 3M bone ash was predicted to plateau at a lower level than Kornegay (1986) predicted.

The response surfaces for bending (3M) and shear (4M) force and stress are shown in Figures 2 and 3, respectively. The predicted shape of the response to diet and time are very similar for the force and stress of both bending and shear, differing mainly in magnitude of response; shear (4M) force was substantial higher than that of bending (3M) force (Table 3 and Figure 2), whereas bending stress was higher than the corresponding values for shear stress (Table 3 and Figure 3).

Both force and stress were predicted to increase linearly over time, in agreement with the effect of time on force and stress predicted by Crenshaw (1986). Predicted force appeared to increase linearly with increasing Ca/P initially, and to become increasing quadratic in response to diet later on (Figure 2). Kornegay (1986) predicted that bone breaking strength (force) would increase quadratically with increasing dietary Ca/P, plateauing at levels of dietary Ca/P in excess of those used in the current study. The predicted response of force to dietary Ca/P in the current study was very similar to curves shown by Kornegay (1986). In contrast to results observed with force, both bending and shear stress, which are adjusted for the area over which the force was applied, were predicted to increase quadratically with increasing dietary Ca/P initially, and to become more linearly related to diet over time (Figure 3).

The response surfaces reported for measures of bone dimension, strength and ash content collectively describe bone development in response to the effects of dietary Ca/P level and time from weaning to market weight. The responses described here relate very closely to the responses expected from the literature, with the few exceptions that are noted. Therefore, these predicted responses should serve as a comprehensive data base for

relating Ca/P and time to bone development of weanling, growing and finishing pigs.

The CAP levels associated with 95 and 98% of maximum 3M and 4M bone length, wet weight, wall thickness, cross-sectional area, bending (3M) or shear (4M) force, bending or shear stress, dry fat-free extracted weight, dry fat-free ash weight and dry fat-free ash percent, and for the corresponding body weight, ADG and ADFI, at 27, 55, 83, 112 and 138 are presented in Tables 6 and 7 (95 and 98% of maximum, respectively). Ninety-five percent of maximum bone length, wet weight and radius of both 3M and 4M bone pairs was achieved with approximately 70, 85 and 71% of NRC recommended Ca/P level, respectively (Table 6), whereas 98% of maximum length, weight and radius required approximately 77, 93 and 75% of NRC recommended Ca/P (Table 7). Wall thickness and cross-sectional area reached 95% of maximum with a Ca/P level of 95 and 91% of NRC recommendations, respectively, for the 3M bones and 111 and 102% of NRC recommended Ca/P for the 4M bones. The different maximums for 3M and 4M bone pairs were confirmed with the 98% maximums for wall thickness and area (Table 7). Maximums for force, stress, dry fat-free extracted weight, dry fat-free ash weight and dry fat-free ash percentage were also reached at different CAP levels for 3M and 4M bone pairs.

The CAP level associated with both 3M and 4M maximum force and stress decreased over time (averaging 122 and 114% Ca/P for 95% maximum 3M and 4M force, respectively, and 138 and 135% Ca/P for 3M and 4M stress when averaged across all time periods), while CAP levels associated with maximization of 3M dry fat-free ash percent appeared to increase quadratically over time (averaged across all time periods, 95% maximum 3M ash percent was obtained with 74% Ca/P). The CAP level required to produce maximum 4M dry fat-free ash decreased over time (averaging 83% Ca/P for 95% of maximum 4M ash across all time periods). Maximum 3M and 4M extracted and ash weights were associated with decreasing CAP levels over time as well, with 4M bones requiring higher CAP levels to maximize extracted and ash weight than the 3M bone pairs (102 vs 97% CAP for 95% maximum 4M vs 3M extracted weight, and 110 vs 100% CAP for 95% maximum 4M and 3M ash weight, when averaged across all time periods). The CAP level required to maximize the other bone criteria were fairly constant across time (Tables 6 and 7).

When the percentage of maximum represented by each CAP level at each time is plotted for bending (3M) and shear (4M) force (Figure 4) and stress (Figure 5), the percentage of maximum associated with each given CAP level increased quadratically with each succeeding slaughter time. For

example, 100% NRC recommended CAP levels produced 85.1,
87.3, 89.0, 90.2 and 90.9% of maximum 4M force at 27, 55,
83, 112 and 138 days on test, respectively. The percentage
of maximum for each CAP level for 4M dry fat-free ash
percentage (Figure 6B) also increased quadratically with
time, with little difference between maximums at 112 and
136 d on test, whereas 3M dry fat-free ash percentage
(Figure 6A) did not demonstrate a consistent pattern over
time; the percentage of maximum attained with each CAP
level differed little from time to time for all other bone
criteria, and are not shown. Additionally, examination of
these curves shows that for force and stress (Figures 4 and
5) and for 4M dry fat-free ash percentage (Figure 6B), the
increases in the percentage of maximum over time for a
given CAP level were greater for the lower CAP levels,
whereas the difference in percentage of maximum at over
time for a given CAP level were smaller for the higher CAP
levels.

Maximization of body weight and ADFI also required
higher CAP levels over time, while the CAP level related to
maximization of ADG increases in linear fashion over time
(Tables 6 and 7). When averaged across all time periods,
95% of maximum BW, ADG and ADFI required CAP levels of 85,
93 and 86% of NRC recommendations, respectively (98%
maximums were obtained with 95, 105 and 96% of NRC

recommended CAP levels). For any given CAP level, the percentage of maximum represented by that CAP level tended to decrease over time for BW, ADG and ADFI. For example, at day 27 on test, a CAP level of 100% would produce nearly 97.7% of maximum ADG, whereas at days 55, 83, 112 and 136 this same CAP level will only produce 97.3, 96.9, 96.6 and 96.4% of maximum ADG, respectively.

The CAP level associated with 95 and 98% maximization of bone length, wet weight, radius (average outside diameter) and dry fat-free ash percent of both the 3M and 4M bone pairs appears to be the same or lower than that required to maximize performance criteria. Bone wall thickness, cross-sectional area, force, stress, dry fat-free extracted weight, and dry fat-free ash weight appear to maximize with CAP levels higher than those required for performance criteria. In general agreement, Crenshaw (1986) and Kornegay (1986) suggested that the effect of increasing levels of Ca and/or P will plateau at a much lower level for growth criteria than for most bone criteria. In the compiled data set from a number of studies conducted from 1969 to 1985, Kornegay (1986) found that daily gain, gain to feed ratio and bone ash reached 97% of maximum when 101, 93 and 100% of NRC recommended Ca/P levels were fed, whereas 97% of maximum breaking strength (force) was obtained only when 128% of NRC Ca/P

levels were fed. In the current study, the maximums obtained for these criteria (Tables 6 and 7) were similar to those expected from the report of Kornegay (1986).

The CAP levels related to 95 and 98% maximization of bone strength (force and stress) at all time periods were clearly higher than the NRC recommended (1979) levels. Other researchers (Guèguen and Perez, 1981; Maxson and Mahan, 1983; Nimmo et al., 1981; Reinhard et al., 1976; van Kempen et al., 1976) who reported that NRC recommended levels were inadequate for maximization of bone development, based their conclusions primarily on bone strength criteria, in agreement with the bone strength results of this study. However, these data also suggest that overall length, weight, thickness and ash of these bones and the performance of the pigs are near maximum at or near the NRC recommended levels. Therefore, the reduction in bone strength found on NRC recommended CAP levels does not appear to be accompanied by decreased size and mineralization of the bones. This result would suggest that maximization of performance characteristics is far more important than maximizing bone strength when determining Ca and P levels for slaughter pigs.

Implications

The results of this research confirm the conclusions drawn from the literature that bone strength is maximized at dietary Ca/P levels higher than those required to maximize performance in postweaning swine to market weight, but offers little evidence that maximization of bone strength is necessary in swine raised for slaughter. A comprehensive data set is presented, describing the effects of CAP and time on a number of bone criteria; these response surfaces will allow prediction of the effect of 70 to 130% of NRC recommended Ca/P on any of these criteria during the weanling, growing and finishing phases.

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TABLE 1. MEAN SQUARES AND SIGNIFICANCE OF EFFECTS OF TRIAL, DIET, SLAUGHTER TIME AND ALL INTERACTIONS ON BONE CRITERIA, ACROSS TRIALS[a]

Source	Effect							Error Mean Square	R^2
	Trial (Tr)	Diet (D)	Tr x D	Time (T)	Tr x T	D x T	Tr x D x T		
DF	2	4	8	4	8	16	32	148[b]	
3M Length, cm	.634**	1.031**	.155**	48.551**	.381	.054	.042	.072	95.7
4M Length	.840**	1.272**	.090	55.132**	.551**	.058	.045	.080	95.7
3M Wet weight, g	63.26**	141.07**	6.31	2343.76**	14.32**	8.68**	2.85	4.96	94.5
4M Wet weight	39.90**	167.27**	5.10	2570.25**	13.40**	10.58*	2.99	5.06	94.6
3M Radius, cm	.0818**	.0571**	.0029	1.1364**	.0075+	.0050	.0035	.0043	90.5
4M Radius	.0447**	.0714**	.0020	1.3085**	.0092**	.0039	.0025	.0045	91.1
3M Wall thickness, cm	.00872**	.01019**	.00023	.03592**	.00036	.00020	.00013	.00031	85.1
4M Wall thickness	.00417**	.01788**	.00020	.04315**	.00077*	.00028	.00022	.00037	84.7
3M Area, cm²	.1653**	.2650**	.0085	1.6500**	.0097	.0085	.0032	.0085	89.1
4M Area	.0375*	.4298**	.0022	2.0616**	.0220*	.0120	.0061	.0096	89.5
3M Bending force, kg	4604**	20626**	434	84701**	832*	1203**	345	399	90.4
4M Shear force, kg	5467**	93242**	697	262263**	2927**	4706**	984	881	93.0
3M Bending stress, kg cm⁻²	8053*	97284**	3839	146161**	3940**	2953**	3590**	2543	77.9
4M Shear stress, kg cm⁻²	2350**	14641**	415*	21513**	1276**	696**	347**	185	87.6
3M Extr. weight[c], g	3.785**	22.628**	.234	151.413**	.737*	1.167**	.140	.313	95.1
4M Extr. weight	6.994**	23.710**	.278	159.337**	1.448**	1.329**	.193	.299	95.3
3M Ash weight[c], g	1.526**	10.111**	.104	58.576**	.308*	.576**	.062	.125	95.1
4M Ash weight	1.193**	10.354**	.092	54.909**	.548**	.579**	.057	.106	95.3
3M Dry fat-free ash, %	.00111*	.01411**	.00040	.02567**	.00195**	.00065*	.00033	.00033	84.0
4M Dry fat-free ash	.01080**	.02776**	.00025	.01886**	.00109**	.00053	.00039	.00036	83.2

Note: bending stress units shown as $kg\ cm^{-2}$; shear stress units shown as $kg\ cm^{-2}$.

aThe significance of effects are as follows: **P<.01, *P<.05, +P<.10.
bDegrees of freedom for error were: 146 for 3M length, wet weight, radius and bending force; 145 for 3M wall thickness, area and bending force; 143 for 3M extr. weight, ash weight and dry fat-free ash percentage.
cExtr. weight is the extracted weight of bone tissue; ash weight is the dry fat-free weight of bone ash.

TABLE 2. LEAST SQUARES MEAN BONE MEASURES FOR
POOLED THIRD METACARPAL AND METATARSAL BONES (3M), BY DIET
AND SLAUGHTER TIME, FOR PIGS FED FIVE LEVELS OF DIETARY
CA/P FROM WEANING TO MARKET WEIGHT

	Ca/P level (% of NRC)					
Time	70	85	100	115	130	SEM
3M Length, cm[abcd]						
27	5.66	5.78	5.76	5.90	5.88	.08
55	6.41	6.69	6.60	6.74	6.70	.08
83	7.02	7.45	7.65	7.60	7.65	.08
112	7.84	8.16	8.16	8.24	8.28	.08
138	8.01	8.55	8.46	8.59	8.60	.08
3M Wet weight, g[abce]						
27	8.47	9.32	9.30	10.61	10.23	.57
55	12.17	14.63	15.19	15.21	15.31	.56
83	15.95	19.74	21.77	20.30	21.62	.57
112	19.80	24.25	25.19	26.94	27.37	.55
138	22.81	28.71	29.86	30.35	29.60	.60
3M Radius, cm[abcd]						
27	1.16	1.20	1.17	1.21	1.20	.02
55	1.27	1.39	1.38	1.36	1.36	.02
83	1.38	1.47	1.52	1.45	1.49	.02
112	1.46	1.54	1.53	1.59	1.57	.02
138	1.54	1.65	1.65	1.65	1.63	.02
3M Wall thickness, cm[abc]						
27	.0853	.0963	.1072	.1153	.1147	.0058
55	.1023	.1239	.1360	.1411	.1431	.0060
83	.1273	.1366	.1520	.1569	.1558	.0058
112	.1362	.1616	.1786	.1851	.1952	.0056
138	.1531	.1607	.1866	.1930	.1880	.0061
3M Cross-sectional area, cm^2[abc]						
27	.318	.376	.400	.450	.442	.029
55	.430	.556	.601	.611	.616	.030
83	.572	.648	.737	.714	.739	.029
112	.633	.788	.854	.929	.957	.028
138	.733	.841	.969	1.000	.946	.031
3M extracted weight, g[abcf]						
27	1.095	1.367	1.486	1.795	1.788	.146
55	1.588	2.375	2.918	2.877	2.936	.145
83	2.423	3.597	4.415	4.222	4.497	.144
112	3.218	4.843	5.485	5.931	6.312	.140
138	4.277	6.005	6.775	7.134	6.970	.155
3M Dry fat-free ash weight, g[abcf]						
27	.571	.740	.798	.982	.981	.096
55	.843	1.333	1.709	1.694	1.749	.096
83	1.344	2.086	2.650	2.548	2.730	.096
112	1.809	2.898	3.295	3.736	3.863	.093
138	2.469	3.579	4.102	4.323	4.269	.102

[a,b]Overall linear (P<.01) or quadratic (P<.01) effect of diet, respectively.
[c,d]Overall linear (P<.01) or quadratic (P<.01) effect of time, respectively.
[e,f]Significant diet by time interaction (P<.05 and P<.01, respectively).

TABLE 3. LEAST SQUARES MEAN BONE BENDING OR SHEAR FORCE
AND STRESS AND DRY FAT-FREE ASH PERCENTAGE, BY DIET AND
SLAUGHTER TIME, OF PIGS FED FIVE LEVELS OF DIETARY CA/P
FROM WEANING TO MARKET WEIGHT

	Ca/P level (% of NRC)					
Time	70	85	100	115	130	SEM
3M Bending Force, kg[abcf]						
27	19.5	25.4	31.1	33.0	37.8	6.2
55	27.2	47.2	55.7	62.7	74.3	6.1
83	46.8	69.9	105.3	103.7	105.1	6.2
112	70.9	99.2	130.4	133.7	156.9	6.0
138	86.6	115.2	158.5	173.8	163.1	6.6
4M Shear Force, kg[abcef]						
27	41.8	50.2	66.5	82.5	104.2	8.8
55	52.8	78.5	108.5	126.3	143.3	8.5
83	69.4	126.7	175.5	174.5	178.2	8.8
112	108.7	183.7	241.5	255.4	302.1	8.5
138	134.3	232.9	298.5	314.5	292.5	8.9
Bending Stress, kg/cm^2[acd]						
27	172.1	185.7	217.0	214.2	245.1	17.4
55	165.0	205.6	241.3	267.4	316.4	17.8
83	195.8	253.6	331.9	364.0	344.9	17.4
112	271.8	294.0	372.8	340.5	394.3	16.8
138	275.4	290.9	364.4	386.3	391.1	18.4
Shear Stress, kg/cm^2[abcf]						
27	62.5	64.3	73.4	85.6	104.2	4.7
55	64.1	69.9	83.3	101.4	107.5	4.6
83	58.9	91.5	111.2	116.5	108.3	4.7
112	83.4	112.2	136.8	129.2	142.9	4.6
138	90.5	134.2	143.3	142.6	139.8	4.8
3M Dry fat-free ash, %[abceg]						
27	52.1	54.1	53.5	54.1	55.0	.7
55	52.8	55.9	58.4	58.7	59.4	.7
83	55.0	57.9	60.0	60.3	60.7	.6
112	56.1	59.8	60.0	62.9	61.2	.6
138	57.4	59.3	60.5	60.6	61.0	.7
4M Dry fat-free ash, %[abce]						
27	47.7	50.5	51.0	54.6	54.4	.7
55	48.9	53.8	55.6	54.8	56.4	.7
83	51.1	55.0	57.4	57.5	57.9	.7
112	51.5	55.4	57.7	58.1	58.5	.7
138	53.7	55.4	57.8	58.7	58.7	.7

[a,b]Overall linear (P<.01) or quadratic (P<.01) effect of diet, respectively.
[c,d,e]Overall linear (P<.01) or quadratic (P<.05 or P<.01) effect of time, respectively.
[f,g]Significant diet by time interaction (P<.01 or P<.05, respectively).

TABLE 4. ASYMPTOTIC MEAN SQUARES, R^2 AND LACK OF FIT
RATIOS FOR BONE MEASUREMENTS OF PIGS FED FIVE LEVELS OF
DIETARY CA/P FROM WEANING TO MARKET WEIGHT

	Model 1[a]		Model 2[b]		Lack of fit F ratio[c]
	MSE	R^2	MSE	R^2	
Length					
3M	.0037361	99.85	.012553	99.07	6.31**
4M	.0048069	99.82	.013114	99.10	4.89*
Wet Weight					
3M	.359860	99.70	.512475	99.23	1.95
4M	.423425	99.67	.754545	98.94	2.76+
Wall Thickness					
3M	.0000050	99.79	.000021	98.46	8.04**
4M	.0000190	99.40	.000047	97.31	4.31*
Cross-sectional Area					
3M	.0002418	99.76	.000517	99.06	3.56*
4M	.0006502	99.48	.001112	98.39	2.60+
Force					
3M	34.9047	99.38	43.6479	98.60	1.56
4M	126.8750	99.29	325.3690	96.74	4.52*
Stress					
3M	378.9550	97.23	702.5350	90.74	2.92+
4M	44.3395	97.64	480.2740	54.08	23.12**
Dry Fat-Free Extracted Weight					
3M	.0235946	99.73	.041415	99.15	2.70+
4M	.0306236	99.65	.096722	98.02	5.86**
Dry Fat-Free Ash Weight					
3M	.0114504	99.67	.018596	99.05	2.40+
4M	.0134758	99.58	.046503	97.40	6.51**
Dry Fat-Free Ash Percent					
3M	.0000552	97.57	.000041	96.73	.43
4M	.0000480	98.18	.000179	87.76	7.14**

[a]Model 1: $Y = A(1 - Be^{[-k \times SCAP]})$, fit within each
slaughter period. Degrees of freedom for regression=15,
for error=10.
[b]Model 2: $Y = A(1 - Be^{[-k \times SCAP]})$, fit across all
slaughter periods, with A and B fit as quadratic functions
of day on test. Degrees of freedom for regression=7, for
error=18.
[c]Significance of lack of fit ratio is denoted: **P<.01,
*P<.05, +P<.10.

TABLE 5. PARAMETER ESTIMATES FOR ASYMPTOTIC RESPONSE SURFACES RELATING CAP AND DAY ON TEST TO BONE MEASUREMENTS OF PIGS FED FIVE LEVELS OF DIETARY CA/P FROM WEANING TO MARKET WEIGHT[a]

	Coefficients of A as a function of time			Coefficients of B as a function of time			
	A_0	A_1	A_2	B_0	B_1	B_2	k
Length							
3M	4.966	.0384	-.000088	.041	.00055	-.0000037	.1418
4M	4.901	.0415	-.000098	.019	.00119	-.0000072	.1990
Wet Weight							
3M	4.887	.2113	-.000199	.184	.00143	-.0000091	.1114
4M	5.655	.1859	-.000020	.255	-.00025	.0000003	.0997
Wall Thickness							
3M	.0898	.0010	-.000002	.344	-.00246	.0000151	.0670
4M	.0931	.0013	-.000002	.347	-.00135	.0000109	.0449
Cross-sectional Area							
3M	.2714	.0067	-.000010	.333	-.00150	.0000093	.0828
4M	.3128	.0069	-.000005	.425	-.00259	.0000148	.0583
Force							
3M	8.727	1.199	.000505	.046	-.00466	.0000193	.0462
4M	46.938	1.856	.000989	.983	-.00570	.0000167	.0577
Stress							
3M	275.0	1.241	-.001412	.704	-.00585	.0000223	.0306
4M	67.4	.681	-.000880	1.000	-.00870	.0000209	.0325
Dry Fat-Free Extracted Weight							
3M	.6534	.0426	.000035	.487	-.00023	-.0000039	.0807
4M	.4737	.0491	-.000014	.553	-.00119	-.0000021	.0682
Dry Fat-Free Ash Weight							
3M	.2115	.0292	.000010	.512	.00047	-.0000851	.0757
4M	.3967	.0236	.000027	.765	-.00492	.0000164	.0577
Dry Fat-Free Ash Percent							
3M	.4894	.0023	-.000011	-.032	.00325	-.0000190	.0961
4M	.5715	-.0003	.000003	.236	-.00269	.0000116	.0566

[a]Asymptotic response surface model:
$$Y = (A_0 + A_1 t + A_2 t^2)\{1 - (B_0 + B_1 t + B_2 t^2)e^{[-k \times SCAP]}\},$$
where t = Day on test at slaughter.

TABLE 6.   PREDICTED CA/P LEVEL, EXPRESSED AS A PERCENTAGE
   OF NRC-RECOMMENDED LEVELS, WHICH PRODUCE 95 PERCENT OF
      MAXIMUM FOR BONE CRITERIA AND PERFORMANCE MEASURES[a]

| Criteria | CAP level associated with 95% of maximum at each day on test | | | | |
	27	55	83	112	136
3M Length	70.4	71.3	71.4	70.7	69.4
4M Length	69.6	71.2	71.6	71.1	69.9
3M Wet weight	83.1	83.9	84.1	83.7	82.9
4M Wet weight	86.1	85.8	85.6	85.3	85.1
3M Radius	70.3	71.4	71.5	71.0	69.5
4M Radius	72.8	72.2	71.7	71.3	71.2
3M Wall thickness	96.2	94.3	93.7	94.5	96.2
4M Wall thickness	111.3	110.4	110.7	112.3	114.3
3M Area	91.6	90.7	90.5	90.9	91.7
4M Area	104.1	102.2	101.4	101.8	103.1
3M Bending force	126.2	123.2	121.1	120.0	120.1
4M Shear force	118.9	116.2	113.7	111.6	110.4
3M Bending stress	149.0	141.7	135.5	131.5	130.7
4M Shear stress	154.6	145.7	135.7	123.9	113.3
3M Extracted weight	98.0	97.6	97.0	96.1	95.2
4M Extracted weight	104.3	103.2	101.9	100.2	98.6
3M DFF ash weight	100.9	100.7	100.2	99.3	98.1
4M DFF ash weight	114.3	111.4	108.8	106.9	106.1
3M DFF ash, %	68.2	76.0	77.9	76.5	71.5
4M DFF ash, %	91.8	86.0	80.9	78.4	79.4
Body Weight	80.6	86.1	87.7	87.0	84.3
ADG	89.9	92.3	94.0	95.2	95.8
ADFI	83.3	88.3	89.2	87.2	81.2

[a]Bone maximums obtained by solving response surface
 equations in Table 5 for 95% maximum at each slaughter
 date.   Performance maximums obtained by solving
 performance response surface equations of Combs et al.
 (1989a) for 95% maximum at each slaughter date.

