Efficiency of Dietary Calcium Use for Skeletal Growth and Mineralization in Young Pigs Fed Diets with Various Phosphorus Concentrations

Nutrient efficiencies are generally greater for animals fed diets with marginal deficiencies. In a preliminary study, however, Ca efficiency was lower in pigs fed diets with a marginal Ca deficiency (J Bone Miner Re 20:S193). The study assessed recovery of skeletal growth in young pigs following a period of Ca-deficiency. The Ca efficiency may have been inadvertently limited by the P concentrations in the diet. This study is designed to assess the effects of 70, 90, 120% of NRC P requirements on Ca efficiency for 75% and 150% of Ca levels. The Ca efficiencies were estimated using BMC values obtained from DXA Scans. Pigs fed 70% P did not gain bone mass over 27 d trials, regardless of Ca level, and pigs fed 120% did not show significant differences in Ca efficiency. Pigs fed 90%, however, exhibited effects that support Ca efficiencies being greater for marginally deficient diets (75% Ca).

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COVER SHEET

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Introduction

Bone formation and maintenance of bone mass is dependent on the composition of feed consumed in mammalian species. Specifically, the formation and resorption of bone is intricately linked to calcium and phosphorus concentrations and ratios in a given diet. The two minerals form a calcium phosphate complex, known as a hydroxyapatite crystal, which is a component of bones and teeth (Cezar, 2007). Increasing the calcium and phosphorus ratios in swine diets result in increased bone strength when phosphorus is at or above the dietary requirement (Hall *et al.*, 1991). Furthermore, imbalances in the coupling of bone formation and resorption can lead to severe conditions, such as osteoporosis, osteomalacia, or osteopetrosis. These bone conditions currently affect both humans and animals.

If a nutrient is limited in the diet, it is expected to be utilized and retained in the body more readily. In other words, the efficiency of retention of a limited nutrient is anticipated to be greater than that of an excess nutrient. This concept of limited nutrient efficiency is quite evident in nature; for example, sheep originated in copper-deficient areas and thus easily reach a level of copper toxicity if they were exposed to feed or grazing fields with more than 20-25 ppm of copper (Huston & Greene, 1999). In this case, the efficiency of copper retention was exceptionally high. Similar deductions can be made about other nutrients, especially the efficiency of calcium because 99% of calcium absorbed resides in the bone, and therefore it is easy to measure (Cezar, 2007).

A recent investigation, however, reported the efficiency of calcium retention was lower when pigs were fed diets that were marginally deficient in calcium than a diet with excess calcium (Smiltneek *et al.*, 2005). As a part of the 10 week study, two groups of young pigs were fed diets with 70% of the NRC calcium requirement for the first four weeks, while two other groups of young pigs were fed diets with 150% of the NRC calcium requirement. The calcium efficiencies for the two deficient treatments were calculated to be 0.30 and 0.38 respectively, whereas the calcium-excess treatments had a calcium efficiency of 0.61 and 0.65 respectively (see figure 1). The efficiency of calcium was not as expected; the 70% groups had lower efficiency of Ca retention than the 150% groups. The calcium efficiencies for the last six weeks were as expected; however, the results mentioned above were not discussed in detail and it is not clear why the efficiency of calcium was significantly less during a time of depletion.

Due to the complex interactions of calcium and phosphorus, it is reasonable to theorize that the levels of phosphorus in the diet played a key role in this obscurity. We hypothesize that phosphorous at or above the NRC requirements will allow adequate absorption and retention of calcium in bone, whereas low phosphorous levels will not allow formation of hydroxyapatite crystals. The current study evaluated bone mass and calcium efficiencies in young pigs fed diets with either 75 or 150% of their calcium requirements (NRC, 1998), each formulated with three levels of dietary phosphorous, in order to determine the effects of phosphorous levels on calcium efficiency in a calcium-depleted diet.

Methods

A total of 42 (Duroc X LR X LW barrows) pigs were weaned at three weeks of age and fed standard UW SRTC diets for approximately two weeks. This time was allotted for the pigs to become accustomed to eating dry feed. Pigs were selected from a contemporary group and randomly assigned to one of six dietary treatments or an initial kill group.

On day 0, all pigs were scanned with a GE Lunar Prodigy Dual Energy X-ray Absorpitometry machine (DXA). Each pig was maintained under anesthesia using Halothane for about 30 minutes. During this time, the DXA machine took bone mineral content measurements of the entire body. The representative six pigs randomly assigned to the initial kill group were killed after their respective DXA scan. The left and right femurs were harvested from each of the six pigs and frozen. The remaining 36 pigs were allowed to recover before being housed in individual pens.

The pigs were then fed their assigned diet for 27 days (see table below for composition of dietary treatments) and scanned again by DXA at two and four weeks. They were allowed continuous access to feed and water throughout the trial, except the pigs were taken off feed 12 hours prior to DXA scans as a safety precaution. Pig weights and feed consumption were determined at weekly intervals. After day 27, all pigs were killed and the femurs were harvested and frozen.