TABLE 7. PREDICTED CA/P LEVEL, EXPRESSED AS A PERCENTAGE
OF NRC-RECOMMENDED LEVELS, WHICH PRODUCE 98 PERCENT OF
MAXIMUM FOR BONE CRITERIA AND PERFORMANCE MEASURES[a]

Criteria	CAP level associated with 98% of maximum at each day on test				
	27	55	83	112	136
3M Length	76.9	77.7	77.8	77.2	75.9
4M Length	74.2	75.8	76.2	75.7	74.5
3M Wet Weight	91.4	92.1	92.3	92.0	91.2
4M Wet Weight	95.3	95.0	94.8	94.5	94.3
3M Radius	73.4	74.5	74.7	74.1	72.7
4M Radius	77.6	77.0	76.5	76.1	76.0
3M Wall Thickness	109.9	108.0	107.4	108.2	109.9
4M Wall Thickness	131.7	130.8	131.1	132.7	134.8
3M Area	102.7	101.8	101.5	101.9	102.8
4M Area	119.8	117.9	117.1	117.5	118.9
3M Bending Force	146.0	143.1	140.9	139.8	139.9
4M Shear Force	134.7	132.0	129.6	127.5	126.3
3M Bending Stress	178.9	171.6	165.4	161.4	160.6
4M Shear Stress	182.8	173.9	163.9	152.1	141.5
3M Extracted weight	109.3	108.9	108.3	107.5	106.6
4M Extracted weight	117.8	116.7	115.3	113.7	112.1
3M DFF ash weight	113.0	112.8	112.3	111.4	110.2
4M DFF ash weight	130.2	127.3	124.7	122.8	121.9
3M DFF ash, %	77.7	85.6	87.4	86.0	81.0
4M DFF ash, %	108.0	102.1	97.1	94.6	95.5
Body Weight	90.5	95.9	97.6	96.9	94.2
ADG	101.5	103.9	105.6	106.8	107.4
ADFI	93.0	98.0	99.0	96.9	90.9

[a]Bone maximums obtained by solving response surface
equations in Table 5 for 98% maximum at each slaughter
date. Performance maximums obtained by solving
performance response surface equations of Combs et al.
(1989a) for 98% maximum at each slaughter date.
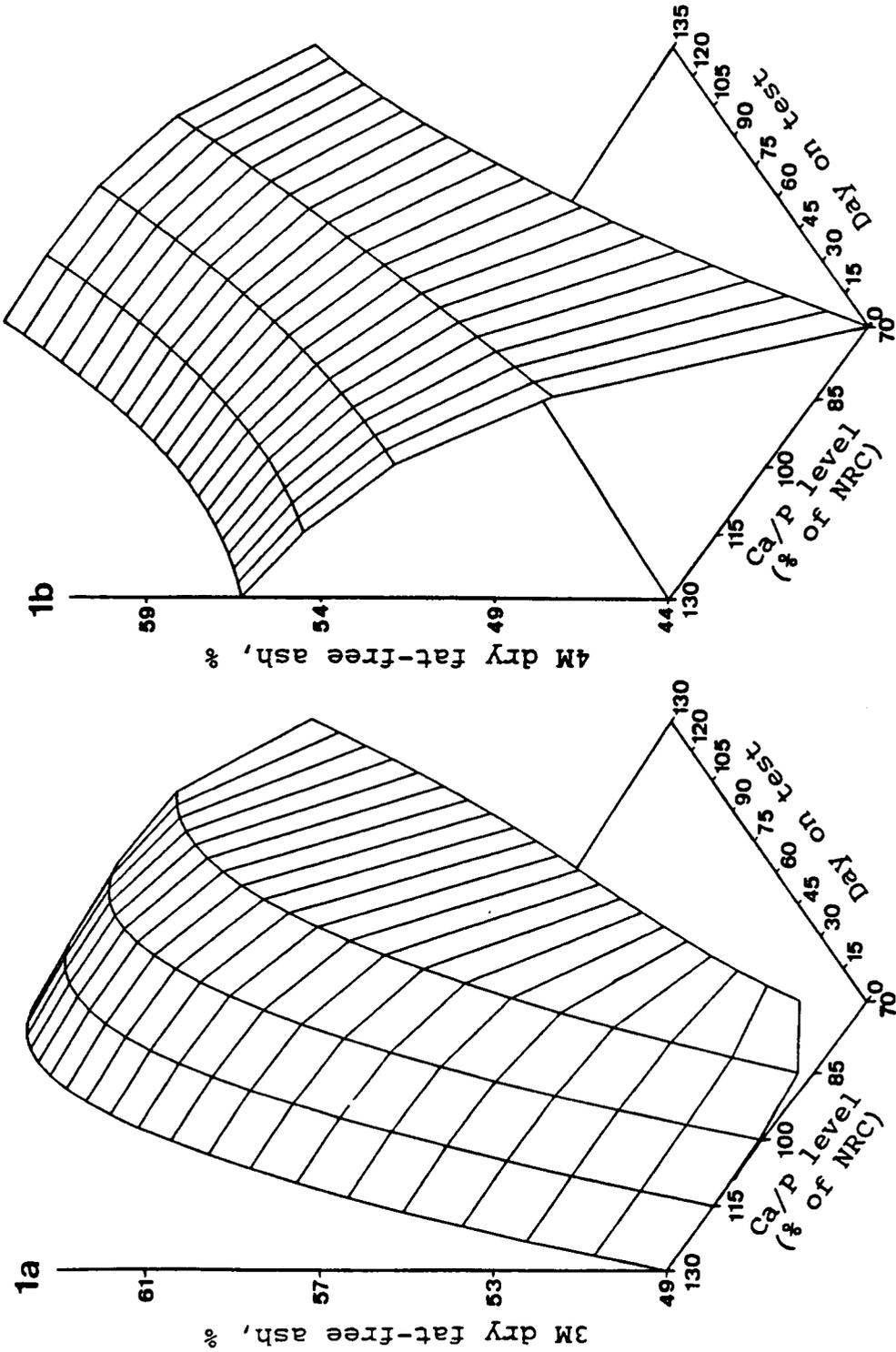
Figure 1. Asymptotic response surface relating the effects of CAP level and time on test to the observed dry fat-free ash percentage of pooled third metacarpal and metatarsal bones (1b) and of pooled fourth metacarpal and metatarsal bones (1b) for pigs fed five levels of dietary Ca/P from weaning to market weight.
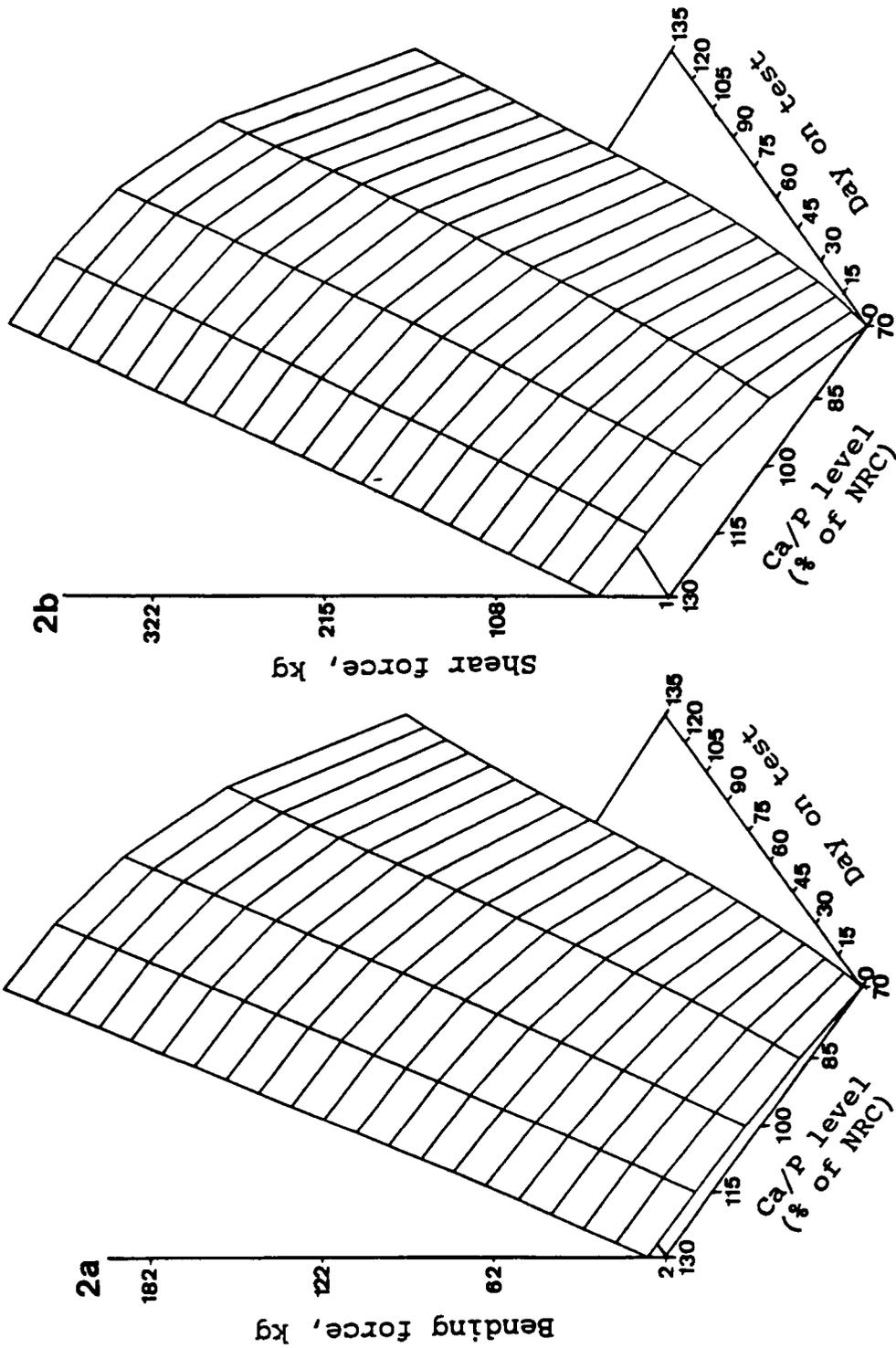
Figure 2. Asymptotic response surface relating the effects of CAP level and time on test to the observed bending force of pooled third metacarpal and metatarsal bones (2a) or shear force of pooled fourth metacarpal and metatarsal bones (2b) for pigs fed five levels of dietary Ca/P from weaning to market weight.
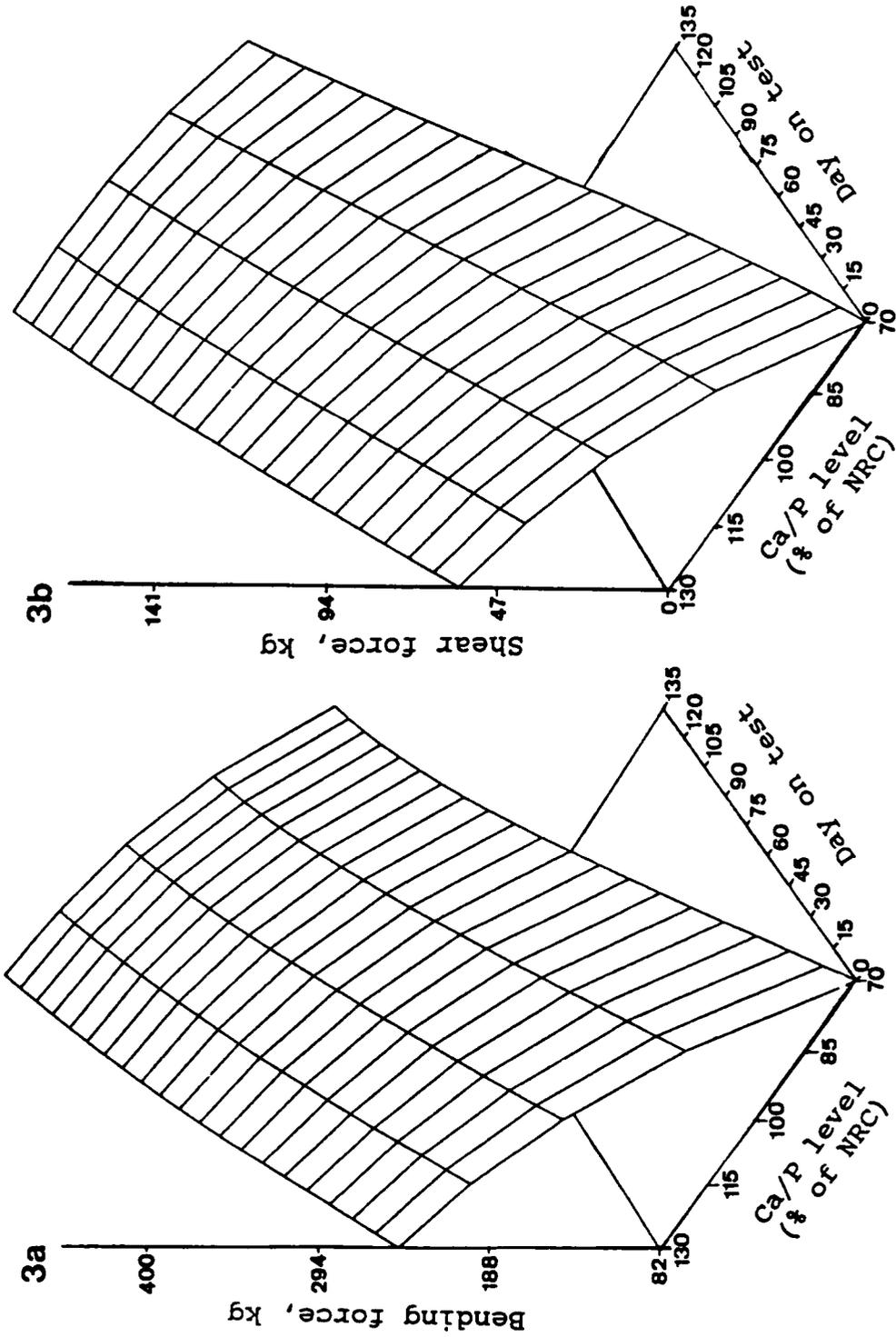
Figure 3. Asymptotic response surface relating the effects of CAP level and time on test to the observed bending stress of pooled third metacarpal and metatarsal bones (3a) or shear stress of pooled fourth metacarpal and metatarsal bones (3b) for pigs fed five levels of dietary Ca/P from weaning to market weight.
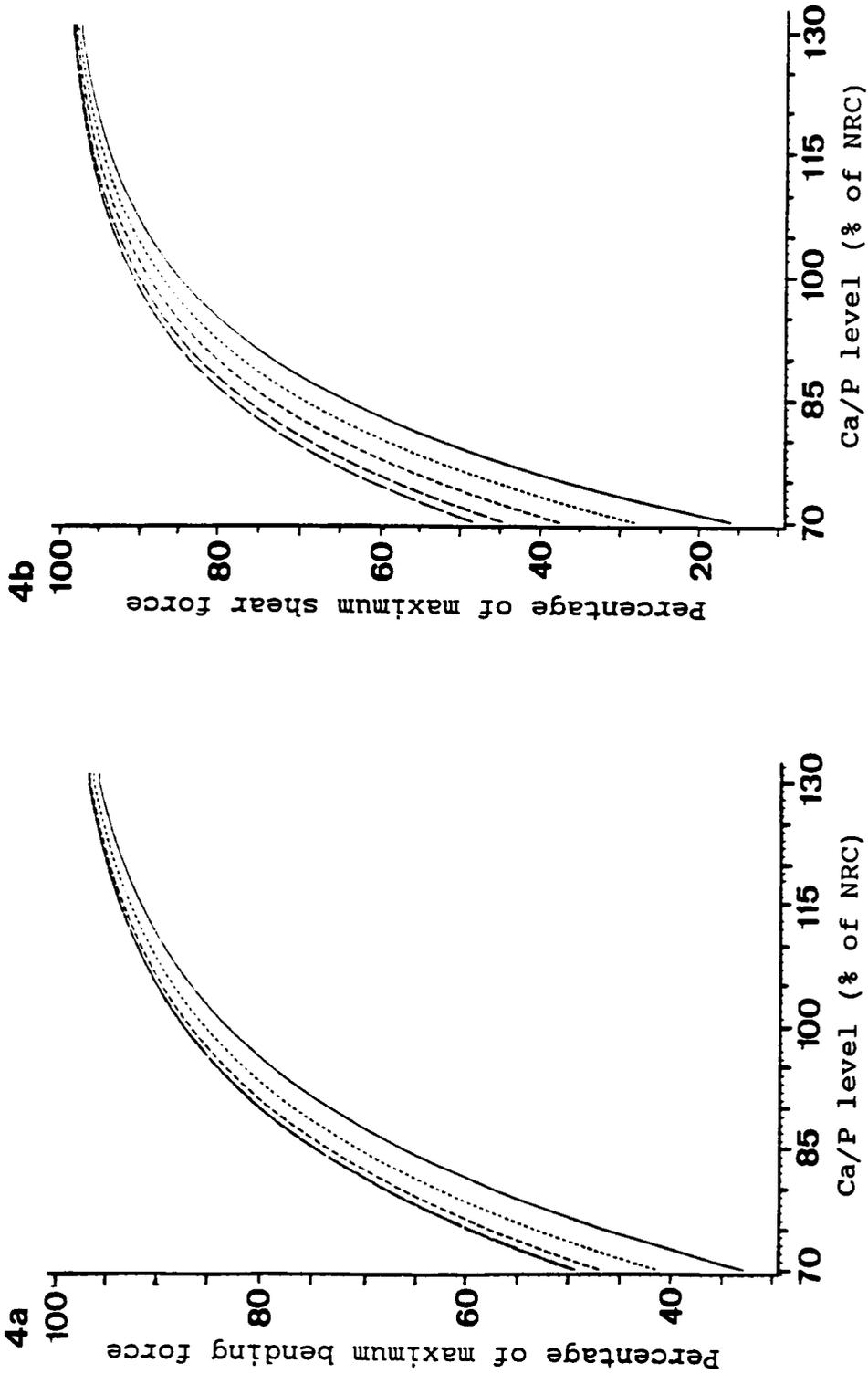
Figure 4. Percentage of maximum bending (4a, pooled third metacarpal and metatarsal) and shear (4b, pooled fourth metacarpal and metatarsal) force associated with each CAP level at 27 (———), 55 (·····), 83 (– – –), 112 (– · –), and 138 (———) days on test for pigs from weaning to market weight.
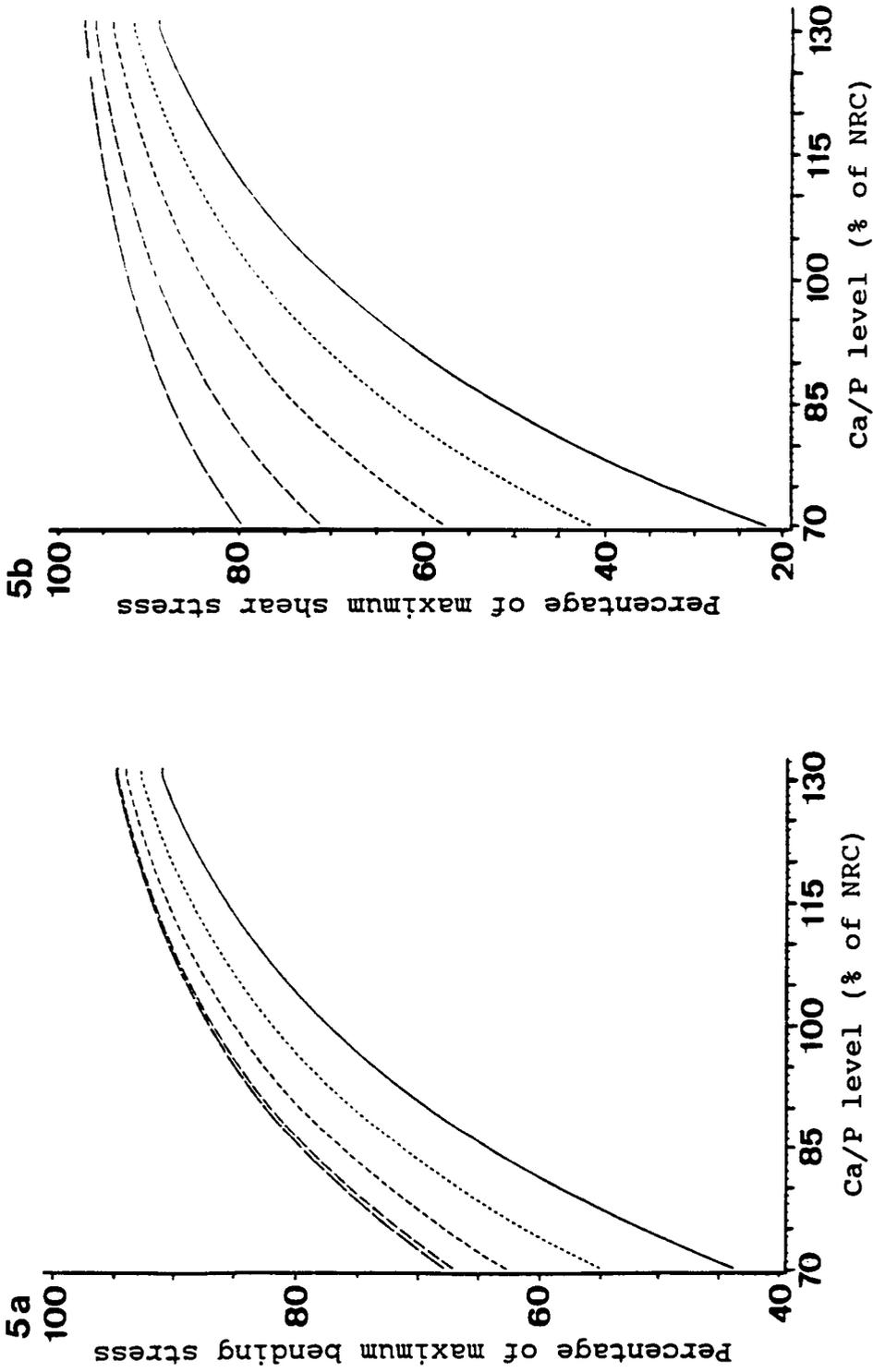
Figure 5. Percentage of maximum bending (5a, pooled third metacarpal and metatarsal) and shear (5b, pooled fourth metacarpal and metatarsal) stress associated with each CAP level at 27 (———), 55 (-·-·-), 83 (-·--·), 112 (—-·), and 138 (———→) days on test for pigs from weaning to market weight.
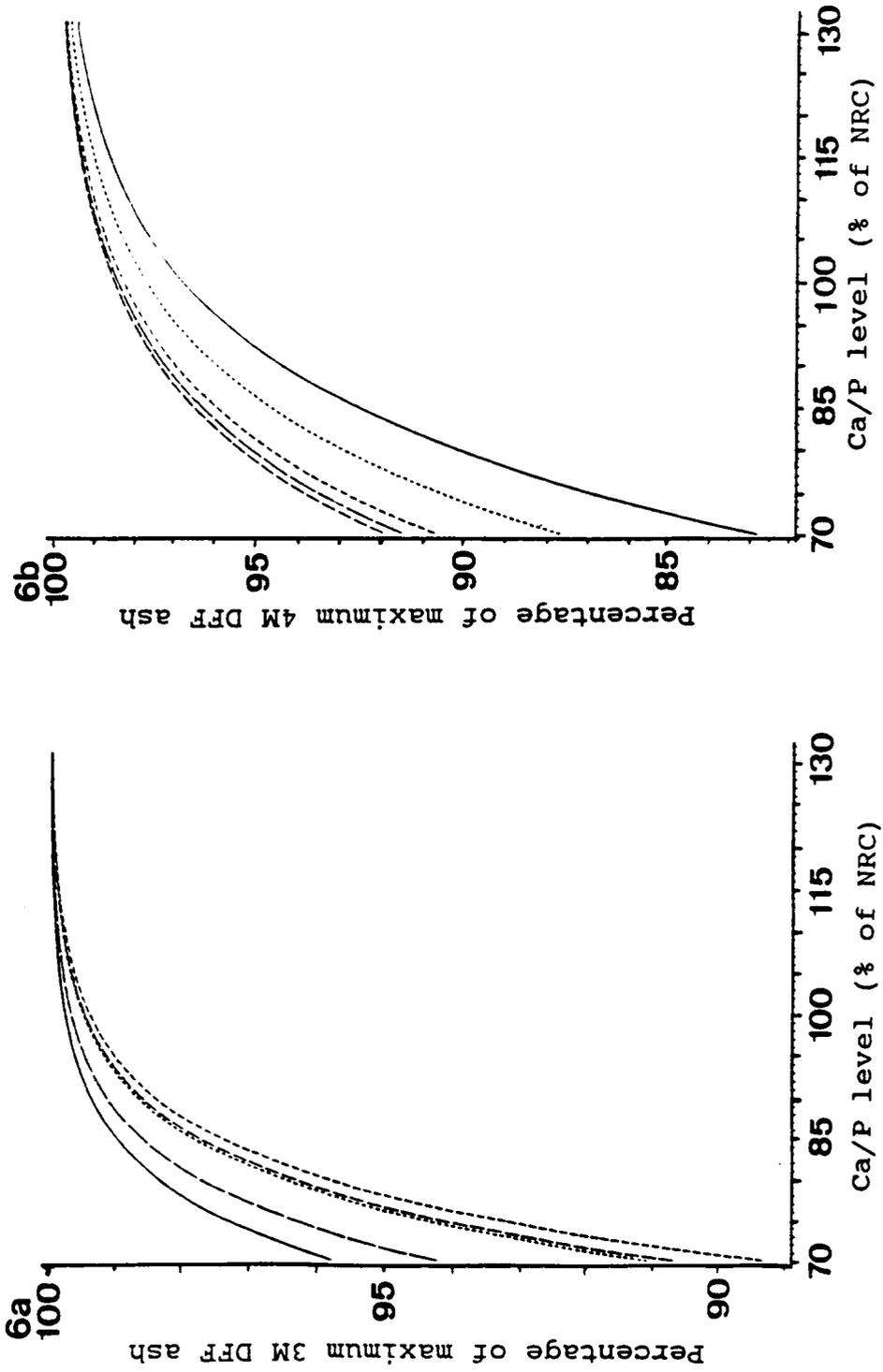
Figure 6. Percentage of maximum pooled third metacarpal and metatarsal dry fat-free ash percentage (6a) and pooled fourth metacarpal and metatarsal dry fat-free ash percentage (6b) associated with each CAP level at 27 (———), 55 (-----), 83 (-·-·-), 112 (—·—), and 138 (———) days on test for pigs from weaning to market weight.

CHAPTER 6

Running head: Evaluation of bone biopsy for swine

EVALUATION OF A BONE BIOPSY TECHNIQUE FOR
DETERMINING THE CALCIUM AND PHOSPHORUS STATUS
OF SWINE FROM WEANING TO MARKET[1,2,3]

ABSTRACT

Bone biopsies were taken from 75 live pigs at 4 wk
intervals and from 251 slaughter pigs to evaluate bone
biopsy as a procedure for determining the Ca/P status of
live pigs fed a wide range of Ca/P levels [70, 85, 100, 115
and 130% of the NRC recommended (1979) dietary Ca and P
levels] from weaning to market. The diet and time effects
on chloroform-methanol ash weight, ash percentage of wet
weight and dry fat-free ash percentage (DFF%) of the biopsy
core did not differ between slaughter and live biopsies,
and generally responded linearly and quadratically (P<.01)
to increasing Ca/P level and time. Biopsies measures were
significantly correlated with third and fourth metacarpal
and metatarsal length, wet weight, wall thickness, area,
force, stress and DFF% for all diets. Least squares (LS)
means and SE of live and slaughter biopsy wet weight,
extracted weight, ash weight and DFF% were compared at each
time in each trial and found not to differ. Also, LS means

for slaughter biopsy DFF% were not found to differ greatly
from pooled third and fourth metacarpal and metatarsal
DFF%. Pooled live and slaughter biopsy DFF% LS means were
found to be lower than the average bone ash for the 70 and
85% NRC recommended Ca/P diets, but did not differ for the
100, 115 and 130% NRC diets. Bone biopsy offers potential
as a reliable non-slaughter procedure for monitoring the
Ca/P status of swine from weaning to market, but needs
further study for use in Ca/P research in swine.

(Key Words: Calcium, Phosphorus, Pigs, Biopsy, Bone Ash)

Introduction

Traditionally, the determination of the Ca and P
status of swine has relied heavily on obtaining bone
samples at slaughter for evaluation of bone ash, bone Ca
and P content or bone strength. There is a large amount of
variation inherent in these bone measures (Crenshaw et al.,
1981), necessitating the use of large numbers of animals to
detect true differences among dietary treatments. Thus, a
high cost is associated with obtaining and maintaining the
large number of animals that are required to detect
differences. Additionally, removal of bones can reduce the
salvage value of the carcass, particularly if the animal is
sacrificed before it reaches market weight, and each animal
only provides a single set of data.

A satisfactory non-slaughter technique has not been

reported for swine (Crenshaw, 1986). Little (1972) described a bone biopsy technique for use in monitoring changes in bone composition of cattle and sheep. In subsequent papers, Little and McMeniman (1973) and Little et al. (1978) used this technique to study P depletion and repletion in gestating and lactating ewes. Use of bone biopsy in nutrition studies of the pig has not been previously reported. The objective of this study was to develop and evaluate a bone biopsy procedure for use as a non-slaughter sampling technique for assessing the Ca/P status of swine from weaning to market.

Experimental Methods

A total of 75 live and 251 slaughter pigs were used in three trials to evaluate bone biopsy as a live animal sampling method for studying the Ca/P status of pigs fed a wide range of Ca/P levels from weaning to market. Five dietary Ca/P levels [70, 85, 100, 115 and 130% of the NRC (1979) recommended Ca/P levels] were fed across four phases of growth from weaning to market at 110 kg (approximately 140 d).

Pigs were grouped by sex and sire into blocks of 5 pigs each and replicates were composed of 5 blocks each. There were 5 replicates in Trial 1, and 3 and 4 replicates, respectively, in Trials 2 and 3. One replicate in each trial was randomly chosen for use in the biopsy portion of

the study, with the remaining replicates used in the slaughter portion of the study. One pig within each block was assigned to each of the five diets. More complete details concerning dietary treatments, housing and management practices are reported by Combs et al. (1989a).

The live pigs were biopsied (live biopsies) on the first day on test and at 4 wk intervals thereafter until the average weight of the group was approximately 110 kg. At that time the pigs were slaughtered and the final biopsy was taken from the hot carcass. The side of the live pig that was biopsied was alternated between sampling times so that the time interval between sampling at the same site was 8 wk. Additionally, one block from each slaughter replicate (slaughter biopsies) was sacrificed at the end of each 4 wk interval and a biopsy was taken from each hot carcass at the location corresponding to the live-animal biopsy.

Pigs were placed under general anesthesia for the live-animal biopsy procedure using 11 to 16 mg·kg^{-1} BW of a 4% solution (40 mg·ml^{-1}) of Bio-Tal (thiamylal sodium)[4] as the anesthetic agent. The anesthetic was injected into the anterior vena cava in the initial sampling period and the

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[4]Bio-Tal is the thiobarbiturate analogue of secobarbital [sodium 5 allyl-5-(1-methyl-butyl)-2-thiobarbiturate]. Product of Bio-Ceutic Division, Boehringer Ingelheim Animal Health, Inc. St. Joseph, Missouri.

two subsequent biopsy periods. During the final two live biopsy periods the injection was made via an external ear vein.

The biopsy procedure was carried out under near-sterile conditions. The biopsy was taken from the medial surface of the proximal tibia, in the area immediately dorsal to the epiphyseal plate (figure 1). The surgical area was clipped[5] and scrubbed with a povidone iodine solution (7.5%)[6]. The area was then rinsed with a 70% isopropyl alcohol solution and patted dry with sterile 4 x 4 cloths[7]. A small incision (approximately 1.75 cm) was made through the skin and tissues covering the bone using a #15 scalpel. A bone core was removed using a 56 mm x 15.9 cm michele laminectomy trephine[8] and immediately placed in air-tight, moisture-proof bag. Then, the incision was closed using a mattress suture[9]. Bone core samples were frozen at -20 C, pending chemical analysis. All tools were maintained in a benzyl-dimethyl ammonium disinfectant

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[5]Oster model A-5 clippers (size 40 blades), Oster Corp. Milwaukee, WI.
[6]Cliniscrub, The Clinipad Corp. Guilford, CT.
[7]Nu-Gauze 4 x 4 - 4 ply. Johnson & Johnson Products Inc. New Brunswick, NJ.
[8]Supplied by Arista Surgical Supply Company, Inc. New York, New York.
[9]3-0 metric (2-0 standard) Ethicon coated, absorbable vicryl, with an FS-1 cutting needle. Supplied by Arista Surgical Supply Company, Inc. New York, New York.

solution[10] between uses and were rinsed in distilled water upon removal from the solution to prevent tissue irritation. All animals were observed for post-operative complications until fully recovered from the anesthesia.

Biopsy samples were thawed and the wet weight of each sample was taken. Samples were then wrapped in unbleached cotton muslin sheeting and tagged with numbered chicken wing tip clips for identification before drying in a forced air oven for 24 hours. The fat contained in the biopsy sample was then extracted by refluxing a 75%:25% chloroform: methanol mixture over the dried samples in a 4 l soxhlet apparatus. Extracted samples were then returned to the forced-air oven and redried for 24 hr. The weight of dry, fat-free bone tissue from each sample was then determined and the entire sample was ashed and cooled completely. Weight of the cooled, ashed sample was then determined and the percentage ash was calculated.

Biopsy measurements analyzed included: dry fat-free ash weight, ash weight as a percentage of the wet weight and dry fat-free ash percentage (DFF% = ash weight/ extracted weight). The dry fat free ash weight of the biopsy core was included in the analysis since the diameter of the biopsy core was fixed by the diameter of the

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[10]Aqueous 17% Zephiran chloride (1:750 dilution). Winthrop Laboratories. New York, NY.

trephine used and the weight of the core sample would be expected to differ more due to the effect of diet on bone wall thickness than due to any sampling error.

For live biopsy data, ANOVA procedures for repeated measures were used to assess main effects of trial, diet, sex and time (the repeated factor) and effects of all two-way interactions involving trial, diet and time. Calculations were performed using the repeated measure option of the GLM procedure of the Statistical Analysis System (SAS Institute Inc., 1985). Orthogonal polynomials of diet means were used to evaluate the shape of the response of the dependent variables to increasing Ca/P intake. Slaughter biopsies were analyzed by ANOVA using a model that included effects of trial, diet, slaughter time, sex and effects of all two-way interactions involving trial, diet and slaughter time. Orthogonal polynomial contrasts were applied to diet and slaughter time means within each slaughter time and across all slaughter periods. Sex effects on live and slaughter biopsy measures were not significant; therefore the effects of sex were excluded from the final models. Combs et al. (1989b) found no effect of sex on third and fourth metacarpal and metatarsal bone criteria used for comparison in this study.

Simple correlations were determined between the measurements obtained from slaughter biopsies and those

obtained from pooled third metacarpal and metatarsal (3M) and pooled fourth metacarpal and metatarsal (4M) bone pairs (Combs et al. 1989b). 3M and 4M measures included bone length, wet weight, wall thickness, cross-sectional area, force, stress, extracted weight, ash weight, and DFF%. Additionally, simple correlation between biopsy core DFF% and pooled bone DFF% (averaged across all four bones) was also determined. Correlations between the final period live biopsy measurements and third and fourth metacarpal and metatarsal bone measurements were also determined. In order to determine if the correlations obtained were dependent on the presence of data from pigs clearly deficient in Ca/P (fed 70% NRC; see Combs et al., 1989a), correlations were also determined without the 70% NRC dietary Ca/P level.

Least squares (LS) means and SE generated from the ANOVA on live and slaughter biopsy measures were plotted to determine if live biopsy measures differed from slaughter biopsy measures within each trial and slaughter time. Additionally, LS means and SE of slaughter biopsy DFF% and of the average of slaughter and live biopsy DFF% were plotted against LS bone DFF% of pooled third and fourth metacarpals and metatarsals to determine if the biopsy ash differed from ash determined on intact metacarpal and metatarsal bones.

Results and Discussion

<u>Diet Effects on Biopsy Cores</u>

The ash weight of slaughter and live biopsy cores increased linearly with increasing dietary Ca/P level (Table 1), and also increased linearly in response to Ca/P intake within each time period. Slaughter and live biopsy ash percentage of wet weight and DFF% increased linearly (P<.01) and quadratically (P<.05) in response to increasing dietary Ca/P. As was the case for the ash weight, most of the diet effect on ash percentages were linear within each time period for both live and slaughter biopsies.

Increased DFF% in response to increasing dietary Ca/P has previously been reported by a number of researchers. Fammatre et al. (1977) found higher sixth rib, femur and metacarpal DFF% in growing pigs fed 140% of NRC recommended Ca/P, when compared with pigs fed 100% NRC Ca/P, although differences were not apparent at the end of the finisher phase. Maxson and Mahan (1983) found that femur, humerus, rib, metacarpal and metatarsal DFF% increased quadratically in response to Ca/P levels from 80 to 180% of NRC recommendations. Nimmo et al. (1980), Kornegay et al. (1981) and Brennan and Aherne (1986) all reported linear increases in bone ash in response to increasing Ca/P above 100% of NRC recommendations, although bone ash appeared to trend toward a quadratic response to diet in all three

cases. Pooled third metacarpal and metatarsal and pooled fourth metacarpal and metatarsal DFF% increased linearly and quadratically with increasing dietary Ca/P levels in the range of 70 to 130% of NRC (1979) recommendations (Combs et al., 1989b). In contrast, other researchers have reported that bone ash was not affected by increasing dietary Ca/P within the range of 60 to 180% of the NRC recommended Ca level and 80 to 200% of NRC recommended P levels (Crenshaw, 1986; Harmon et al., 1974; Liptrap et al., 1970; Schroeder et al., 1974).

Little (1972) and Little and McMeniman (1973) reported that biopsy ash, expressed as a percentage of wet weight, was more sensitive to dietary P than biopsy ash expressed as a percentage of dry fat-free bone. To the contrary, no apparent advantage for the expression of ash weight as a percentage of wet weight instead of as a percentage of DFF bone was observed in the current study.

Time and Interaction Effects on Biopsy Cores

The ash weight, ash percentage of wet weight and DFF% of live biopsy core samples increased at a decreasing rate (P<.01) from weaning to market (Table 1). Slaughter biopsy ash weight, ash percentage of wet weight and DFF% increased linearly (P<.01) over time. Bone ash has previously been reported to increase in a linear and/or quadratic manner from weaning to market weight (Crenshaw, 1986; Hickman et

al., 1983; Lepine et al., 1985; Maxson and Mahan, 1983).

Pooled third metacarpal and metatarsal and pooled fourth metacarpal and metatarsal DFF% also increased at a decreasing rate from weaning to market in pigs fed 70 to 130% of NRC (1979) recommended dietary Ca/P levels (Combs et al., 1989b). Overall, both linear and quadratic responses (P<.01) of average bone DFF% to increasing dietary Ca/P, as well as to time, are reflected in the averages presented in Table 2. Contrary to results observed with biopsy core samples, quadratic diet effects were observed within many time periods for these average bone ash values.

Time by linear diet interactions were significant (P<.05 or P<.01) for ash weight and DFF% of live biopsy cores. Both linear and quadratic diet by time effects were significant (P<.01) for ash percentage of wet weight. For slaughter biopsy measurements, only ash percentage of wet weight had a significant linear diet by time effect (P<.05). Crenshaw (1986) found no significant interaction of dietary Ca or P and time for the ash weight of third metatarsal bones, whereas the increase over time for the percentage of DFF ash could be altered by dietary Ca level.

Summary of Diet and Time Effects on Biopsy Cores

Although biopsy core DFF% may respond somewhat differently than bone DFF% within each slaughter period, the overall diet and time effects are similar in this study. Additionally, it appears that there are few differences between slaughter biopsy core measures and those of live biopsy cores obtained by repeated sampling of the same animals. In the present study, the overall diet and time effects observed for biopsy core samples are consistent with the overall diet and time effects on the intact bones of these pigs, as well as the effects summarized from published literature.

Correlations

Overall, slaughter biopsy DFF% correlated strongly (P<.001) with 3M and 4M force (r=.55 and .57, respectively) and stress (r=.52 and .55, respectively; Table 3). The overall correlation of slaughter biopsy DFF% with physical measurements of the bone (bone length, wet weight, wall thickness and cross-sectional area) were generally lower, but consistent (P<.001) between 3M and 4M bone pairs. The overall correlation between slaughter biopsy DFF% and 3M DFF% was .48 (P<.001), and a value of r=.55 was obtained for the slaughter biopsy DFF% and 4M DFF% (P<.001). Averaged across all bones, the correlation of slaughter biopsy DFF% with bone DFF% was .56 (P<.001).

The correlations between live biopsy core measures and bone measures could only be determined at final, market-weight slaughter. The correlation of live biopsy DFF% with 3M and 4M length, wet weight and radius were similar to those for slaughter biopsies (Table 3). The overall correlations between live biopsy DFF% and all other bone measures were slightly lower than between slaughter biopsy DFF% and the bone measures.

The correlations between biopsy DFF% and measures of bone dimension and strength are higher than have been reported for ash measurements with these bone criteria. Cromwell et al. (1972) found that the simple correlation between total ash percentage of the second and fifth metacarpals and third and fourth metacarpal measures were r=.13 for length, .17 for wall thickness, .18 for weight and .29 for breaking force; turbinate ash percentage was also reported to be correlated with breaking force at r=.21.

When simple correlations were determined without the 70% NRC dietary Ca/P level, the correlations between slaughter biopsy core percentages and bone measures were often equal or slightly lower than previously seen when the lowest Ca/P level was included (Table 3). The correlations increased in magnitude between slaughter biopsy core weights and bone wall thickness, area and average bone

DFF%; correlations were lower in magnitude between
slaughter biopsy core weights and bone length, wet weight
and stress when the 70% NRC diet was excluded. Changes in
the correlations between live biopsy core measures and bone
measures, resulting from excluding the 70% NRC Ca/P diet,
did not show as consistent a pattern as observed for
slaughter biopsies (Table 3), although generally, the
magnitude of the coefficients decreased when the lowest
Ca/P diet was excluded.

The simple correlations reported in this study
indicate that both slaughter and live biopsy core extracted
weight and core ash weight are correlated significantly
with bone length, wet weight, wall thickness, area, force,
stress, extracted weight, ash weight and DFF%. When
expressed as a percentage of wet weight, the percent of ash
of both slaughter and live biopsy cores are correlated with
bone wall thickness, area, force, stress, extracted weight,
ash weight and DFF%, while slaughter biopsy ash, as a
percent of wet weight, is also correlated with bone length,
wet weight and radius. The DFF% of slaughter and live
biopsy samples correlate strongly with bone length, wet
weight, wall thickness, area, force, stress, extracted
weight, ash weight and DFF%.

These data indicate that the composition of both
slaughter and live biopsy cores are related to the

composition profiles of third and fourth metacarpals and
metatarsals. This would suggest that the biopsy core
obtained by the procedure described herein could provide
bone tissue that is consistent with what would be obtained
for intact bones, warranting further evaluation of the
biopsy procedure as a live animal sampling technique.

Least Squares Mean Comparisons

The SE of the least squares (LS) means for slaughter
and live biopsy ash weights overlapped in at least two of
the three trials for nearly all diets at all slaughter
times (Table 4). When ash weight was expressed as a
percentage of wet weight, the LS means and SE for the
slaughter biopsies and the live biopsies overlapped less
consistently, particularly for the early time periods. The
DFF% of slaughter and live biopsy cores were more
consistent, overlapping in all trials in four of the
slaughter periods for each diet.

The greater consistency of ash, expressed as a
percentage of dry fat-free bone, when compared to ash
expressed as a percentage of wet weight, is contrary to the
observations of Little (1972) and Little and McMeniman
(1973). These researchers found ash expressed as a
percentage of fresh bone tissue was a more sensitive
indicator of skeletal mineralization than DFF%.

Consistency of the biopsy extracted weights and ash

weights, and of the DFF% between slaughter and live biopsy cores has been demonstrated. This suggests that the profiles of the independent slaughter biopsies were similar to those of the live biopsies obtained by repeated measure on the same animal at each time, and warranting a comparison of biopsy DFF% with that of bone DFF%.

LS means and SE of slaughter biopsy DFF% was compared at each slaughter time to corresponding LS means and SE for bone DFF%, averaged across the third and fourth metacarpals and metatarsals (Table 5). At initial slaughter, the DFF% of biopsy and bone samples overlapped only in Trial 3. At later time periods, the LS means and SE for slaughter biopsy and bone ash overlapped in at least two of the three trials, with the exception of the 130% dietary Ca/P level at day 27, and the 85% dietary Ca/P level at day 55 and 112.

Slaughter and live biopsy DFF% were then pooled for each diet and slaughter time and compared to the average bone ash (Figure 2). The LS mean DFF% and SE of the pooled biopsies overlapped the LS means and SE of the bone ash at the initial slaughter time (not shown). For the 70% dietary Ca/P level, biopsy and bone ash did not differ at day 138, whereas overlap of LS means and SE were observed at days 27 and 83 for the 85% dietary Ca/P level. Biopsy and bone ash were similar at days 27, 112 and 138 for the

three highest dietary Ca/P levels, at days 55 and 83 for

115% dietary Ca/P and at day 83 for the highest dietary

Ca/P level. When averaged across slaughter periods, biopsy

ash and bone ash were not different for the three highest

Ca/P levels; biopsy DFF% was lower than bone DFF% for pigs

fed diets containing 70 and 85% of NRC recommended dietary

Ca/P.

The LS mean comparisons indicate that bone core

samples obtained by repeated biopsy of the proximal tibia

of live growing-finishing pigs have similar ash weights and

DFF% when compared to biopsy samples obtained from proximal

tibia of the hot carcass of slaughter pigs. This appears

to hold true at all time periods from weaning to market,

and for pigs fed diets containing 70 to 130% of the NRC

recommended Ca/P levels. When compared to third and fourth

metacarpal and metatarsal intact bone ash, the DFF% of

biopsy cores for pigs fed diets containing 100 to 130% of

NRC recommended Ca/P levels appear to be similar to the DFF

ash of the intact bone, although biopsy ash of pigs fed

diets containing 70 or 85% of NRC Ca/P is lower than that

of bone ash.

Evaluation of Surgical Procedure

Up to three biopsies were taken from the proximal

tibia of each leg of the live pigs, with intervals of 8 wk

between sampling at the same site. The anesthetic dosage

required to induce a surgical plane of anesthesia averaged between 11 and 16 mg·kg^{-1}. In general, the per kg dosage required was higher at 4 wk on test (pigs 8 to 10 wk of age) than for the initial surgery, then decreased at subsequent sampling periods. A surgical plane of anesthesia was reached within 3 to 5 min of injection, and was sufficient to allow preparation of the surgical site and subsequent surgery. In general, recovery from the effects of the anesthetic began within 15 min of injection, and, by 1 hr post-injection, pigs were feeding and no lingering anesthesia effects were apparent.

During the course of three trials, only one pig was removed from test due to a physical complication of the biopsy procedure. In that instance, a pig on the lowest Ca/P level suffered a broken tibia (at the biopsy site) 2 wk after the third surgery when being moved to the scale area. Subsequent necropsy of this animal revealed that the bone wall thickness of the tibia was only 10% as thick as would be expected for a pig of that age (approximately 15 wk of age). Two pigs each in Trials 1 and 2 died during surgery due to an adverse reaction to the anesthetic; one pig in each trial died during the fourth surgery, while the other two pigs were lost during the fifth surgery. Respiratory depression is listed as one of the potential side effects of thiamylal sodium, the anesthetic agent

used. Three other pigs were removed from test for problems
that were shown by post-mortem evaluation to be unrelated
to dietary treatments or surgical procedures.

Cross-sections were made through the biopsy site of
the tibias of the biopsy pigs following slaughter, and the
biopsy sites were examined histologically. Bone
regeneration and remodeling at the biopsy site was evident,
and no histological abnormalities in bone repair were
detected.

From a physical standpoint, the biopsy technique was
very successful. Of the 75 pigs initially allotted for the
live-biopsy study, 67 pigs completed the trial. Stiffness
was noted for most pigs for 1 to 2 d after surgery, but no
differences in feed intake or gain for biopsy pigs compared
to non-biopsied pigs were found (Combs et al., 1989a).

Implications

The research reported herein indicates the effects of
feeding 70 to 130% of NRC recommended Ca/P level to pigs
from weaning to market are similar for bone cores obtained
by biopsy of the proximal tibia, and for third and fourth
metacarpal and metatarsal bone ash. Biopsy core measures
also correlate significantly with bone dimensional,
strength and ash measures. LS mean comparisons indicate
that biopsy core DFF% may relate more closely to intact
bone ash when dietary Ca/P levels of 100% of the NRC

recommendations or greater are fed to growing-finishing

pigs. Overall, these results suggest that use of biopsy as

a live-animal sampling technique in Ca/P studies warrants

further consideration.

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TABLE 1. LEAST SQUARES MEAN LIVE AND SLAUGHTER BIOPSY CORE MEASURES FOR PIGS FROM WEANING TO MARKET WEIGHT, BY DIET AND DAY ON TEST[a]

Live measures — Ca/P level (% of NRC)

Day on Test	70	85	100	115	130	SEM
	Live ash weight, g[b]					
0	20.7	25.5	28.0	23.2	23.7	3.2
27	23.4	21.7	29.4	36.0	46.4	4.7e
55	20.1	21.6	30.7	33.1	42.5	4.3e
83	21.7	25.6	39.5	55.3	46.9	5.0e
112	-	-	-	-	-	-
138	33.4	42.2	48.4	82.0	77.5	7.2e
	Live ash, % of wet weight[h]					
0	28.4	24.0	26.9	23.3	27.4	1.5
27	22.9	25.8	29.8	35.8	32.6	1.2ef
55	33.0	30.2	34.9	39.8	38.1	1.4e
83	25.4	28.3	33.3	37.7	36.6	1.5e
112	-	-	-	-	-	-
138	26.1	33.1	39.1	39.7	38.8	2.0ef
	Live dry fat-free ash percentage[j]					
0	51.1	51.0	51.8	49.8	50.9	1.0
27	46.8	49.3	52.8	54.3	53.7	1.1e
55	51.1	48.2	51.1	54.7	53.3	1.1e
83	47.8	50.8	53.9	56.5	57.5	1.1e
112	-	-	-	-	-	-
138	52.4	56.5	58.9	61.0	59.8	.8eg

Slaughter measures — Ca/P level (% of NRC)

Day on Test	70	85	100	115	130	SEM
	Slaughter ash weight, g[c]					
0	-	-	-	-	-	-
27	19.1	24.0	30.6	37.7	36.6	10.0e
55	26.6	23.5	42.6	56.2	56.0	9.9e
83	18.2	32.5	44.5	51.4	53.9	10.3e
112	70.4	55.2	97.6	112.8	107.1	10.0
138	30.1	48.2	73.2	85.3	67.1	10.3ef
	Slaughter ash, % of wet weight[i]					
0	-	-	-	-	-	-
27	27.6	33.7	33.3	31.4	33.4	1.9
55	29.7	27.2	31.6	35.3	34.1	2.0d
83	21.8	34.0	36.9	37.1	40.0	2.0eg
112	27.9	31.9	41.1	41.2	38.6	2.3ef
138	34.5	36.9	41.0	40.5	43.8	2.0e
	Slaughter dry fat-free ash, %[k]					
0	-	-	-	-	-	-
27	47.8	51.3	52.8	53.2	52.9	1.3e
55	48.5	47.2	53.8	57.8	55.1	1.3e
83	50.5	56.4	55.3	57.3	58.9	1.3e
112	50.4	51.9	57.5	58.9	58.5	1.5e
138	54.0	55.7	57.5	59.2	60.2	1.3e

[a]No estimate of least squares means at slaughter time 4 could be obtained for live biopsy pigs due to missing cells in repeated measure design. Initial slaughter biopsy averages were: ash weight = 26.1±5.7; ash % = 31.3±1.1; and dry fat-free ash % = 51.5+.7.

[b]Overall linear (P<.01) effect of diet and overall linear (P<.01) and quadratic (P<.01) effect of time; significant linear diet by time interaction (P<.01).

[c]Overall linear effects of both time and diet (P<.01).

[d,e]Linear effect of diet (P<.05 and P<.01, respectively).

[f,g]Quadratic effect of diet (P<.05 and P<.01, respectively).

[h]Overall linear (P<.01) and quadratic (P<.01) effect of diet and overall linear (P<.01) and quadratic (P<.01) effect of time; significant linear and quadratic diet by time interactions (P<.01).

[i]Overall linear (P<.01) and quadratic (P<.01) effects of diet and linear (P<.01) effect of time; significant quadratic diet by time interaction (P<.05).

[j]Overall linear (P<.01) and quadratic (P<.05) effect of diet and overall linear (P<.01) and quadratic (P<.01) effect of time; significant linear diet by time interaction (P<.01).

[k]Overall linear (P<.01) and quadratic (P<.05) effects of diet and linear (P<.01) effect of time.

TABLE 2. LEAST SQUARES MEAN POOLED DFF ASH PERCENTAGES FOR THIRD AND FOURTH METACARPAL AND METATARSAL BONES, FROM PIGS USED TO OBTAIN SLAUGHTER AND LIVE BIOPSY CORES, BY DIETARY CA/P LEVEL AND DAY ON TEST ACROSS TRIALS[a,b]

| Day on Test | Diet | | | | | SEM |
	70	85	100	115	130	
Avg. Dry fat-free ash, %[c]						
27	49.9	52.4	52.3	54.1	54.8	.4[d]
55	50.8	54.7	57.0	56.7	57.8	.4[de]
83	53.1	56.4	58.7	59.0	59.2	.4[de]
112	53.6	57.4	58.6	59.6	59.7	.4[de]
138	55.7	57.6	59.3	59.6	60.2	.4[df]
Live pig[g]	56.1	59.2	58.7	60.4	60.3	.4[de]

[a]Avg. is the average of third and fourth metacarpal and metatarsal bone ash percentage.

[b]Initial slaughter pig average was $52.0 \pm .2\%$.

[c]Overall linear (P<.01) and quadratic (P<.01) effects of both diet and time; significant diet by time interaction (P<.05).

[d]Linear effect of diet (P<.01).

[e,f]Quadratic effect of Ca/P (P<.01 and P<.05, respectively).

[g]Live pig values were determined at day 138 on test for pig receiving repeated live biopsies.

TABLE 3. SIMPLE CORRELATIONS FOR OVERALL RELATIONSHIP OF SLAUGHTER AND LIVE BIOPSY CORE MEASURES WITH MEASURES OF THIRD AND FOURTH METACARPAL AND METATARSAL BONE[a,b]

| | | Core Ash Weight | | | | Core Ash Percent | | | | Core DFF Ash Percent | | | |
| | | Slaughter | | Live | | Slaughter | | Live | | Slaughter | | Live | |
Criteria	Diets[c]:	1-5	2-5	1-5	2-5	1-5	2-5	1-5	2-5	1-5	2-5	1-5	2-5
3M Length, cm		.44	.28*	.46	--	.33	--	.37	--	.43	.27*	.46	--
4M Length, cm		.44	.31*	.46	--	.31	.26*	.35	--	.43	.33**	.45	--
3M Wet weight, g		.46	.41**	.48	.28*	.37	.32**	.38	--	.48	.38**	.47	--
4M Wet weight, g		.45	.45	.47	.30*	.38	.35**	.39	--	.49	.44**	.48	--
3M Wall thickness, cm		.46	.52	.49	.40**	.35	.55	.31	.34*	.48	.65	.44	.47**
4M Wall thickness, cm		.44	.53	.46	.44**	.41	.58	.38	.39**	.54	.68	.50	.54
3M Area, cm²		.47	.51	.49	.40**	.37	.48	.35	--	.50	.58	.46	.37**
4M Area, cm²		.45	.51	.47	.41**	.41	.51	.40	.27*	.53	.63	.50	.46**
3M Force, kg		.53	.54	.56	.43**	.45	.69	.43	.57	.55	.69	.52	.52
4M Force, kg		.55	.56	.58	.49**	.47	.62	.43	.41**	.57	.71	.52	.52
3M Stress, kg/cm²		.46	.42**	.48	.28*	.47	.68	.46	.57	.52	.62	.46	.43**
4M Stress, kg/cm²		.54	.46	.58	.31*	.47	.57	.41	.34*	.55	.59	.49	.30*
3M DFF ash, %		.44	.36**	.43	--	.38	.46	.33	.33*	.48	.48	.41	.33*
4M DFF ash, %		.35	.45	.35	.32*	.45	.39**	.37	--	.55	.55	.45	.27*
Avg. DFF ash, %		.42	.48	.43	.38**	.45	.50	.38	.30*	.56	.61	.46	.39**

aAbbreviations are as follows: 3M = average of third metacarpal and metatarsal; 4M = average of fourth metacarpal and metatarsal; Avg. = average of third and fourth metacarpal and metatarsal. 3M force and stress were determined by bending tests, whereas 4M force and stress were determined by shear tests.

bCorrelations are significant at P<.0001 unless otherwise noted: **P<.01, *P<.05.

c1-5 = included all five diets (N = 247 and 67, for slaughter and biopsy, respectively); 2-5 = included only diets 2 through 5 (N = 204 and 55, for slaughter and biopsy, respectively).

TABLE 4. NUMBER OF TRIALS WHERE OVERLAP OF LEAST SQUARES MEANS AND STANDARD ERRORS OF SLAUGHTER AND LIVE BIOPSY CORE MEASURES OCCURRED, FOR PIGS FED FIVE LEVELS OF DIETARY CA/P FROM WEANING TO MARKET WEIGHT, BY DIET AND SLAUGHTER TIME[a]

	Diet				
Day on Test	70	85	100	115	130
Ash weight, g					
0	3	3	2	3	3
27	3	3	3	3	3
55	3	3	2	2	3
83	3	3	2	3	2
138	3	3	2	3	3
Ash weight, % of wet weight					
0	1	0	2	0	2
27	2	1	2	1	3
55	2	2	2	1	2
83	2	3	3	3	3
138	2	2	3	3	3
Dry fat-free ash percentage, %					
0	2	3	3	3	2
27	3	3	3	3	3
55	3	3	2	2	3
83	3	2	3	3	3
138	3	3	3	3	3

[a]Least squares mean live biopsy core measures could not be estimated at day 112 on test due to missing cells in the repeated measure analysis, therefore no comparison could be made at this time period.

TABLE 5. NUMBER OF TRIALS WHERE OVERLAP OF LEAST SQUARES
MEANS AND STANDARD ERRORS OF SLAUGHTER BIOPSY CORE DRY FAT-
FREE ASH PERCENTAGE AND AVERAGE BONE DRY FAT-FREE ASH
OCCURRED, FOR PIGS FED FIVE LEVELS OF DIETARY CA/P FROM
WEANING TO MARKET WEIGHT, BY DIET AND SLAUGHTER TIME[a]

Slaughter Time	Diet				
	70	85	100	115	130
0	1	1	1	1	1
27	2	3	3	3	-
55	2	1	2	3	2
83	2	2	2	3	2
112	2	1	3	3	3
138	3	3	2	2	3

[a]Average bone ash is the average of the third and fourth
metacarpals and metatarsals.
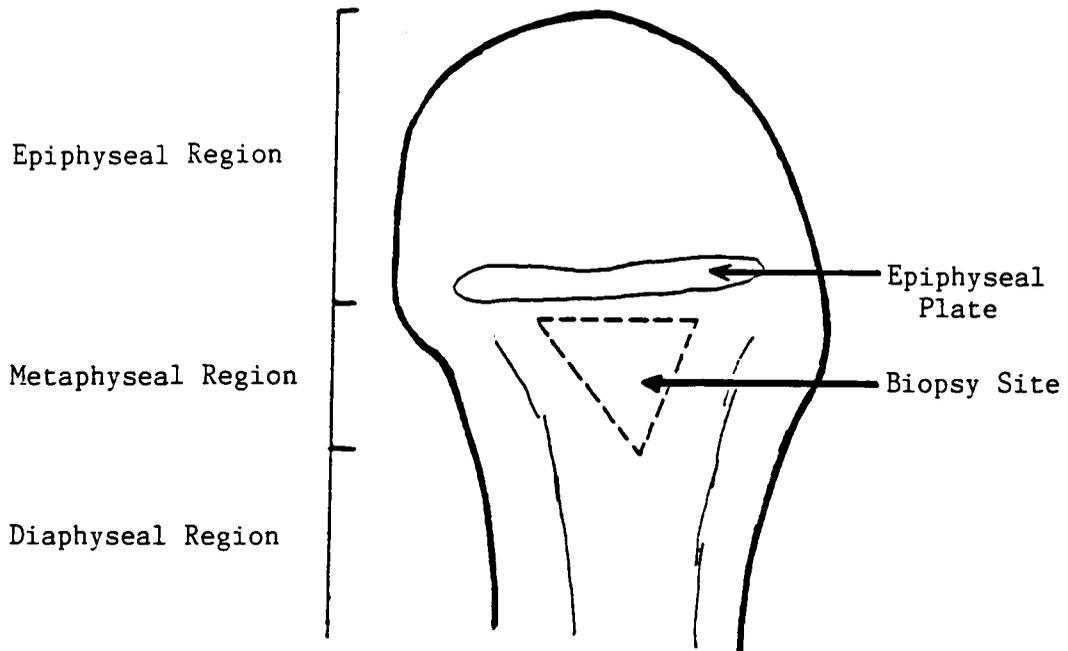
Figure 1. Biopsy site was the medial surface of the proximal tibia. Bone core was removed from the metaphyseal region, in the area denoted by the triangle.

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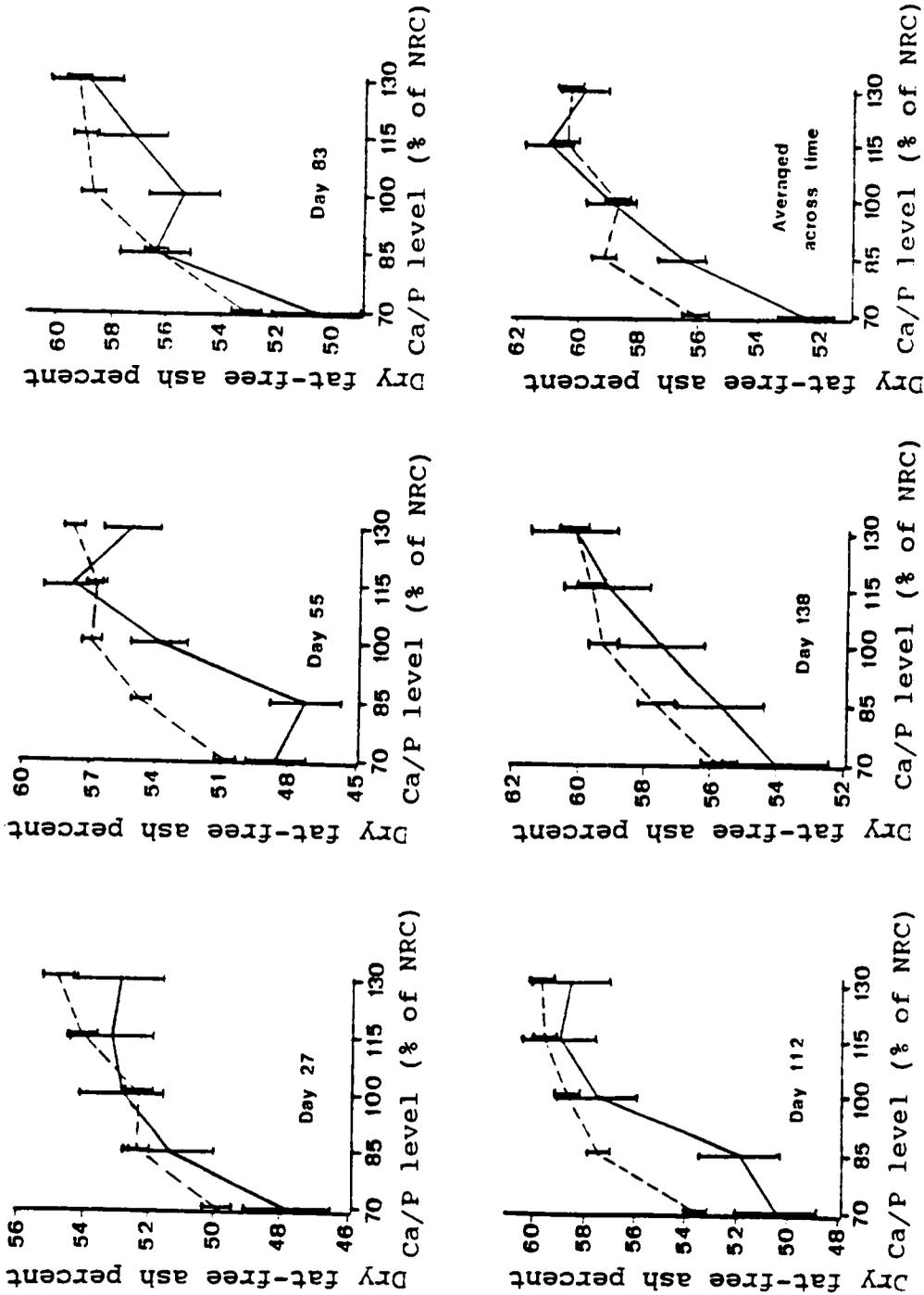
Figure 2. Overlap of LS means and SE for DFF ash percentage of pooled
slaughter and live biopsy cores (——), and LS means and SE for DFF ash
percentage of pooled third and fourth metacarpal and metatarsal bone (----),
by day on test at slaughter and averaged across all slaughter periods.

CHAPTER 7

Running Head: Effect of P on Performance and Bone

THE EFFECT OF VARYING PHOSPHORUS LEVELS WITH
A CA:P RATIO OF .9:1 ON PERFORMANCE AND BONE
CRITERIA OF SWINE FROM WEANING TO MARKET[1,2]

ABSTRACT

One hundred twenty five cross-bred pigs (avg initial wt, 10 kg) were used to determine the performance and bone characteristics of pigs fed 70, 85, 100, 115 and 130% of NRC recommended dietary P levels in .9:1 Ca:P ratios from weaning to market. Proportion of dolomitic limestone and dicalcium phosphate were varied in the corn-soybean meal diet to obtain desired Ca/P levels. The pigs were weaned at 4 wk of age and blocked by sex and sire into 5 replicates of 5 blocks each, with 5 pigs per diet per block. One of the replicates was randomly chosen for use in a evaluation of a biopsy procedure as a live-animal sampling technique. One block in each of the other replicates was slaughtered every 4 wk following the start of the trial. Pigs were weighed every 2 wk and performance criteria were determined for every 2 wk period. BW and ADG increased at a decreasing rate (P<.05) with increasing dietary P level and linearly over time (P<.01), whereas

ADFI responded linearly (P<.01) to diet and increased at a decreasing rate (P<.05) over time. No significant effect of diet on EFU was found, but EFU decreased at a decreasing rate(P<.01) over time. Third and fourth metacarpal and metatarsal measures of bone dimension generally increased at a decreasing rate (P<.05) with both dietary P and time. Bone force and stress increased linearly (P<.01) with both diet and time. Dry fat-free ash percentage increased at a decreasing rate (P<.01) with diet, and linearly over time (P<.01). Diet effects on slaughter and live biopsy core measures were found to be similar, but least squares means comparisons of slaughter and live biopsy core measures, and of biopsy core DFF% and bone DFF% showed little resemblance between either of the two groups. The effects of diet and time on performance and bone criteria when .9:1 Ca:P ratios were used were similar to what was found when Ca:P ratios of 1.2:1 were maintained.

(Key Words: Performance, Bones, Biopsy, Ca:P ratio,
Phosphorus, Pigs.)

Introduction

The Ca:P ratio has been shown to affect the response of pigs to dietary Ca and P levels. Chapman et al. (1962) found the Ca:P ratio to be most critical when minimal levels of P were used in the diet, and that the P level had more effect on the response criteria than did the Ca level. The Ca:P ratio appears to be more critical in the early

portion of the growth period, and when dietary Ca and P
levels are lower than recommended, a narrower Ca:P ratio
may provide more desirable performance (Combs and Wallace,
1962). Both wide (>2:1) and imbalanced (<.9:1) Ca:P ratios
have been shown to depress performance of growing pigs,
regardless of the Ca and P level (Hall et al., 1985;
Nielsen et al., 1971; Reinhart and Mahan, 1985).

The objectives of this study were to examine the
effects of increasing dietary P levels from 70 to 130% of
the NRC (1979) recommendations, with a .9:1 Ca:P ratio, on
performance and bone criteria of pigs from weaning to
market, and to compare the results obtained to those when
Ca:P ratios were maintained at 1.2:1 for dietary P levels
from 70 to 130% of NRC.

Experimental Procedures

One hundred twenty five crossbred pigs were used to
determine the effects on performance and various bone
criteria of pigs fed a wide range of dietary P levels in
.9:1 Ca:P ratios from weaning to market. Five dietary
treatments were used representing 70, 85, 100, 115 and 130%
of NRC recommended P levels (NRC, 1979). Proportion of
dolomitic limestone and dicalcium phosphate were varied in
the corn-soybean meal diet to obtain desired Ca and P
levels. The same relative Ca and P levels for each dietary
treatment were maintained across four phases, based on NRC

recommended nutrient density changes. Table 1 gives
composition of the control (100% NRC) diet for each phase.

Crossbred pigs were weaned at approximately 4 wk of
age and fed a standard starter diet (appendix Table 9),
meeting NRC (1979) requirements for all nutrients, until
the average weight of the group was 10 kg. At that time,
pigs were blocked by sex and sire; each block contained
five pigs and there were five blocks in each of four
replicates. One pig from each block within a replicate was
randomly assigned to each of the five dietary treatments,
resulting in five pigs per treatment in each replicate. At
the time of allotment, one block per replicate was randomly
assigned to each of five slaughter times. One additional
replicate (25 pigs) was randomly selected for periodic
biopsy. Pigs in the biopsy replicate contributed gain data
throughout the trial; no significant differences in
performance of biopsy pigs were observed. Pigs used for
the live biopsy procedure were excluded from the analysis
of bone effects to prevent any possible confounding of
effects of bone repair and remodeling with diet and time
effects on the bone criteria.

Pigs were weighed bi-weekly and feed intake per pen
during the preceding 2 wk was recorded. There were 10
periods in this trial and the length of the trial was 135
d. An initial BW was also taken, which gave 11 possible

weights in the trial. At the end of wk 4, and every 4 wk subsequently, one block of pigs per replicate was slaughtered, for a total of five slaughter periods. Additional details concerning analysis of the feed and management of the animals can be found in Combs et al. (1989a) and in various sections of the Appendix.

At slaughter, the third and fourth metacarpals and metatarsals from one side of the pig were removed. The right or left side was alternately sampled from one slaughter time to the next, so as to coincide with the side that was being biopsied on live pigs at that sampling time. Bones were frozen immediately after slaughter in air-tight plastic bags, were later thawed and freed of extraneous tissue for determination of dimension measurements, and were then refrozen in air-tight plastic bags to prevent dessication pending bending and shear testing.

For the bending and shear tests, force was applied to the bones at a speed of 10 mm·min^{-1} and resulting force curves were plotted at a chart speed of 50 mm·min^{-1}. Maximum force required to cause bending or shear failure was electronically measured and recorded. Afterwards, proximal halves of bones were dried and crushed, and the fat was then extracted according to the procedures outlined by Combs et al. (1989c).

For analysis of performance and bone criteria, a

series of ANOVA models were fit to evaluate discrete effects of diet, pen within diet, period and their interactions and orthogonal polynomial contrasts for the effect of diet were determined. The observed diet and time effects were then compared to those observed when 70 to 130% of NRC recommended P levels were fed in a 1.2:1 Ca:P ratio (Combs et al., 1989a,c). The performance and bone criteria considered included: body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and efficiency of feed utilization (EFU = Gain:feed); and bone length, wet weight, wall thickness, cross-sectional area, bending or shear force and stress, extracted weight (proximal half), ash weight (proximal half), and the dry fat-free ash percentage (DFF%).

Effects of sex on performance and bone criteria were evaluated by fitting an ANOVA model including diet and sex effects within each period. The effects of sex were never significant in two successive periods for any performance or bone criterion. Therefore, effects of sex were excluded from the final model for performance and bone criteria. Combs et al. (1989a) found no effects of biopsy or sex on performance criteria when 1.2:1 Ca:P ratios were used, and no effects of sex on bone criteria were reported for the higher Ca:P ratios by Combs et al. (1989c).

For evaluation of a live biopsy technique, live pigs

were biopsied on the first day on test and at 4 wk intervals thereafter until the average weight of the group was approximately 110 kg (live biopsies). At that time the pigs were slaughtered and the final biopsy was taken from the hot carcass. The side of the live pig biopsied was alternated from sampling time to sampling time so that the time interval between sampling at the same site was 8 wk. Additionally, a biopsy was taken from the hot carcass of each slaughter pig (slaughter biopsies) at the location corresponding to the live-animal biopsy. Details of procedures used to obtain and analyze biopsy core samples are described by Combs et al. (1989d).

Four pigs fed the 70% NRC P diet died before the end of the trial; two pigs on each of the two highest P levels were also lost prior to the end of the trial. Of these pigs, two on the 70% NRC P diet, both pigs on the 115% NRC P diet, and one of the pigs on the 130% NRC died due to adverse reactions to the anesthesia during surgery. The other pigs died due to causes unrelated to diet or the biopsy procedure, as indicated by post-mortem examination.

Biopsy measurements analyzed included: dry fat-free ash weight, ash weight as a percentage of the wet weight and dry fat-free ash percentage (DFF% = ash weight/ extracted weight). Repeated measures ANOVA on live biopsy samples (repeated in time), relating the effects of diet

and sex to the observed measures, was performed and
orthogonal polynomial contrasts of the effects of diet on
these measures were made. Slaughter biopsies were analyzed
by ANOVA of the effects of diet, slaughter time and sex and
orthogonal polynomial contrasts of the overall effects of
diet and slaughter time, and the effects of diet at each
time were made. Effects of sex on live and slaughter
biopsy measures were not significant, therefore the effects
of sex was excluded from the final models. Combs et al.
(1989d) found no effect of sex on biopsy core measures when
1.2:1 Ca:P ratios were fed.

Simple correlations were determined between the
measurements taken on the slaughter biopsies and
measurements taken on pooled third metacarpals and
metatarsals (3M) and pooled fourth metacarpals and
metatarsals (4M) bone pairs (length, wet weight, wall
thickness, cross-sectional area, force, stress, extracted
bone weight, bone ash weight, and bone DFF%).
Additionally, the simple correlations between biopsy core
DFF% and bone DFF%, average across all four bones, were
also determined. Correlations between the final period
live biopsy measurements and third and fourth metacarpal
and metatarsal bone measurements were also determined. In
order to determine if the correlations obtained were
dependent on the presence of data from pigs clearly

deficient in P (fed 70% NRC), correlations were also determined without the 70% NRC dietary P level.

Least squares (LS) means and SE generated from the ANOVA on live and slaughter biopsy measures were plotted to determine if live biopsy measures differed from slaughter biopsy measures within each slaughter time. Additionally, LS means and SE of slaughter biopsy DFF% and of the average of slaughter and live biopsy DFF% were plotted against LS bone DFF% of pooled third and fourth metacarpals and metatarsals to determine if the biopsy ash differed from ash determined on intact metacarpal and metatarsal bones.

Results and Discussion

Evaluation of Slaughter carcasses. Slaughter carcasses were visually appraised for gross skeletal abnormalities, particularly beading of the ribs, as described by Combs et al. (1989a). The overall incidence of beaded ribs was 20%. For this trial, the majority of the observed cases of beaded ribs were associated with the lowest P level (65% of the total cases; 64% of the pigs fed this diet) and 27% of the observed cases were in pigs fed the second diet. Rib beading has been shown to accompany deficiency of both Ca and P (Miller et al., 1962, 1964). The high incidence of beaded ribs in pigs fed the 70% NRC recommended P diet indicates the lower levels of Ca/P used in this study were deficient with respect to the needs of the pig.

Performance Analysis. BW and ADG demonstrated overall
increases at a decreasing rate (P<.05) in response to
increasing dietary P level, with Ca:P ratios of .9:1
(Tables 2 and 3, respectively); ADFI increased linearly
(P<.01) with increasing P (Table 4). Overall diet effects
on EFU were only nearly significant (P<.11), with a
quadratic increase in response to increasing dietary P
level (Table 5). When Ca:P ratios of 1.2:1 were fed, BW,
ADG and ADFI increased increased at a decreasing rate with
increasing dietary P level, whereas diet effects on EFU
only tended to be linear (Combs et al., 1989a). In
agreement with present results, Libal et al. (1969) found
that for the overall grower-finisher period, gain increased
as the P level was increased from .3 to .7% of the diet,
when Ca was maintained at .35% of the diet. To the
contrary, Nielsen et al. (1971) reported that Ca:P ratios
less than .9:1 depress performance of growing pigs,
regardless of the Ca and P level used. When the Ca:P ratio
remains between .9 and 2:1, Ca levels as low as .25% of the
diet and P levels as low as .44% of the diet were not found
to depress gain in weanling pigs (Combs et al., 1962; Combs
and Wallace, 1962; Mahan, 1982). Combs and Wallace (1962)
concluded that when dietary Ca and P levels are lower than
recommended, the narrower Ca:P ratio provides more
desirable gain. As was seen in this study, others have

reported that there were no effects of Ca and/or P level or Ca:P ratio on efficiency (Combs et al., 1962; Crenshaw et al., 1981; Fammatre et al., 1977; Kesel et al., 1983; Kornegay et al., 1981; Lepine et al., 1985; Nimmo et al., 1980; Thomas and Kornegay, 1981). In contrast to present results, Koch et al. (1984), Mahan (1982) and Reinhart and Mahan (1985) found that efficiency improved with increasing dietary P level.

When 70 to 130% of NRC recommended P levels were fed in a .9:1 Ca:P ratio, BW and ADG increased linearly ($P<.01$) over time (period - Tables 2 and 3, respectively), whereas ADFI increased and EFU decreased at a decreasing rate ($P<.05$; Tables 4 and 5, respectively). For 1.2:1 Ca:P ratios, increases at a decreasing rate for BW from weaning to market were observed, whereas ADG and ADFI increased quadratically at a decreasing rate with time during the growing-finishing period, and EFU declined quadratically at a decreasing rate over the same period (Combs et al., 1989a).

Bone Analysis

The overall effect of dietary Ca and P, in a .9:1 Ca:P ratio, on 3M and 4M bone length and wet weight, and on 3M cross-sectional area was to produce increases at a decreasing rate ($P<.05$) in these criteria (Table 6). Both 3M and 4M dry fat-free ash percentage also increased at a

decreasing rate (P<.01) as dietary P level was increased
(Table 7). 3M and 4M wall thickness (Table 6), extracted
weight and ash weight (Table 7), force and stress (Table
8), and 4M cross-sectional area (Table 6) increased
linearly (P<.01) with increasing dietary P level. When
dietary P was increased from 70 to 130% of NRC
recommendations in a 1.2:1 Ca:P ratio, all bone criteria
increased both linearly and quadratically with increasing
dietary P, with the exception of bending stress which
increased linearly (Combs et al., 1989c). In reviewing Ca
and P research, Kornegay (1986) concluded that, in general,
the weight, length and outside diameters of bone were not
very responsive to dietary Ca and P levels, in contrast to
present results. Kornegay further concluded that wall
thickness increased with increasing Ca or P, in agreement
with present results. Increased bone ash in response to
increasing P level at a constant Ca level has been reported
(Combs et al., 1962; Koch et al., 1984; Mahan, 1981; Parker
et al., 1975; Reinhart and Mahan, 1985). Libal et al.
(1969) and Parker et al. (1975) found bending force
increased linearly with increasing dietary P level, as was
seen in the current study. In contrast, both linear and
quadratic increases in force in response to dietary P level
have also been reported (Crenshaw, 1986; Koch et al.,
1984).

When Ca:P ratios of .9:1 were used, both 3M and 4M bone length, and 3M wall thickness and cross-sectional area increased at a decreasing rate (P<.01) from weaning to market (Table 6). 3M and 4M wet weight (Table 6), extracted weight, ash weight and dry fat-free ash percentage (Table 7), and force and stress (Table 8), as well as 4M wall thickness and cross-sectional area (Table 6), increased in linear (P<.01) manner over the entire growth period. For 1.2:1 Ca:P ratios, the wet weight, wall thickness, cross-sectional area, stress, dry fat-free extracted weight and dry fat-free ash weight of 3M and 4M bones, and the 3M bending force all increased linearly with time from weaning to market. Bone length, and dry fat-free ash percentage of both 3M and 4M bones, and 4M shear force and stress increased at a decreasing rate over time from weaning to market for the 1.2:1 Ca:P ratios (Combs et al., 1989c).

Comparison of Bending and Shear Tests. For Ca:P ratios of .9:1, both bending and shear stress increased linearly (P<.01) with increasing dietary Ca:P level (Table 9). In contrast, when 1.2:1 ratios were used, diet effects were linear and quadratic for shear stress and linear for bending stress (Combs et al., 1989b). Both bending and shear stress were linearly related to time (P<.01) for the .9:1 Ca:P ratios (Table 10), as was also seen when Ca and P

were fed in 1.2:1 ratios. Also consistent with what was observed for the 1.2:1 Ca:P ratios, diet by time interactions were significant for 4C and 4T shear stress, but not for 3C and 3T bending stress. When the weight of the pigs at slaughter was included in the model as a covariate, diet, time and diet by time interactions became nonsignificant for all bones (P>.10), as was also observed for the higher Ca:P ratios (Combs et al., 1989b). The standard errors for diet associated with the two shear measures (4C and 4T) were smaller relative to the mean and the range of values than those associated with the two bending measures (Table 9); there was no clear-cut advantage of bending or shear standard errors for time (Table 10). For the higher Ca:P ratios, standard errors for both diet and time associated with the two shear measures were also smaller relative to the mean and to the range of values, than those associated with the two bending measures (Combs et al., 1989b).

In both models, the largest R^2 was obtained for 4T; the R^2 for 3T bending and 4C shear were similar in both models (Table 11). When the average R^2 of the two bending bones was compared with the average of the two shear bones, the average R^2 of the two shear bones was consistently higher than that of the two bending bones. The R^2 in this study averaged 64% for the bending (3C, 3T) stress

equations and 73% for the shear (4C, 4T) stress equations,
indicating that more of the variation in shear stress can
be explained by the model than can be explained for bending
stress. These trends were similar to what was observed
when Ca:P ratios of 1.2:1 were used (Combs et al., 1989b).

When the CV were averaged across bending (3C, 3T) and
shear (4C, 4T) bones, the shear stress measures had lower
average CV than the bending stress measures for both
models, indicating the residual variation was smaller for
shear stress, relative to the mean, than for bending stress
(Table 11). Similar findings were observed when dietary Ca
and P were fed in a ratio of 1.2:1 (Combs et al., 1989b).

The partial correlation of 3C and 3T bending stress
was averaged .72 when dietary Ca:P ratios were .9:1 (Table
12); 4C and 4T shear stress were only correlated at r=.25.
This indicated that the bending results were more
repeatable within the same animal than were the shear
results. These correlations were slightly higher than were
reported in Combs et al. (1989b) for the two bending or
shear bones when the dietary Ca:P ratios were 1.2:1.
Correlations of either bending bone with either shear bone
averaged .32. The significance of the correlations among
and within bending and shear stress measures, indicate that
there is consistency between the results of the bending and
shear tests; although, as indicated earlier, the bending

results may be more repeatable. The partial correlations of the weight corrected model were less than those obtained for the first model, and with the exception of the correlation between the two bending bones, these correlations were not significant (P>.10).

As indicated in the report of Combs et al. (1989), shear tests are conducted in the same fashion as bending tests, but subsequent calculation of stress is much easier for shear than is the case for bending. In the current report, diet by time interactions and standard errors favor shear stress, while R^2 and CV only tended to favor shear stress and the correlations favor bending stress. Therefore, the results obtained in this study only tend to support the conclusions of Combs et al. (1989b) that shear tests may offer a more desirable test of bone strength than do the bending tests.

Biopsy Analysis. Both slaughter and live biopsy core ash weight increased linearly (P<.01) with increasing dietary P levels when the Ca:P ratio was .9:1 (Table 13). This was consistent with what was observed when Ca:P ratios of 1.2:1 were maintained (Combs et al., 1989d). Live biopsy ash weight and slaughter biopsy ash weight increased at a decreasing rate (P<.01) from weaning to market when Ca:P ratios of .9:1 were used. Similarly, for Ca:P ratios of 1.2:1, live biopsy ash weight responded linearly and

quadratically to time, whereas slaughter biopsy ash weight increased linearly over time for the higher Ca:P ratios (Combs et al., 1989d). Bone DFF%, averaged across third and fourth metacarpals and metatarsals, also increased at a decreasing rate (P<.01) with increasing dietary P level.

The diet and time effects on ash as a percentage of wet weight and DFF% were the same for both .9 and 1.2:1 Ca:P ratios. Both live and slaughter biopsy core percentages increased at a decreasing rate (P<.05) in response to increasing dietary P (Table 13). Live biopsy core percentages increased in at a decreasing rate (P<.01) in relation to time, whereas slaughter biopsy core percentages increased in linear (P<.01) fashion over time. 3M DFF% increased at a decreasing rate (P<.05) over time, whereas the response of 4M and the average of 3M and 4M bone DFF% increased only linearly (P<.01) from weaning to market. In general, the effects of diet and time on live biopsy core DFF% appeared to behave like diet and time effects on 3M DFF%, whereas slaughter biopsy core DFF% diet and time effects more closely resembled the effects on 4M or average bone DFF%.

The correlations between slaughter biopsy core ash weight and both 3M and 4M bone measures were consistent (Table 15), whereas no correlations of live biopsy cores and bone measures were significant. Slaughter biopsy core

ash weight averaged r=.29 and r=.24 with force and stress, respectively, lower than was found when dietary Ca:P ratios of 1.2:1 were used (Combs et al., 1989d). Correlations of slaughter core ash weight with bone DFF% was also lower than seen for the higher Ca:P ratios, averaging r=.34. In general, when the lowest dietary P level was excluded, correlations between slaughter biopsy ash weight and bone measures changed very little.

The correlations of slaughter and live biopsy core percentages (ash as a percent of wet weight and DFF%) were generally higher when Ca:P ratios were .9:1 (Table 15) than was observed for the 1.2:1 ratio (Combs et al., 1989d), both with and without the lowest dietary P level. Slaughter biopsy core percentages generally had r=.60 to .70 for the relationship with bone measures. Live biopsy core percentages were generally similar to those for slaughter core percentages, although biopsy core ash as a percentage of wet weight did not correlate well with force, stress or DFF%, contrary to what was observed for Ca:P ratios of 1.2:1.

In general, slaughter and live biopsy core percentages correlated well with bone measures, whereas live biopsy ash weight did not correlate well with bone measures. The correlations observed between ash weight and bone measures were usually lower when .9:1 Ca:P ratios were used, whereas

correlations of core percentages with bone criteria were lower when 1.2:1 Ca:P ratios were used.

The LS means and SE of slaughter and live biopsy core ash weight overlapped for four of five diets at initial slaughter, and for all diets at days 115 and 135, but did not overlap for any diet at days 26 and 86; no estimates of LS live biopsy core measures could be made at day 59 due to missing cells in the repeated measure analysis. For slaughter and live biopsy ash percentage of wet weight, LS means and SE overlapped for three diets at initial slaughter, and for the four higher diets at day 26. Diets 1, 2, 4 and 5 overlapped at day 86, and diets 1 and 4 overlapped at day 135, but no diets overlapped at day 115. LS means and SE for DFF% of slaughter and live biopsy cores overlapped for all diets at final slaughter, but only one or two of the five diets overlapped at any other time period, with no consistent pattern. Therefore, it would appear that the slaughter and live biopsy cores differ when dietary Ca:P ratios of .9:1 were used, contrary to the conclusion drawn when 1.2:1 Ca:P ratios were used (Combs et al., 1989d). Additionally, slaughter biopsy DFF% least squares means and standard errors hardly ever overlapped with average bone DFF%, nor did biopsy core DFF%, averaged across biopsy and slaughter, overlap with average bone DFF%. Therefore, based on least squares means and standard

error comparisons, one would have to conclude that the biopsy technique does not produce consistent bone samples when the dietary Ca:P ratios are .9:1, contrary to the results obtained when dietary Ca:P ratios were maintained at 1.2:1.

Implications

As one might expect when Ca:P ratios are maintained at .9:1, little difference in the effect of dietary P level or time on performance or bone criteria were found when compared to dietary Ca:P ratios of 1.2:1. These results suggest that the ratio of .9:1 is not particularly deleterious on performance or bone criteria over a range of dietary P from 70 to 130% of NRC recommended levels. A biopsy procedure, which was found to offer promise when dietary Ca:P ratios were held at 1.2:1, produced inconsistent results when the Ca:P ratios were maintained at .9:1.

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TABLE 1. PERCENTAGE COMPOSITION OF CONTROL DIET (100% NRC)

Ingredient	Phase[a]			
	1	2	3	4
Ground corn	72.07	77.67	77.97	83.15
Soybean meal, 49% CP	24.89	19.86	19.81	14.81
Dicalcium phosphate[b]	1.15	.98	.70	.53
Dolomitic limestone[b]	.89	.89	.92	.91
Vitamin-Se premix[c]	.35	.20	.20	.20
TM premix[d]	.05	.05	.05	.05
Salt	.35	.35	.35	.35
Antibacterial[e]	.25	-	-	-
Calculated values				
P	.57	.52	.47	.42
Ca	.52	:47	.42	.37
Analyzed values				
P	.59	.62	.53	.50
Ca	.54	.50	.32	.37

[a]Diets were changed according to phase as follows: Phase 1 - 10 to 20 kg; phase 2 - 20 to 35 kg; phase 3 - 35 to 60 kg; phase 4 - 60 to 100 kg.

[b]Proportions were altered to obtain 70, 85, 115 and 130% of basal diet levels for diets 1, 2, 4 and 5, respectively, with some adjustments in corn and soybean meal.

[c]Supplied (per kg diet): 4400 IU vitamin A, 440 IU vitamin D_3, 11 IU vitamin E, 1.1 mg vitamin K, 4.4 mg riboflavin, 22 mg pantothenic acid, 22 mg niacin, 22 ug vitamin B_{12}, 440 mg choline, 440 ug biotin, .3 mg Se during phase 1; during subsequent phases, provides: 3520 IU vitamin A, 352 IU vitamin D_3, 8.8 IU vitamin E, .88 mg vitamin K, 3.52 mg riboflavin, 17.6 mg pantothenic acid, 17.6 mg niacin, 17.6 ug vitamin B_{12}, 352 mg choline, 352 ug biotin, and .1 mg Se.

[d]Supplied (per kg diet): 100 mg zinc, 50 mg iron, 27.5 mg manganese, 5.5 mg copper and .75 mg iodine.

[e]Supplied (per kg diet): 110 ppm chlortetracycline, 55 ppm procaine penicillin and 110 ppm sulfamethazine.

TABLE 2.   LEAST SQUARES MEAN BODY WEIGHT (KG), BY DIET AND
   PERIOD, FOR PIGS FED FIVE DIETARY P LEVELS IN A .9:1
          CA:P RATIO FROM WEANING TO MARKET WEIGHT

	P level (% of NRC)[ab]					
Period[c]	70	85	100	115	130	SEM
0	10.8	10.8	10.8	10.9	10.9	.3
1	18.9	19.3	19.5	19.5	19.8	.5
2	22.9	22.9	24.1	23.5	23.4	.6
3	35.6	37.6	39.3	39.3	39.1	1.1
4	41.5	44.9	46.9	47.6	45.2	1.3
5	50.0	55.8	57.2	58.4	58.2	1.5
6	56.7	65.3	65.9	69.5	68.5	1.8
7	64.4	77.9	77.5	83.4	82.3	2.1
8	74.6	89.6	89.2	95.2	95.5	2.5
9	85.0	101.7	105.4	110.3	112.3	2.8
10	88.4	101.1	111.0	116.8	112.6	5.2

[a]Linear (P<.01) and quadratic (P<.01) effect of diet.
[b]Significant diet by time interaction (P<.01).
[c]Linear (P<.01) effect of time.

TABLE 3.   LEAST SQUARES MEAN AVERAGE DAILY GAIN (G), BY
DIET AND PERIOD, FOR PIGS FED FIVE DIETARY P LEVELS IN A
.9:1 CA:P RATIO FROM WEANING TO MARKET WEIGHT

Period[b]	P level (% of NRC)[a]					SEM
	70	85	100	115	130	
1	507	532	541	539	555	59
2	440	400	518	447	405	49
3	605	715	728	758	752	78
4	489	611	637	687	508	64
5	539	674	659	684	725	72
6	555	791	731	933	861	85
7	531	793	900	930	958	90
8	602	832	834	848	940	89
9	643	753	832	831	891	91
10	833	741	921	839	1,144	176
Avg	574	684	730	770	774	27

[a]Linear (P<.01) and quadratic (P<.05) effect of diet.
[b]Linear (P<.01) effect of time.

TABLE 4. LEAST SQUARES MEAN AVERAGE DAILY FEED INTAKE (G),
BY DIET AND PERIOD, FOR PIGS FED FIVE DIETARY P LEVELS IN A
.9:1 CA:P RATIO FROM WEANING TO MARKET WEIGHT

| | P level (% of NRC)[ab] | | | | | |
Period[c]	70	85	100	115	130	SEM
1	957	966	977	969	1,031	84
2	894	901	938	923	939	78
3	1,038	1,085	1,022	1,067	1,098	91
4	2,031	1,970	1,679	2,170	1,897	166
5	1,646	2,420	2,004	2,137	2,212	178
6	2,177	3,031	2,787	3,197	2,952	242
7	2,172	3,001	3,293	3,490	3,417	263
8	1,950	2,314	2,792	3,282	3,198	231
9	3,122	3,608	3,977	4,786	4,443	341
10	3,321	2,337	3,432	3,056	4,187	500
Avg	1,931	2,163	2,290	2,508	2,537	68

[a]Linear (P<.01) effect of diet.
[b]Significant diet by time interaction (P<.05).
[c]Linear (P<.01) and quadratic (P<.05) effect of time.

TABLE 5.   LEAST SQUARES MEAN EFFICIENCY OF FEED
UTILIZATION (GAIN:FEED), BY DIET AND PERIOD, FOR
PIGS FED FIVE DIETARY P LEVELS IN A .9:1 CA:P
RATIO FROM WEANING TO MARKET WEIGHT

	P level (% of NRC)[a]					
Period[b]	70	85	100	115	130	SEM
1	.531	.552	.555	.557	.539	.051
2	.498	.451	.553	.489	.437	.045
3	.585	.653	.711	.711	.680	.062
4	.241	.317	.379	.324	.252	.028
5	.330	.292	.329	.325	.329	.030
6	.260	.266	.263	.299	.291	.026
7	.233	.267	.276	.271	.280	.025
8	.315	.395	.300	.260	.294	.029
9	.216	.212	.212	.216	.203	.020
10	.247	.320	.268	.268	.274	.046
Avg	.346	.372	.385	.372	.358	.012

[a]Quadratic (P<.01) effect of diet; overall diet effect only
approaches significance (P<.11).
[b]Linear (P<.01) and quadratic (P<.01) effect of time.

TABLE 6. LEAST SQUARES MEAN MEASURES OF BONE DIMENSION FOR POOLED THIRD METACARPAL AND METATARSAL (3M) AND POOLED FOURTH METACARPAL AND METATARSAL (4M) BONES BY DIET AND DAY ON TEST AT SLAUGHTER (DAY), FOR PIGS FED FIVE DIETARY P LEVELS IN A .9:1 CA:P RATIO FROM WEANING TO MARKET WEIGHT

| | P level (% of NRC) | | | | | | P level (% of NRC) | | | | | |
Day	70	85	100	115	130	SEM	70	85	100	115	130	SEM
	3M Length, cm^{ac}						4M Length, cm^{bc}					
26	5.64	5.75	5.63	5.70	5.75	.12	5.73	5.92	5.79	5.79	5.94	.14
59	6.24	6.58	6.54	6.70	6.71	.12	6.43	6.66	6.65	6.83	6.84	.12
86	6.98	7.39	7.72	7.48	7.42	.12	7.01	7.50	7.83	7.61	7.50	.15
115	7.56	7.74	7.85	8.02	7.77	.12	7.63	7.71	7.96	8.09	7.79	.14
135	7.98	7.95	8.09	8.27	7.99	.11	8.08	8.06	8.16	8.70	8.11	.13
	3M Wet weight, g^{bd}						4M Wet weight, g^{bd}					
26	8.46	9.16	9.51	9.24	9.68	.78	8.18	9.10	9.32	9.24	9.51	.88
59	11.70	13.04	13.80	14.61	13.86	.79	11.87	12.83	13.48	14.33	13.73	.80
86	14.57	17.80	20.13	19.62	20.03	.79	14.74	17.69	20.16	19.74	19.44	.84
115	18.14	19.69	21.88	23.10	22.27	.78	18.30	19.76	21.93	23.56	22.30	.92
135	20.86	21.97	23.73	26.93	25.19	.77	20.69	22.14	23.80	26.29	25.67	.85
	3M Radius, cm^{bc}						4M Radius, cm^{ef}					
26	1.152	1.171	1.209	1.205	1.202	.029	1.106	1.139	1.195	1.177	1.185	.032
59	1.262	1.314	1.332	1.372	1.357	.029	1.225	1.294	1.307	1.347	1.314	.030
86	1.381	1.421	1.484	1.484	1.479	.029	1.443	1.406	1.466	1.455	1.427	.035
115	1.437	1.466	1.553	1.570	1.521	.029	1.395	1.432	1.533	1.540	1.497	.034
135	1.517	1.527	1.550	1.631	1.583	.029	1.512	1.517	1.566	1.612	1.603	.031
	3M Wall thickness, cm^{ce}						4M Wall thickness, cm^{de}					
26	.0798	.0950	.1026	.1008	.0981	.0078	.1011	.1187	.1204	.1207	.1262	.0101
59	.1054	.1128	.1179	.1224	.1117	.0079	.1233	.1286	.1382	.1435	.1331	.0093
86	.1130	.1234	.1423	.1403	.1509	.0079	.1275	.1385	.1494	.1341	.1566	.0109
115	.1251	.1449	.1363	.1477	.1599	.0078	.1715	.1628	.1492	.1787	.1910	.0106
135	.1202	.1368	.1477	.1593	.1729	.0077	.1517	.1833	.1745	.1814	.1876	.0097
	3M Cross-sectional area, cm2^{bc}						4M Cross-sectional area, cm2^{de}					
26	.304	.364	.404	.395	.386	.038	.482	.528	.572	.616	.551	.042
59	.434	.481	.511	.543	.491	.039	.592	.622	.695	.629	.706	.049
86	.506	.570	.678	.667	.710	.039	.746	.737	.736	.860	.876	.048
115	.582	.678	.685	.746	.770	.038	.731	.856	.858	.917	.948	.044
135	.595	.671	.734	.833	.868	.038	.741	.889	.881	.883	1.003	.045

^{a}Linear (P<.05) and quadratic (P<.05) effect of diet.
^{b}Linear (P<.01) and quadratic (P<.05) effect of diet.
^{c}Linear (P<.01) and quadratic (P<.01) effect of time.
^{d}Linear (P<.01) effect of time.
^{e}Linear (P<.01) effect of diet.
^{f}Linear (P<.01) and quadratic (P<.05) effect of time.

TABLE 7. LEAST SQUARES MEAN BONE EXTRACTED WEIGHT, ASH
WEIGHT AND DRY FAT-FREE ASH PERCENTAGE FOR POOLED THIRD
METACARPAL AND METATARSAL (3M) AND POOLED FOURTH METACARPAL
AND METATARSAL (4M) BONES BY DIET AND DAY ON TEST AT
SLAUGHTER (DAY), FOR PIGS FED FIVE DIETARY P LEVELS
IN A .9:1 CA:P RATIO FROM WEANING TO MARKET WEIGHT

	P level (% of NRC)					
Day	70	85	100	115	130	SEM
3M Extracted weight, g^{ab}						
26	1.113	1.354	1.448	1.438	1.701	.190
59	1.679	1.997	2.279	2.500	2.150	.193
86	2.096	2.877	3.609	3.583	3.780	.193
115	2.603	3.574	4.146	4.658	4.784	.211
135	3.297	4.027	4.728	5.603	5.535	.188
4M Extracted weight, g^{abc}						
26	.922	1.129	1.338	1.297	1.421	.222
59	1.534	1.863	2.029	2.390	2.011	.202
86	2.088	2.595	3.419	3.443	3.709	.237
115	2.549	3.411	3.644	4.243	4.297	.231
135	2.769	3.398	4.267	5.055	5.501	.212
3M Ash weight, g^{ab}						
26	.586	.728	.814	.814	.895	.125
59	.904	1.155	1.322	1.479	1.263	.127
86	1.169	1.656	2.130	2.136	2.255	.127
115	1.369	2.102	2.431	2.824	2.945	.138
135	1.847	2.366	2.828	3.395	3.392	.123
4M Ash weight, g^{abd}						
26	.479	.602	.714	.673	.762	.132
59	.767	1.005	1.112	1.333	1.130	.121
86	1.144	1.400	1.914	1.952	2.119	.142
115	1.348	1.909	2.152	2.512	2.572	.138
135	1.529	1.961	2.513	3.016	3.306	.127
3M Dry fat-free ash, %^{be}						
26	53.6	53.9	55.8	56.3	53.5	1.3
59	52.9	57.4	57.6	59.0	59.1	1.0
86	54.7	57.4	58.9	59.4	59.6	1.0
115	56.2	59.1	57.9	60.2	61.4	.9
135	56.7	59.5	60.2	60.8	61.2	1.0
4M Dry fat-free ash, %^{be}						
26	52.4	54.6	52.5	53.2	53.6	1.4
59	49.1	53.5	54.6	55.6	56.5	1.1
86	50.4	54.0	55.9	56.4	56.9	1.2
115	50.8	57.5	60.4	59.0	59.6	1.1
135	55.4	58.1	59.1	59.7	59.2	1.1

[a]Linear (P<.01) effect of diet.
[b]Linear (P<.01) effect of time.
[c]Significant diet by time interaction (P<.05).
[d]Significant diet by time interaction (P<.01).
[e]Linear (P<.01) and quadratic (P<.01) effect of diet.

TABLE 8.   LEAST SQUARES MEAN BONE FORCE AND STRESS
FOR POOLED THIRD METACARPAL AND METATARSAL BENDING (3M)
AND POOLED FOURTH METACARPAL AND METATARSAL SHEAR (4M)
BONES BY DIET AND DAY ON TEST AT SLAUGHTER (DAY),
FOR PIGS FED FIVE DIETARY P LEVELS IN A .9:1
CA:P RATIO FROM WEANING TO MARKET WEIGHT

| | | | P level (% of NRC) | | | |
Day	70	85	100	115	130	SEM
3M Bending force, kg[abc]						
26	21.3	25.4	28.0	28.0	32.7	8.3
59	32.5	41.5	48.7	53.2	44.9	8.5
86	36.5	57.6	73.8	76.8	83.1	8.5
115	54.4	68.4	76.0	98.2	123.8	8.3
135	53.4	82.2	102.3	122.3	136.2	8.2
4M Shear force, kg[abd]						
26	53.3	67.8	67.6	61.8	71.6	13.0
59	56.7	88.5	94.1	106.1	92.5	11.9
86	79.5	91.3	133.6	162.5	162.0	23.9
115	104.0	136.6	134.9	186.9	223.6	13.6
135	96.6	151.5	205.7	224.1	289.3	12.5
3M Bending stress, kg/cm^2[ab]						
26	195.1	195.7	189.6	192.5	233.0	25.4
59	188.0	213.6	240.9	240.8	231.8	25.8
86	181.4	233.5	261.0	266.8	280.7	25.8
115	219.3	235.2	240.9	284.4	374.7	25.4
135	195.5	270.7	303.7	311.6	344.0	24.1
4M Shear stress, kg/cm^2[abd]						
26	76.3	78.7	72.5	69.4	75.4	7.6
59	56.9	82.3	81.4	86.6	85.6	6.9
86	66.2	73.6	95.8	129.0	115.2	8.1
115	65.2	89.1	91.7	108.4	128.3	7.9
135	68.1	90.8	119.3	123.6	147.5	7.2

[a]Linear (P<.01) effect of diet.
[b]Linear (P<.01) effect of time.
[c]Significant diet by time interaction (P<.05).
[d]Significant diet by time interaction (P<.01).

TABLE 9. LEAST SQUARES MEAN STRESS (KG·CM^{-2}) FOR THIRD METACARPAL (3C) AND METATARSAL (3T) BENDING TEST AND FOURTH METACARPAL (4C) AND METATARSAL (4T) SHEAR TEST AS INFLUENCED BY DIETARY P LEVEL

| Bone | P level (% of NRC) | | | | | SEM | Range/ SEM |
	70	85	100	115	130		
3C	233.0	254.7	295.5	296.7	335.9	16.1[a]	6.4
3T	166.0	193.4	203.1	217.6	253.3	12.3[a]	7.1
4C	70.7	87.2	99.9	107.1	107.7	5.5[a]	6.7
4T	62.0	79.1	90.1	97.8	108.5	4.7[a]	9.9

[a]Linear (P<.01) effect of diet.

TABLE 10. LEAST SQUARES MEAN STRESS (KG·CM^{-2}) FOR THIRD METACARPAL (3C) AND METATARSAL (3T) BENDING TEST AND FOURTH METACARPAL (4C) AND METATARSAL (4T) SHEAR TEST FOR SLAUGHTER PERIODS FROM WEANING TO MARKET

| Bone | Day on test | | | | | SEM | Range/ SEM |
	26	59	86	115	135		
3C	232.6	259.3	289.4	310.0	324.4	16.0[a]	5.7
3T	166.0	185.8	205.9	228.3	247.5	12.3[a]	6.7
4C	87.6	80.8	98.0	94.3	111.9	5.5[a]	5.7
4T	61.9	76.3	91.9	97.8	109.7	4.7[a]	10.3

[a]Linear effect of time (P<.01).

TABLE 11. R^2 VALUES AND CV OBTAINED FROM FIXED
EFFECTS MODELS FOR THIRD METACARPAL (3C) AND
METATARSAL (3T) BENDING STRESS AND FOURTH
METACARPAL (4C) AND METATARSAL (4T) SHEAR STRESS

| Model | Bending Stress | | | Shear Stress | | |
	3C	3T	Avg.	4C	4T	Avg.
			R^2, %			
1[a]	49	56	53	56	72	64
2[b]	76	75	76	77	84	81
Avg.	63	66	64	67	78	73
			CV, %			
1	22.5	23.4	23.0	22.8	20.7	21.8
2	19.3	22.0	20.7	20.3	19.1	19.7
Avg.	20.9	22.7	21.8	21.6	19.9	20.7

[a]Model 1: Stress = Diet + Time + Diet x Time.
[b]Model 2: Stress = Diet + Time + Weight + Weight^2 +
Diet x Time + Weight x Diet +
Weight x Time + Weight x Diet x Time.

TABLE 12.   ALL POSSIBLE PARTIAL CORRELATIONS OF THIRD
METACARPAL (3C) AND METATARSAL (3T) BENDING STRESS AND
FOURTH METACARPAL (4C) AND METATARSAL (4T) SHEAR
STRESS FOR BOTH MODELS OF STRESS

Model[a]	Correlation					
	3C-3T	3C-4C	3C-4T	3T-4C	3T-4T	4C-4T
1	.74[b]	.44[b]	.37[b]	.44[b]	.39[b]	.39[b]
2	.70[b]	.18	.26[c]	.21[d]	.22[d]	.10
Avg.	.72	.31	.32	.33	.31	.25

[a]Refer to Table 11 for definition of effects in each model.
[b,c,d]Correlation is different from zero (P<.01, P<.10
and P<.15, respectively).

TABLE 13. LEAST SQUARES MEAN LIVE AND SLAUGHTER BIOPSY CORE MEASURES FOR PIGS FROM WEANING TO MARKET WEIGHT, BY DIET AND DAY ON TEST[a]

Day on Test	Live ash weight, g[b] — P level (% of NRC)						Slaughter ash weight, g[b] — P level (% of NRC)					
	70	85	100	115	130	SEM	70	85	100	115	130	SEM
0	9.1	20.7	13.2	27.6	15.3	4.6	-	-	-	-	-	-
26	9.5	22.4	24.1	29.6	39.0	6.1e	38.7	45.8	43.8	55.5	55.8	6.4ef
59	-	-	-	-	-	-	8.3	14.7	34.0	35.2	36.9	4.5e
86	24.7	30.0	46.2	46.9	39.8	5.7d	87.7	119.8	111.8	74.6	148.6	17.6
115	28.1	38.2	31.8	34.1	38.2	7.1	20.6	38.1	44.0	49.0	42.6	11.4
135	39.1	68.4	63.2	59.4	69.5	12.3	49.6	64.4	54.3	72.0	74.1	7.6d

Day on Test	Live ash weight, % of wet weight[h]						Slaughter ash weight, % of wet weight[l]					
	70	85	100	115	130	SEM	70	85	100	115	130	SEM
0	15.4	18.8	18.5	16.6	23.2	3.0	-	-	-	-	-	-
26	23.6	15.0	21.9	30.7	32.8	2.1ef	16.2	18.9	25.9	26.1	28.6	2.2e
59	-	-	-	-	-	-	15.5	22.8	25.3	31.0	36.8	3.2e
86	21.7	27.0	33.9	40.5	31.2	3.9d	27.0	26.0	25.8	35.2	33.8	2.8d
115	12.4	18.9	21.7	22.0	22.2	2.9d	23.7	33.4	38.2	32.7	41.7	2.9e
135	24.5	24.3	28.9	34.0	32.8	2.8d	26.5	31.0	35.6	37.1	42.0	2.3e

Day on Test	Live dry fat-free ash percentage[j]						Slaughter dry fat-free ash, %[k]					
	70	85	100	115	130	SEM	70	85	100	115	130	SEM
0	33.3	45.5	38.3	45.0	47.1	3.6d	-	-	-	-	-	-
26	42.1	35.6	44.1	50.5	54.8	2.6e	40.9	45.0	51.6	51.7	53.1	1.9e
59	-	-	-	-	-	-	33.5	44.2	49.7	53.6	52.7	2.8ef
86	49.0	49.4	57.8	60.4	54.6	1.8ef	48.4	48.4	50.3	53.5	56.7	1.6e
115	32.4	39.9	44.1	44.7	45.4	2.7e	42.7	51.9	52.5	52.2	57.1	1.5e
135	46.8	50.1	54.9	56.2	57.8	2.8e	45.3	51.5	54.7	55.6	57.3	1.3ef

[a] No estimate of LS means at day 59 could be obtained for live biopsy pigs due to missing cells in repeated measure design.

[b] Overall linear (P<.01) effect of diet and linear (P<.01) and quadratic (P<.01) effect of time; significant linear diet by time interaction (P<.05).

[c] Overall linear effects of both time and diet (P<.01).

[d,e] Linear effect of diet (P<.05 and P<.01, respectively).

[f,g] Quadratic effect of diet (P<.05 and P<.01, respectively).

[h] Overall linear (P<.01) and quadratic (P<.01) effect of diet and linear (P<.01) and quadratic (P<.01) effect of time; significant linear and quadratic diet by time interactions (P<.01).

[i] Overall linear (P<.01) and quadratic (P<.01) effects of diet and linear (P<.01) effect of time; significant quadratic diet by time interaction (P<.05).

[j] Overall linear (P<.01) and quadratic (P<.05) effect of diet and linear (P<.01) and quadratic (P<.01) effect of time; significant linear diet by time interaction (P<.01).

[k] Overall linear (P<.01) and quadratic (P<.05) effects of diet and linear (P<.01) effect of time.

TABLE 14. LEAST SQUARES MEAN POOLED DRY FAT-FREE ASH
PERCENTAGES FOR THIRD METACARPAL AND METATARSAL AND
FOURTH METACARPAL AND METATARSAL BONES, FROM PIGS
USED TO OBTAIN SLAUGHTER AND LIVE BIOPSY CORES, BY
DIETARY P LEVEL AND DAY ON TEST[a,b]

Day on Test	P level (% of NRC)					SEM
	70	85	100	115	130	
Avg. Dry fat-free ash, %[c]						
26	53.0	54.6	54.4	54.8	53.5	1.2
59	51.0	55.5	56.1	57.3	57.8	.9[de]
86	52.5	55.7	57.4	58.0	58.3	.9[d]
115	53.5	58.4	59.2	59.6	60.5	.9[de]
135	56.0	58.8	59.7	60.3	60.2	.9[df]
Live pig[g]	55.3	59.0	59.5	60.6	59.4	.7[d]

[a]Avg. is the average of third and fourth metacarpal and metatarsal bone ash.
[b]Initial pig average was 52.0+.2%.
[c]Overall linear and quadratic effects of diet (P<.01) and linear (P<.01) effect of time.
[d]Linear effect of diet (P<.01).
[e,f]Quadratic effect of diet (P<.05 and P<.01, respectively).
[g]Live pig values were determined for repeat biopsy pigs at day 138 on test.

TABLE 15. SIMPLE CORRELATIONS FOR OVERALL RELATIONSHIP OF SLAUGHTER AND LIVE BIOPSY CORE MEASURES WITH MEASURES OF THIRD AND FOURTH METACARPAL AND METATARSAL BONE[a,b].

Criteria	Diets[c]:	Core Ash Weight[c] Slaughter		Core Ash Percent Slaughter		Core Ash Percent Live		Core DFF Ash Percent Slaughter		Core DFF Ash Percent Live	
		1-5	2-5	1-5	2-5	1-5	2-5	1-5	2-5	1-5	2-5
3M Length, cm		.45	.47	.68	.70	.63**	.55*	.61	.64	.67**	–
4M Length, cm		.48	.49	.67	.69	.62**	.54*	.61	.65	.65**	–
3M Wet weight, g		.42	.43	.70	.70	.76**	.73**	.59	.60	.72**	.62*
4M Wet weight, g		.43	.43	.68	.69	.76**	.72**	.58	.59	.75**	.63*
3M Wall thickness, cm		.37**	.40**	.73	.74	.50*	–	.64	.65	.52*	.62*
4M Wall thickness, cm		.26*	.28**	.64	.64	.75**	.71**	.58	.60	.75**	.76**
3M Area, cm²		.40	.42	.72	.73	.67**	.68**	.62	.63	.61*	.69**
4M Area, cm²		.33**	.34**	.68	.68	.85**	.83**	.60	.61	.79**	.79**
3M Force, kg		.31**	.32**	.71	.70	–	–	.58	.57	.55*	–
4M Force, kg		.27**	.27*	.71	.70	.53*	–	.59	.56	.67**	.59**
3M Stress, kg/cm²		.23*	.24*	.66	.66	–	–	.58	.57	–	–
4M Stress, kg/cm²		.24*	.24*	.67	.67	–	–	.57	.54	–	–
3M DFF ash, %		.35**	.33**	.74	.76	–	–	.67	.65	.77**	.57*
4M DFF ash, %		.30**	.28**	.69	.73	–	–	.62	.61	.56*	–
Avg. DFF ash, %		.34**	.32**	.74	.76	–	–	.67	.65	.69**	–

[a]Abbreviations are as follows: 3M = average of third metacarpal and metatarsal; 4M = average of fourth metacarpal and metatarsal. Avg. = average of third and fourth metacarpal and metatarsal. 3M force and stress were determined by bending tests, whereas 4M force and stress were determined by shear tests.

[b]Correlations are significant at P<.0001 unless otherwise noted: **P<.01, *P<.05.

[c]No correlations were significant between biopsy core ash weight and the bone measures.

[d]1-5 = included all five diets (N = 98 and 17, for slaughter and biopsy, respectively); 2-5 = included only diets 2 through 5 (N = 76 and 16, for slaughter and biopsy, respectively).

CHAPTER 8

SUMMARY AND CONCLUSIONS

In order to address the stated objectives of this
study, three trials were conducted, using a total of 300
swine fed 70, 85, 100, 115 or 130% of NRC (1979)
recommended Ca/P levels from weaning (avg BW 10 kg) to
market weight (avg BW 110 kg). The proportion of dicalcium
phosphate and calcitic limestone in corn-soybean meal diets
was adjusted to provide the desired dietary Ca/P levels in
a 1.2:1 Ca:P ratio. A fourth trial was also conducted,
involving 125 pigs, but dolomitic limestone was used in
place of calcitic limestone, yielding five dietary P levels
of 70, 85, 100, 115 or 130% of NRC recommendations in a
.9:1 Ca:P ratio.

The effects of dietary Ca/P level and time on test on
performance criteria (body weight, average daily gain,
average daily feed intake and efficiency of feed
utilization) were reported Chapter 3. Dietary and time
effects on bone measures [length, wet weight, radius
(average outside diameter), wall thickness, cross-sectional
area, bending or shear force, bending or shear stress, and
the extracted weight, dry fat-free ash weight and dry fat-

free ash percentage of the proximal halves of pooled third metacarpals and metatarsals (3M), and of pooled fourth metacarpals and metatarsals (4M)] were reported in Chapter 5.

The Ca/P level required to maximize the performance and bone criteria were compared in Chapter 5. The Ca/P level associated with 95 and 98% of maximum bone length, wet weight, radius and dry fat-free ash percentage of both 3M and 4M bone pairs appears to be the same or lower than that required to maximize the performance criteria; performance criteria reached near maximum for Ca/P levels approximating the NRC recommendations. Bone wall thickness, cross-sectional area, force, stress, extracted weight, and dry fat-free ash weight appears to require higher Ca/P levels to reach near maximum than is required for performance criteria. Further comparison of performance and bone criteria, by determining the correlations among all measures, would also add to the body of knowledge concerning the Ca and P status of swine from weaning to market, and is suggested as a follow-up analysis on the study reported.

Asymptotic response surfaces, relating the effects of Ca/P level and time from weaning to market weight to least squares mean performance (Chapter 3) and bone measures (Chapter 5) were derived. Although the time trends for the

efficiency of feed utilization were strong, the effects of Ca/P level on this variable were not consistent; therefore, it was concluded that no response surface could be derived for efficiency of feed utilization. No exact test of lack of fit was available due to non-independence of the least squares means, but lack of fit was estimated to be significant for the response surface models of all performance and most bone criteria. In all cases, there was evidence that the magnitude of the lack of fit detected was small, indicating that the predicted performance and/or bone measures accurately represented the effects of Ca/P and time on these criteria despite apparent lack of fit.

The bone biopsy technique was evaluated by comparing the bone cores obtained by repeated sampling of live animals to bone cores obtained from slaughter animals at each of five slaughter periods from weaning to market, and by comparing the dry fat-free ash percentage of biopsy bone cores to the dry fat-free ash percentage of the proximal halves of pooled third and fourth metacarpal and metatarsal bones (Chapter 6). The effects of feeding 70 to 130% of NRC recommended Ca/P levels to swine from weaning to market weight on dry fat-free ash were similar for biopsy bone cores and pooled bones. Biopsy core measures also correlated significantly with bone dimensional, strength and ash measures. Least squares mean comparisons indicated

that biopsy core dry fat-free ash percentage may relate more closely to intact bone ash when dietary Ca/P levels of 100% of NRC recommendations or greater are fed. Overall, it was concluded that the use of biopsy as a live-animal sampling technique in Ca/P studies warrants further investigation.

Bending and shear stress, determined by three-point bending testing third metacarpal and metatarsal bones or shear testing fourth metacarpal and metatarsal bones, was also compared (Chapter 4) for use in measuring bone strength. Shear testing was found to be a more desirable method of determining bone strength, when compared to bending testing because: 1) it was less subject to confounding by other effects than bending; 2) simpler calculations were required than for bending tests; 3) dietary and time effects were more readily detected due to reduced variation; 4) higher R^2 were obtained for shear models than for bending models, indicating a greater proportion of the variance in shear could be explained; and 5) lower CV were associated with shear stress than with bending stress, indicating less variation relative to the size of the mean. Partial correlations indicated that bending stress was more repeatable across bones from the same animal than was the case for shear stress. Further comparison of bending and shear testing is indicated. In

the study reported here, third (inside) metacarpals and metatarsals were used for bending tests and fourth (outside) metacarpals and metatarsals were used for shear testing. This may have resulted in confound differences between inside and outside bones with the diet and time effects observed for bending or shear stress. Therefore, in future comparison of bending and shear tests, the choice of bones used for the tests should be such that this possible confounding is avoided.

The performance and bone characteristics observed when .9:1 Ca:P ratios were used were compared to the results obtained for the 1.2:1 ratios. Although no exact tests could be made, the response of performance and bone criteria to diets and time were similar for both Ca:P ratios. Evaluation of the bone biopsy technique when a Ca:P ratio of .9:1 was used gave variable results and offered little support for use of the technique under these conditions, contrary to what was suggested when the 1.2:1 Ca:P ratio was used. Eight of the 25 live biopsy pigs died during the .9:1 Ca:P trial due to various causes unrelated to dietary treatment, with subsequent reduction in animal numbers in later periods. This likely contributed to the observed variability encountered for the biopsy comparison. This would suggest that the biopsy technique may still warrant further evaluation for use when Ca:P ratios of .9:1

are used in the diet.

Three overall objectives of this study were proposed initially. These were: 1) to determine the relationships among measures of the Ca and P status of swine fed a wide range of Ca/P levels in the diet from weaning to market weight; 2) to establish response curves for use in evaluating the Ca and P status of swine for a wide range of Ca/P levels from weaning to market; and 3) to evaluate a bone biopsy technique as a means of assessing the Ca and P status of swine at any stage of postweaning growth without requiring slaughter of the animal to obtain bone samples. From the foregoing discussion, it can be concluded that each of these stated objectives were met in this study. Additionally, further study of some aspects has been suggested that would clarify or offer additional information in regard to these objectives.

APPENDIX

Dietary Treatments

Five dietary treatments were used in the study. The
basal diet (diet 3) contained NRC-recommended Ca/P levels
(NRC, 1979). Diets 1, 2, 4 and 5 contained 70, 85, 115 and
130% of the diet 3 level, respectively. Calcium carbonate
and dicalcium phosphate were used to provide NRC-
recommended Ca/P levels in the basal corn-soy diet. The
proportion of these mineral supplements were varied to
obtain the other dietary treatments. The amount of plant
phosphorus remained constant across all diets within a
phase, and was similar for all phases.

The relative percentage calcium and phosphorus levels
for each dietary treatment were maintained across four
phases, based on the NRC-recommended nutrient density
changes. The weanling-growing-finishing period was divided
as follows: Phase 1 - 10 to 20 kg (NRC recommended levels
of 0.65% Ca and 0.55% P); Phase 2 - 20 to 35 kg (0.60% Ca
and 0.50% P); Phase 3 - 35 to 60 kg (0.55% Ca and 0.45% P);
Phase 4 - 60 to 100 kg (0.50% Ca and 0.40% P). The pigs
were changed to the next phase diets when the average
weight of the entire group reached the next higher weight
classification. The dietary Ca/P level of each diet was

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decreased in each of the phases to its respective percentage of the diet 3 NRC-recommended level, so as to maintain a constant percentage of diet 3 level in the diets throughout the entire growing-finishing period.

Due to an error, dolomitic limestone was used in trial 4 instead of calcitic limestone. This resulted in lower calcium levels than were used in other trials, although the phosphorus was approximately the same.

Appendix table 1 contains the diets used in each of the four phases, with the exception that dolomitic limestone replaced calcitic limestone in equal amounts for trial 4 (see also text table 1 in Chapters 3 and 7).

Feed Analysis

The diets were analyzed for their mineral content and the analyzed values were compared to the calculated values for each diet in each phase. Duplicate samples of each batch of each diet were ashed at 475 C for 6 hr, then the ash was dissolved in 5 ml concentrated HCl and allowed to sit for 30 min. Ten ml of deionized-distilled water was then added and the solution was allowed to sit for an additional 20 min. Following dilution to 50 ml with deionized-distilled water, the samples were filtered and the mineral content of each sample was determined simultaneously on a model ICAP 9000 inductively coupled plasma spectrometer (ICP).

The analyzed values of the batches of each diet were significantly different than the calculated values when expressed on a percentage basis (i.e. $mg \cdot kg^{-1}$ diet) and differed within batches of the same diet. When the analyzed values, expressed as the average daily intake by averaging across batches of the same diet and using the actual feed intake of each pen, were compared to the calculated averaged daily intakes, the differences were minimized.

Therefore, it was determined that the proper diet variable used in subsequent analysis of responses should be based on average daily intake. Appendix tables 2 and 3 contain the analyzed and calculated dietary values, respectively, expressed on a percentage of the diet basis. Appendix table 4 shows the average daily feed intakes (ADFI) averaged across all pens on each diet, presented by trial and phase. Tables 5 through 8 of the appendix give the average daily intake of Ca and P, averaged across pens on each diet, by each phase, for trials 1 through 4, respectively.

Facilities and Management

Pigs were weaned at 4 to 5 weeks of age. The weaned pigs were given access to a standard starter diet (Appendix table 9), calculated to meet NRC-recommended levels for all nutrients. When the average weight of the entire group of

pigs reached 10 kg, the pigs were blocked by sire (breed sire) and sex and the blocks were randomly assigned to replicates. Each block contained five pigs and there were five blocks per replicate. One pig from each block within a replicate was randomly assigned to each of the five dietary treatments, resulting in five pigs per treatment in each replicate. At the time of allotment, one block per replicate was randomly assigned to each of five slaughter times. Ten pigs in trials 1 and 2 and six pigs in trial 3 were slaughtered at the beginning of the trial. One additional replicate in each trial (25 pigs per trial) was randomly selected for periodic biopsy.

Following allotment to treatment, pigs were housed in nursery facilities for the first four weeks of the trials. Nursery facilities used at the Tidewater Research Center contained .9 m x 1.2 m (.22 $m^2 \cdot pig^{-1}$) floor pens with plastic coated wire floors. Two matched nurseries were used in Blacksburg for trial 2. These nurseries contained .9 m x 1.2 m (.22 $m^2 \cdot pig^{-1}$) double-decked cages with plastic coated wiring flooring. Upper and lower decks were separated by sheets of galvanized metal to prevent contamination of lower pens. In addition to these Blacksburg nurseries, a third environmentally-controlled nursery was also used for trial 4. The double deck cages in this third nursery were 1.2 m x 1.2 m, providing .29

$m^2 \cdot pig^{-1}$.

Growing-finishing facilities at Tidewater consisted of 1.5 m x 3 m solid concrete floor pens, while those at Blacksburg were 1.25 m x 3 m totally slotted concrete pens. Pigs were moved to these grower-finisher facilities at the end of the fourth week on test (approximately 9 to 10 wk of age and 20 to 25 kg body weight). Initially, at least .45 $m^2 \cdot pig^{-1}$ was provided in these pens, increasing to approximately 1.85 $m^2 \cdot pig^{-1}$ at market weight.

Pigs were self-fed in both the nursery and grower and had water available ad libitum at all times. The animals were weighed every 2 weeks and the feed intake for the pen was determined.

Preparation and Testing of Metacarpals and Metatarsals

Metacarpals and metatarsals were cut out of the feet at slaughter, using #60 Bard-Parker Disposable Autopsy knives. The bones were then frozen at -20 C immediately after slaughter in air-tight plastic bags. At a later time, the bones were thawed so that extraneous tissue could be removed. The autopsy knives were used to scrape the adhering membrane and other tissue off the bone surface.

The bones were then weighed and the dimensions of each bone were measured. Overall length of each bone was determined and the width of the bone shaft at the narrow, wide and perpendicular dimensions of the bone shaft was

measured using a dial caliper (see Figure 1, Chapter 4).
The bones were then refrozen in moisture-proof bags pending
bending or shear tests.

The bones were thawed immediately prior to testing and
maintained under refrigeration until testing was completed.
Force was applied to the bones at a speed of 10 mm·min^{-1}
for both bending and shear tests, and the resulting force
curve was plotted at a chart speed of 50 mm·min^{-1}. Maximum
force required to cause a bending or shear failure was
electronically measured and recorded in pounds of force.
The three-point bending jig used had a 2-point spread of
3.175 cm (1 1/4 in).

Following bending or shear testing, the bones were cut
in half at the midpoint of the overall length with a band-
saw (Figure 1, Chapter 4). The wall thickness at the
midpoint of the narrow, wide and perpendicular dimensions
was measured and averaged to determine a value for the wall
thickness at the midpoint of the overall bone length.

The area of the bone tissue at the midpoint plane,
perpendicular to the overall bone length, was assumed to
that of a quarter circle, due to the shape of the bone
tissue on this plane. The radius was assumed to be the
average of the narrow and perpendicular bone shaft
dimensions. Therefore, the area of bone tissue in this
plane was the area of a quarter circle using the outside

radius [(wide + narrow)/2] minus the area of the inner

quarter circle formed by the difference between the outside

radius and the wall thickness, and added to the area formed

by the width of the two "radii" forming the quarter circle

times the length of the radius minus the wall thickness

(see the section "Derivation of Equations" in the

appendix).

In order to compare the force required to produce

bending failure to that required to cause shear failure,

the common unit of stress was calculated for both bending

and shear forces.

Shear stress ($Stress_s$) was calculated according to the

formula:

$Stress_s$ = Maximum force / (2 * Area).

Bending stress ($Stress_b$) was calculated according to

the formula:

$$Stress_b = \frac{0.106 * Maximum\ force * Length}{[(0.44 * r^2 * t) - (1.32 * r * t^2)]}$$

Where: r = radius, t = wall thickness and Length refers to
    the spread between the lower 2 points of the 3-point
    bending jig.

The derivation of the bending stress equation and the

other pertinent equations are presented in the section

"Derivation of Equations" in the Appendix.

Following bending and shear testing and determination

of wall thicknesses, the proximal half of the bones was

placed in a forced-air oven at 70 C for 2 days. The bone tissue was then wrapped in brown wrapping paper and crushed into fine pieces with a hammer and returned to the forced air oven for an additional 4 days. The crushed bone tissue was then wrapped in cheese cloth[1] and tagged with a numbered chicken wing-clip for identification.

The fat contained in the crushed bone sample was then extracted by refluxing a 75%:25% chloroform: methanol mixture over crushed and dried samples in 4 liter soxhlet apparatus. Quantitative determination of the fat portion was not possible since procedures used to dry and crush the bone tissue resulted in loss of some fat tissue prior to extraction. Extracted samples were then returned to the forced-air oven and redried for 24 hours. The weight of dry, fat-free bone tissue from each sample was then weighed and the entire sample was ashed in a muffle furnace at 600 C until a consistent, white ash was obtained (approximately 24 hr). Ashed samples were removed from the muffle furnace to a dessicator and allowed to cool completely. The weight of cooled, ashed sample was then determined and the percent ash was calculated.

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[1]100% cotton, open weave, 25 x 12 threads in^{-1}.

Anesthesia and Surgery

Pigs were place under general anesthesia for the biopsy procedure. Bio-Tal (thiamylal sodium)[1] was used at the anesthetic agent. The dosage used, ranged from 11 mg kg^{-1} (5 mg·lb^{-1}) to a maximum of 17.6 mg kg^{-1} (8 mg·lb^{-1}), [i.e. 1 ml of a 4% (40 mg·ml^{-1}) Bio-Tal solution per 17.6 kg body weight to a maximum of 1 ml of the 4% solution per 11 kg body weight].

Bio-Tal is the thiobarbiturate analogue of secobarbital [sodium 5 allyl-5-(1-methyl-butyl)-2-thiobarbiturate], and can only be administered under the direction of a graduate veterinarian. Bio-Tal is considered an ultra-short-acting thiobarbiturate, resulting in rapid induction of a surgical plane of anesthesia that is sufficient for interferences of 10 to 15 min duration. Bio-Tal is metabolized primarily via the liver, and full recovery from the effects is complete by 3 hr following a single dose, with minimal excitement and post-anesthetic ataxia is of short duration. Potential adverse reactions to Bio-Tal include circulatory depression, thrombophlebitis, pain at the injection site, respiratory depression (including apnea, laryngospasm, bronchospasm), salivation, emergence delirium, injury to the nerves

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[1]Bio-Tal is the thiobarbiturate analogue of secobarbital [sodium 5 allyl-5-(1-methyl-butyl)-2-thiobarbiturate]. Product of Bio-Ceutic Division, Boehringer Ingelheim Animal Health, Inc. St. Joseph, Missouri.

adjacent to the injection site, skin rashes, urticaria, nausea and emesis in some species.

The anesthetic was injected in to the anterior vena cava to induce anesthesia in pigs in the initial sampling period and the two subsequent biopsy periods. The injection was made via the ear external ear vein in the pig during the final two live biopsy periods.

Once a surgical plane of anesthesia had been induced, the biopsy sight was shaved using an Oster model A-5 clippers with size 40 blades. The area was then scrubbed with Betadine solution, followed by a 70% isopropyl alcohol rinse. The area was then dabbed dry using clean 4 x 4's. A small incision (approximately 1.75 cm) was made through the skin and tissues covering the bone, using a #15 scalpel, and a bone core was removed using a 56 mm (7/32 in) x 15.9 cm (6 1/4 in) michele laminectomy trephine[2]. Following extraction of the bone core, the incision was closed using 3-0 metric (2-0 standard) Ethicon coated, absorbable vicryl, with an FS-1 cutting needle, in a mattress suture. All tools were maintained in a aqueous 17% Zephiran chloride (benzyl-dimethyl ammonium mixture) solution (1:750 dilution) for disinfecting between uses and were rinsed in distilled water upon removal from the solution to prevent tissue irritation.

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[2]Supplied by Arista Surgical Supply Company, Inc. New York, New York.

The side biopsied was alternated between the right and left from sampling time to sampling time so that the time interval between sampling at the same sight was 8 wk.

The biopsy was taken from the medial surface of the proximal tibia, in the area immediately dorsal to the epiphyseal plate (see figure 1, Chapter 6). The procedure was carried out under near-sterile conditions.

Derivation of Stress Equations

Calculation of Area - Area based on the assumption that the bone is shaped as a quarter circle. The area formula subtracts off the area of the marrow cavity from that of the entire quarter circle. This requires that the area of an inner quarter circle with radius (r - t) be subtracted off the the area of the entire circle with radius r, then the area of the "legs" minus the "corners" be added back:

Area of entire quarter circle:

$$\frac{\pi * r^2}{4}$$
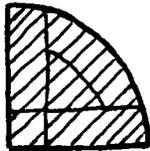
- Area of inner quarter circle:

$$\frac{\pi * (r - t)^2}{4}$$
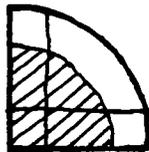
+ Area of the "legs" minus the "corners":
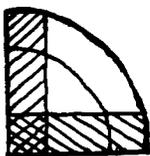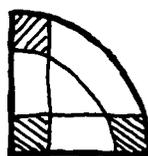
$$2 * r * t \quad - \quad 3t^2 \quad = \quad 2rt - 3t^2$$
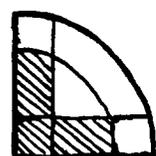
When simplified using a value of 3.14 for pi, the formula for the area of the bone becomes:

$$AREA = \{\frac{(3.14 * r^2) - [3.14(r - t)^2]}{4} +$$

$$[(2 * r * t) - (3 * t^2)]\}$$

Where: r = radius, t = wall thickness

Calculation of Shear Stress (according to the formula of Harner and Wilson, 1985; see Chapter 4 for reference).

$$Stress_s = \frac{Maximum\ Force}{2 * Area}$$

Derivation and Calculation of Bending Stress:

Crenshaw et al. (1981) gives the stress of a flexure test such as a three-point bending test as:

$$Stress = \frac{Force\ (kg) * Length\ (cm) * C\ (cm)}{4 * I\ (cm^4)}$$

Where: Force = maximum force in bending test.
        Length = length between the two fulcra points supporting the bones.
        C = the distance from the neutral axis to the extreme outer fiber.
        I = moment of inertia.

Based on this equation, and the assumption that the third metacarpal and metatarsal bones are shaped as a quarter circle at the point at which force is applied, the working bending stress equation can be derived as follows:

$$C = \frac{4 * r}{3 * \pi} = 0.424 * r\ ,\ where\ r = radius.$$

$$I = (I_{out} - I_{in})$$

Where: $$I_{out} = r^4(\frac{\pi}{16} - \frac{4}{9\pi})$$

$$= 0.05488\ r^4$$

$$I_{in} = (r - 2t)^4(0.05488)$$

$$\cong 0.05488(r^4 - 8 * r^3 * t + 24 * r^2 t^2)$$

Where: t = wall thickness

Therefore:

$$I = 0.05488\{r^4 - r^4 + (8 * r^3 * t) - (24 * r^2 * t^2)\}$$

$$= 0.05488\{(8 * r^3 * t) - (24 * r^2 * t^2)\}$$

$$= 0.44 * r^3 * t - 1.32 * r^2 * t^2$$

Using these calculated values, it can be shown that the formula for bending stress when the bone is assumed to be in the shape of a quarter circle is:

$$Stress_b = \frac{\frac{Force * Length}{4} * (0.424 * r)}{\{(0.44 * r^3 * t) - (1.32 * r^2 * t^2)\}}$$

$$Stress_b = \frac{0.106 * Force * Length}{\{(0.44 * r^2 * t) - (1.32 * r * t^2)\}}$$

Where, for the current study, the value for Length was held constant at 3.175 cm.

Procedures Used for and Results of
Statistical Analysis of Performance Criteria

Four performance criteria [weight (WT), average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (EFF)] were analyzed for the effects of biopsy, sex, CAP ratio and the day on test corresponding to the midpoint of each weighing period (date). Four trials were conducted, but due to an error, dolomitic limestone was used instead of calcitic limestone in trial 4. Therefore, only data from the first three trials were pooled for combined trial analysis of effects.

The CAP ratio used was a ratio of the analyzed average daily intake of calcium plus phosphorus across the entire length of each of the trials divided by the calculated NRC average daily intake of calcium plus phosphorus recommended during the same time. The recommended NRC average daily calcium plus phosphorus was calculated using the expected ADG and ADFI corresponding to the NRC-recommended calcium and phosphorus levels in each of four dietary phases used in the study. Appendix table 10 shows the average CAP values for each diet in each trial. Each weighing period was approximately 14 days in length, and there were 11 weigh-periods in trials 1 and 3, and 10 weigh-periods in trials 2 and 4. In addition, the initial WT was also included, giving 12 possible weights in trials 1 and 3 and 11 weights in trials 2 and 4. The lengths of trials 1, 2,

3 and 4 were 138, 140, 131 and 135 days, respectively.
Therefore, the time on test corresponding to the midpoint
of each weigh-period were unique to each trial (Appendix
table 11).

The effects of biopsy on the four performance criteria
were evaluated by analysis of variance (ANOVA) using a
model including diet and biopsy effects within each trial
and period (15 total degrees of freedom for each trial-
period model). The effects of sex on the performance
criteria were evaluated in similar fashion within trial and
period (15 total degrees of freedom). The effects of
biopsy within each trial and period were nonsignificant
(P>.10). The effects of sex were never significant in two
successive periods for any of the performance criteria, and
sex effects were significant in only three periods (P<.05)
for ADG, marking the highest number of significant cases.
It was concluded that sex did not have a consistent effect
on performance either, therefore, effects of both sex and
biopsy were not included in the final model.

In order to determine whether diet and time effects
existed in the three trials, the first analysis was done
using a fixed effects model including diets, numbered 1
through 5, and periods, numbered 1 through 10 or 11.
Within each trial, analysis of variance of the effects of
diet, pen within diet, period and diet by period

interaction on the four performance criteria was performed. Linear, quadratic and cubic orthogonal contrasts of diet effects on the response criteria were also done to determine what type of relationship existed with diet. In order to clarify the time relationship, the period effect of the first model was replaced with linear, quadratic and cubic date effects.

Based on the consistency of results by trial, the data of the first three trials were pooled. The analysis of variance of the effects of trial, diet, pen within trial and diet, date (linear, quadratic and cubic), and all interactions on the response criteria was performed, and linear, quadratic and cubic orthogonal contrasts on diet were made.

Based on all previous analyses of variance, the following strategy was followed to derive the response surface for WT, ADG, ADFI and EFF in relation to the dietary CAP ratio and date. The analysis of variance for the effects of trial, diet, pen within trial and diet, period and interactions was considered first (Table 2, Chapter 3), and least squares trial by diet by period least squares means were generated. Asymptotic models were considered as response surface models. As a first step (Model 1) the asymptotic model $Y = A[1-Be^{(-k \times SCAP)}]$ was fit within each trial and period for WT, ADG and ADFI least

squares means (generated from the model shown in Table 2, Chapter 3). In this model:

A = maximum performance

B = percentage reduction in Y between maximum (A) and the performance for the lowest CAP level.

k = the rate at which maximum performance is approached - i.e., k is the proportion by which the distance to maximum is reduced by each unit increase in CAP.

SCAP = the scaled CAP ratio, where SCAP=(CAP - minimum CAP), for each trial.

A, B and k estimates were plotted by date for each trial. These plots indicated that A was nearly linear for WT and ADFI, and was clearly quadratic for ADG. Estimates of B for WT, ADG and ADFI were clearly quadratic across period for each trial, while there was no clear pattern discernible for k, although k did vary. The values of A were very consistent across trials, while B and k were less consistent; therefore, it was assumed that the main trial differences were due to differences in A, but that the data could at least be pooled across periods. Total error sums of squares were calculated by summing the error sums of squares of all periods and trials. The mean square error of this model was used as the estimate of mean square error in the lack of fit tests for the asymptotic models.

Using the estimates of A from the trial-period model, the WT, ADG and ADFI responses were divided by the trial-

period maximum to determine the percentage of maximum (Y/A)
for each datum. Second degree polynomial regressions of
estimates of A on the day on test corresponding to the
midpoint of each weigh-period, were performed for each
trial to describe predicted maximum performance (A), as a
function of time (i.e., $\bar{A} = \alpha_0 + \alpha_1 t + \alpha_2 t^2$, where t = time
on test at the midpoint of each weigh-period).

The next asymptotic model fit, within each trial,
was: $(Y/\bar{A}) = [1 - (B_0 + B_1 t + B_2 t^2)e^{(-k \times SCAP)}]$, with B
fit as a linear and quadratic function of time on test at
the midpoint of each weigh-period. For WT and ADFI, B_0 was
assumed to be zero, so the linear and quadratic function of
B was assumed to equal: $(B_1 t + B_2 t^2)$.

The Y/$\bar{A}$ asymptotic model was also fit across trials,
to get estimates of $\bar{B}$ and k across trials. The values of A
were determined within each trial for this model. In the
combined trial model, SCAP = (CAP - lowest CAP in any
trial). The fourth model fit, and used as the asymptotic
response surface, was $(Y/\bar{A}) = [1 - \bar{B}e^{(-k \times SCAP)}]$, where
the $\bar{A}$, $\bar{B}$ and k parameters were fit across trials; A and B
were fit as linear and quadratic functions of time.

Error sums of squares for the Y/A models were
calculated by multiplying Y/A by $\bar{A}$ to derive an estimate of
Y, squaring the deviation of Y from the actual values and
summing these deviations. Total sums of squares used to

calculate R^2 for the models was obtained from the model
$Y = A[1-Be^{(-k \times SCAP)}]$ fit across all trials and periods.

Lack of fit on each model was calculated by dividing the difference in error sums of squares, over the difference in error degrees of freedom, by the estimate of mean square error. Although not an exact test due to non-independence of least squares means, it does
give an estimate of the reduction in fit observed with each subsequent simplification of the model. R^2, for each model was calculated by dividing the difference between total sums of squares and error sums of squares of each model by the total sums of squares.

Overall maximum ADG, ADFI and EFF were also determined for this study. Overall average values for each diet in each trial (5 values per trial) were computed, and fit to the asymptotic model $Y = A[1-Be^{(-k \times SCAP)}]$. The CAP level associated with 90, 95, and 98% maximum was determined by solving the equation $\{1 - Be(-k \times SCAP)\}$ for CAP, where CAP = (SCAP + lowest CAP). The derivation of this solution was as follows:

$X = \{1 - Be^{(-k \times SCAP)}\}$, where X = percentage of maximum.

1. $X + Be^{(-k \times SCAP)} = 1$

2. $Be^{(-k \times SCAP)} = 1 - X$

$$3. \quad e^{(-k \times SCAP)} = \frac{1 - X}{B}$$

$$4. \quad inv_e \ (-k \times SCAP) = \left[\frac{1 - X}{B} \right]$$

$$5. \quad e^{\left[\frac{1 - X}{B} \right]} = -k \times SCAP$$

$$6. \quad \frac{e^{\left[\frac{1 - X}{B} \right]}}{-k} = SCAP$$

Since SCAP = (CAP - lowest CAP), and the lowest CAP level in the study was 75.1 in trial 1, then:

$$7. \quad 75.1 + \frac{e^{\left[\frac{1 - X}{B} \right]}}{-k} = CAP$$

Ninety, 95 and 98% of maximum was substituted into this equation, in turn, for each performance variable, along with the corresponding estimates of B and k, and the equation was solved for the CAP level. Table 6 shows the CAP values associated with 90, 95 and 98% of maximum for overall ADG, ADFI and EFF.

Appendix Tables

APPENDIX TABLE 1. DIETARY TREATMENTS USED FOR CALCIUM AND
PHOSPHORUS STUDY IN BOTH BLACKSBURG AND AT THE TIDEWATER
RESEARCH CENTER. INTERNATIONAL FEED NUMBER APPEARS IN
PARENTHESES AFTER EACH INGREDIENT. [DOLOMITIC LIMESTONE
(6-02-633) WAS USED IN TRIAL 4 INSTEAD OF THE GROUND
CALCITIC LIMESTONE]

Phase I Diets (10 to 20 kg)

			Diet		
Ingredient	1	2	3	4	5
Ground Corn (4-02-935)	73.15	72.62	72.07	71.52	70.94
SBM, 49% (5-04-612)	24.70	24.79	24.89	24.98	25.08
Dicalcium phosphate (6-28-335)	.26	.70	1.15	1.60	2.11
Ground limestone (6-02-632)	.89	.89	.89	.90	.87
Vitamin premix[a]	.25	.25	.25	.25	.25
TM premix[b]	.05	.05	.05	.05	.05
Selenium premix[c]	.10	.10	.10	.10	.10
Salt	.35	.35	.35	.35	.35
ASP-250	.25	.25	.25	.25	.25
	100.00	100.00	100.00	100.00	100.00

Calculated Values:

Protein	18.40%	18.00%	18.00%	18.00%	18.00%
Calcium	.47	.57	.66	.76	.86
Total Phosphorus	.41	.49	.57	.65	.74
Available P	.05	.13	.21	.29	.38
Plant P	.36	.36	.36	.36	.36
Ca-P Level (%NRC)	70%	85%	100%	115%	130%

TABLE 1. CONTINUED

Phase II Diets (20 to 35 kg)

	Diet				
Ingredient	1	2	3	4	5
Ground Corn (4-02-935)	78.67	78.15	77.67	77.17	76.68
SBM, 49% (5-04-612)	19.69	19.78	19.86	19.94	20.03
Dicalcium phosphate (6-28-335)	.14	.59	.98	1.43	1.82
Ground limestone (6-02-632)	.90	.88	.89	.86	.87
Vitamin-Se premix[d]	.20	.20	.20	.20	.20
TM premix[b]	.05	.05	.05	.05	.05
Salt	.35	.35	.35	.35	.35
	100.00	100.00	100.00	100.00	100.00

Calculated Values:

Protein	16.00%	16.00%	16.00%	16.00%	16.00%
Calcium	.44	.53	.61	.70	.78
Total Phosphorus	.37	.45	.52	.60	.68
Available P	.03	.11	.18	.26	.33
Plant P	.35	.34	.34	.34	.35
Ca-P Level (%NRC)	70%	85%	100%	115%	130%

TABLE 1. CONTINUED

Phase III Diets (35 to 60 kg)

	Diet				
	1	2	3	4	5
Ground Corn (4-02-935)	78.82	78.42	77.97	77.53	77.04
SBM, 49% (5-04-612)	19.66	19.73	19.81	19.88	19.96
Dicalcium phosphate (6-28-335)	.03	.31	.70	1.09	1.49
Ground limestone (6-02-632)	.89	.93	.92	.90	.91
Vitamin-Se premix[d]	.20	.20	.20	.20	.20
TM premix[b]	.05	.05	.05	.05	.05
Salt	.35	.35	.35	.35	.35
	100.00	100.00	100.00	100.00	100.00

Calculated Values:

Protein	16.00%	16.00%	16.00%	16.00%	16.00%
Calcium	.41	.49	.57	.64	.73
Total Phosphorus	.35	.40	.47	.54	.62
Available P	.01	.06	.13	.20	.27
Plant P	.34	.34	.34	.34	.35
Ca-P Level (%NRC)	70%	85%	100%	115%	130%

TABLE 1. CONTINUED

Phase IV Diets (60 to 100 kg)

	Diet				
	1	2	3	4	5
Ground Corn (4-02-935)	83.88	83.54	83.15	82.73	82.33
SBM, 49% (5-04-612)	14.68	14.74	14.81	14.88	14.95
Dicalcium phosphate (6-28-335)	.03	.20	.53	.87	1.21
Ground limestone (6-02-632)	.81	.92	.91	.92	.91
Vitamin-Se premix[d]	.20	.20	.20	.20	.20
TM premix[b]	.05	.05	.05	.05	.05
Salt	.35	.35	.35	.35	.35
	100.00	100.00	100.00	100.00	100.00

Calculated Values:

Protein	14.00%	14.00%	14.00%	14.00%	14.00%
Calcium	.37	.45	.51	.59	.66
Total Phosphorus	.33	.36	.42	.48	.55
Available P	.01	.04	.10	.16	.22
Plant P	.32	.32	.32	.32	.33
Ca-P Level (%NRC)	70%	85%	100%	115%	130%

[a]Supplies (per kg diet) 1760000 IU vitamin A, 1760300 IU vitamin D_3, 4400 IU vitamin E, 440 mg vitamin K, 1760 mg riboflavin, 8800 mg panothenic acid, 8800 mg niacin, 8800 ug vitamin B_{12}, 176 g choline, 176 mg biotin.

[b]Contains 20% zinc, 10% iron, 5.5% manganese, 1.1% copper and .15% iodine.

[c]Contains 40.04 mg selenium per kg of premix.

[d]Supplies 1760000 IU vitamin A, 176000 IU vitamin D_3, 4400 IU vitamin E, 440 mg vitamin K, 1760 mg riboflavin, 8800 mg panothenic acid, 8800 mg niacin, 8800 ug vitamin B_{12}, 176 g choline, 176 mg biotin and 40.04 mg selenium per kg of premix.

APPENDIX TABLE 2.   FEED ANALYSIS RESULTS: ANALYZED
DIETARY PHOSPHORUS AND CALCIUM, EXPRESSED AS A
PERCENTAGE OF THE DIET

	Diet									
	1		2		3		4		5	
Phase	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca
Trial 1										
1	.36	.45	.44	.47	.44	.58	.72	.86	.79	.99
2	.37	.45	.44	.46	.52	.64	.62	.72	.65	.70
3	.34	.39	.39	.46	.46	.49	.53	.60	.60	.66
4	.34	.38	.36	.42	.42	.48	.47	.53	.51	.53
Trial 2										
1	.44	.54	.52	.66	.61	.75	.65	.75	.68	.80
2	.39	.50	.47	.52	.52	.60	.65	.81	.70	.80
3	.39	.42	.42	.42	.50	.52	.54	.60	.62	.75
4	.39	.48	.39	.34	.48	.60	.51	.57	.51	.53
Trial 3										
1	.45	.96	.51	.55	.59	.68	.67	.85	.80	.99
2	.37	.43	.44	.40	.53	.55	.60	.70	.65	.68
3	.36	.40	.38	.50	.47	.48	.54	.57	.62	.71
4	.31	.33	.39	.49	.43	.47	.49	.57	.56	.71
Trial 4										
1	.42	.40	.49	.43	.59	.54	.60	.56	.72	.69
2	.43	.31	.50	.38	.62	.50	.64	.50	.75	.63
3	.43	.28	.50	.35	.53	.32	.60	.43	.66	.54
4	.37	.29	.40	.26	.50	.37	.53	.42	.58	.49

APPENDIX TABLE 3. FEED ANALYSIS RESULTS:
CALCULATED DIETARY PHOSPHORUS AND CALCIUM,
EXPRESSED AS A PERCENTAGE OF THE DIET

	Diet									
	1		2		3		4		5	
Phase	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca
Trial 1, 2 and 3										
1	.41	.47	.49	.57	.57	.66	.65	.76	.74	.86
2	.37	.44	.45	.53	.52	.61	.60	.70	.68	.78
3	.35	.41	.40	.49	.47	.57	.54	.64	.62	.73
4	.33	.37	.36	.45	.42	.51	.48	.59	.55	.66
Trial 4										
1	.41	.33	.49	.43	.57	.52	.65	.62	.74	.72
2	.37	.30	.45	.39	.52	.47	.60	.56	.68	.64
3	.35	.27	.40	.34	.47	.42	.54	.50	.62	.58
4	.33	.24	.36	.30	.42	.37	.48	.44	.55	.51

APPENDIX TABLE 4.   MEAN AVERAGE DAILY FEED INTAKE FOR EACH DIET BY TRIAL AND PHASE (KG FEED$\cdot$PIG$^{-1}\cdot$DAY^{-1})

Phase	Diet				
	1	2	3	4	5
Trial 1					
1	0.56	0.67	0.68	0.66	0.68
2	1.45	1.60	1.75	1.70	1.69
3	1.54	1.94	1.98	2.03	1.95
4	2.18	2.69	2.62	2.84	2.81
Trial 2					
1	0.55	0.63	0.60	0.61	0.64
2	1.44	1.84	1.85	1.79	2.05
3	1.75	2.20	2.39	2.27	2.41
4	2.59	2.49	3.13	3.01	2.54
Trial 3					
1	0.76	0.84	0.85	0.82	0.81
2	1.18	1.37	1.48	1.42	1.50
3	1.59	2.28	2.25	2.29	2.27
4	2.22	3.47	3.30	3.28	3.27
Trial 4					
1	0.63	0.64	0.64	0.65	0.70
2	0.88	0.92	0.88	0.91	0.93
3	1.93	2.46	2.10	2.46	2.31
4	1.94	2.44	2.88	3.29	3.52

APPENDIX TABLE 5. MEAN AVERAGE DAILY INTAKES OF
ANALYZED AND CALCULATED CA AND P IN TRIAL 1 BY
PHASE (G CA OR P·PIG^{-1}·DAY^{-1})

	Diet				
	1	2	3	4	5
Phase 1					
Ca, analyzed	2.53	3.14	3.92	5.64	6.77
P, analyzed	2.05	2.95	2.98	4.73	5.41
Ca, calculated	2.67	3.78	4.47	4.98	5.85
P, calculated	2.29	3.25	3.85	4.27	5.09
Phase 2					
Ca, analyzed	6.52	7.30	11.14	12.25	11.45
P, analyzed	5.35	7.03	9.00	10.59	10.61
Ca, calculated	5.33	7.20	9.11	10.25	11.02
P, calculated	6.37	8.42	10.70	11.81	12.75
Phase 3					
Ca, analyzed	6.03	8.97	9.70	12.20	12.96
P, analyzed	5.30	7.62	9.02	10.65	11.71
Ca, calculated	6.35	9.45	11.20	12.98	14.21
P, calculated	5.35	7.74	9.32	10.99	12.01
Phase 4					
Ca, analyzed	8.33	11.40	12.53	15.00	14.94
P, analyzed	7.49	9.74	11.11	13.20	14.33
Ca, calculated	8.09	12.07	13.46	16.71	18.42
P, calculated	7.24	9.76	11.08	13.75	15.37

APPENDIX TABLE 6. MEAN AVERAGE DAILY INTAKES OF
ANALYZED AND CALCULATED CA AND P IN TRIAL 2 BY
PHASE (G CA OR P$\cdot$PIG$^{-1}\cdot$DAY^{-1})

	Diet				
	1	2	3	4	5
Phase 1					
Ca, analyzed	2.94	4.16	4.48	4.56	5.16
P, analyzed	2.41	3.26	3.63	3.94	4.40
Ca, calculated	2.59	3.58	3.96	4.62	5.51
P, calculated	2.22	3.07	3.40	3.96	4.79
Phase 2					
Ca, analyzed	7.24	9.52	11.07	14.50	16.44
P, analyzed	5.65	8.66	9.64	11.67	14.31
Ca, calculated	6.36	9.71	11.37	12.46	16.06
P, calculated	5.32	8.30	9.68	10.81	13.87
Phase 3					
Ca, analyzed	7.37	9.26	12.44	13.60	18.02
P, analyzed	6.80	9.26	11.90	12.33	14.91
Ca, calculated	7.23	10.73	13.53	14.53	17.52
P, calculated	6.09	8.79	11.26	12.30	14.80
Phase 4					
Ca, analyzed	12.41	8.57	18.71	17.26	13.56
P, analyzed	10.13	9.73	15.14	15.25	12.98
Ca, calculated	9.58	11.73	16.07	17.73	16.66
P, calculated	8.57	9.03	13.23	14.60	13.90

APPENDIX TABLE 7.   MEAN AVERAGE DAILY INTAKES OF
ANALYZED AND CALCULATED CA AND P IN TRIAL 3 BY
PHASE (G CA OR P·PIG^{-1}·DAY^{-1})

	Diet				
	1	2	3	4	5
Phase 1					
Ca, analyzed	7.23	4.62	5.80	6.97	7.99
P, analyzed	3.39	4.25	4.99	5.47	6.48
Ca, calculated	3.58	4.76	5.62	6.22	6.91
P, calculated	3.07	4.09	4.83	5.33	6.01
Phase 2					
Ca, analyzed	5.06	5.48	8.06	9.87	10.20
P, analyzed	4.32	6.07	7.77	8.58	9.74
Ca, calculated	5.21	7.24	9.07	9.89	11.73
P, calculated	4.36	6.18	7.72	8.58	10.13
Phase 3					
Ca, analyzed	6.41	11.43	10.88	13.11	16.21
P, analyzed	5.78	8.78	10.53	12.27	14.13
Ca, calculated	6.59	11.13	12.75	14.66	16.53
P, calculated	5.55	9.12	10.60	12.41	13.96
Phase 4					
Ca, analyzed	7.27	16.87	15.49	18.67	23.07
P, analyzed	6.97	13.42	14.22	16.08	18.17
Ca, calculated	8.24	15.53	16.97	19.33	21.42
P, calculated	7.37	16.87	15.49	18.67	23.07

APPENDIX TABLE 8. MEAN AVERAGE DAILY INTAKES OF ANALYZED AND CALCULATED CA AND P IN TRIAL 4 BY PHASE (G CA OR P$\cdot$PIG$^{-1}\cdot$DAY^{-1})

	Diet				
	1	2	3	4	5
Phase 1					
Ca, analyzed	2.50	2.79	3.48	3.60	4.80
P, analyzed	2.65	3.13	3.80	3.87	5.00
Ca, calculated	2.09	2.73	3.35	3.98	4.99
P, calculated	2.56	3.12	3.67	4.20	5.18
Phase 2					
Ca, analyzed	2.76	3.47	4.40	4.54	5.85
P, analyzed	3.76	4.53	5.52	5.82	6.98
Ca, calculated	2.62	3.54	4.17	5.09	6.00
P, calculated	3.25	4.12	4.62	5.49	6.29
Phase 3					
Ca, analyzed	5.39	8.56	6.77	10.49	12.37
P, analyzed	8.29	12.21	11.64	14.73	15.18
Ca, calculated	5.24	8.35	8.82	12.22	13.46
P, calculated	6.71	9.82	9.90	13.33	14.20
Phase 4					
Ca, analyzed	5.55	6.46	10.52	13.78	17.28
P, analyzed	7.23	9.80	14.36	17.38	20.37
Ca, calculated	4.68	7.37	10.62	14.56	18.04
P, calculated	6.43	8.85	12.17	15.95	19.28

APPENDIX TABLE 9. COMPOSITION OF STARTER DIET
FED FROM WEANING UNTIL THE AVERAGE WEIGHT OF ALL
PIGS REACHED 10 KG. INTERNATIONAL FEED NUMBER
APPEARS IN PARENTHESES AFTER EACH INGREDIENT

Ingredient	Percent of Diet
Ground Corn (4-02-935)	66.90
SBM, 49% (5-04-612)	29.50
Dicalcium phosphate (6-28-335)	1.40
Ground limestone (6-02-632)	1.00
Vitamin-Se premix	.50
TM premix	.05
Salt	.40
ASP-250	.25
	100.00

APPENDIX TABLE 10. AVERAGE CAP RATIO FOR
EACH DIET IN EACH TRIAL

	Diet				
Trial	1	2	3	4	5
	----------- Percentage of NRC -----------------				
1	75.1	83.7	95.7	117.6	127.4
2	87.2	92.4	112.9	122.4	133.6
3	80.0	88.9	98.3	116.4	135.5
4	70.1	78.2	93.7	102.4	119.7[a]

[a]Due to error, dolomitic limestone was used instead of calcitic limestone in trial 4, resulting in low calcium intakes and a skewed calcium:phosphorus ratio.

APPENDIX TABLE 11. DAY ON TEST CORRESPONDING
TO THE MIDPOINT OF EACH WEIGH-PERIOD (TIME
ON TEST) FOR EACH TRIAL

	Trial			
Period	1	2	3	4
1	6.5	7.0	7.0	8.5
2	20.5	21.0	17.0	21.5
3	34.5	35.0	23.0	36.5
4	48.0	49.0	33.0	54.0
5	62.0	63.0	47.5	66.5
6	74.0	77.0	62.5	80.0
7	80.0	91.0	76.0	86.0
8	89.5	105.0	90.0	93.5
9	103.5	119.0	105.5	108.0
10	118.0	133.0	115.0	122.0[a]
11	132.5		124.0	

[a]Final period date for biopsy pigs was 134.5.

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