Dietary Treatments (% reflects percentage of Ca and P requirements for 10 to 20 kg pigs, i.e., 0.70% Ca and 0.60% P):

Treatment	% Ca	% P
1	75	70
2	75	95
3	75	120
4	150	70
5	150	95
6	150	120

Results

At day 0, the weight of the initial kill pigs averaged 13.4 kg with a standard deviation (SD) of ± 2.0 kg. The remaining 36 pigs averaged 13.20 kg ± 0.42 kg. The growth of the pigs increased according to the diet they were assigned. At the end of 27 days, pigs fed treatments 1 and 4 exhibited the least weight gain in comparison to pigs fed treatments 2 and 5, which were relatively intermediate, and pigs fed treatments 3 and 6, which had the most weight gain (see figure 2). The following are raw weight data values in kilograms:

TRT #	Day 0	SD	Day 7	SD	Day 14	SD	Day 21	SD	Day 27	SD
1	12.66	1.30	15.03	1.30	18.70	2.13	24.13	3.02	28.57	2.98
2	12.96	1.14	15.60	1.85	20.30	1.54	26.90	1.46	32.13	2.63
3	13.09	1.05	16.23	1.37	21.60	1.20	27.63	1.62	34.13	1.50
4	13.08	1.35	15.23	1.60	18.07	2.05	22.13	2.88	25.00	3.74
5	13.58	1.32	16.73	1.71	20.30	1.41	25.87	2.06	31.83	1.49
6	13.81	1.29	16.93	1.40	21.60	2.42	27.40	3.08	33.83	3.57

In correlation with the weights are the bone mineral content (BMC) values of the pigs. The outcomes were similar comprehensively; pigs fed treatments 1 and 4 had the least BMC, pigs fed treatments 2 and 5 were intermediate, and pigs fed treatments 3 and 6 had the greatest BMC (see figures 3-6). The BMC values are as follows in grams:

TRT #	BMC (g) Day 0	SD	BMC (g) Day 14	SD	BMC (g) Day 27	SD
0	214	32	N/A	N/A	N/A	N/A
1	195	31	168	25	158	41
2	210	13	227	17	385	20
3	196	14	241	19	446	21
4	210	19	205	41	162	25
5	217	15	233	12	335	17
6	232	21	319	34	586	60

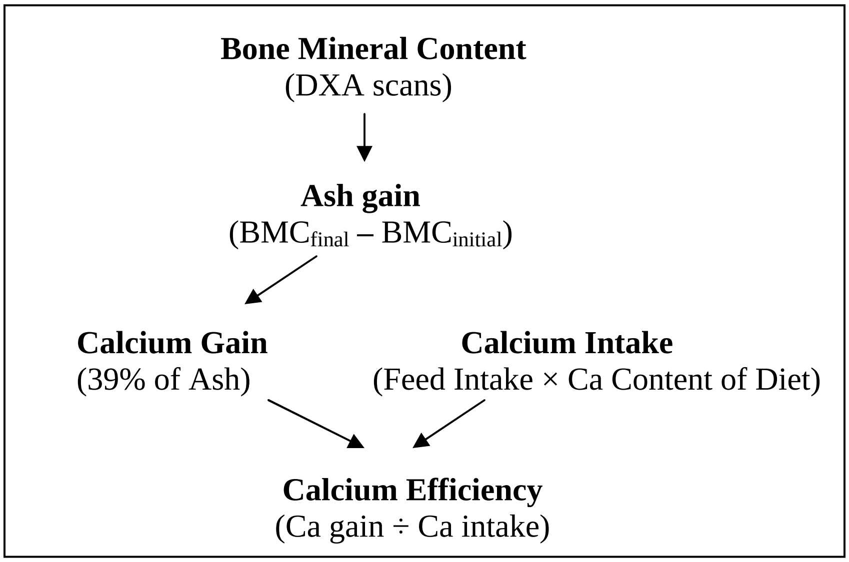
Discussion

Weight gain and bone mineral content are indicative of calcium deposition, although weight gain is less reliable than BMC because it includes growth of soft body tissue and fat. It is, therefore, more important to focus on bone mineral content values when determining calcium efficiencies. Relatively speaking, it is simple to measure the amount of calcium gained because 99% of calcium that is absorbed and stored in the body resides in the skeleton. Measuring the BMC through DXA scans over a time interval is an accurate method to estimate total body retention of calcium.

The DXA machine has a fan beam that moves at one cm wide scans perpendicular to the longitudinal axis of the pig. Numerous functions are performed simultaneously that allow calculations of different elements and minerals, thereby allowing scientists to quantify differences in soft tissue, fat, and bone. X-ray scans of the pig show brighter pixels for skeletal tissue and fainter pixels for soft tissue (Radiological Society of North America, 2007). The program allows one to analyze certain regions of interest or the entire body. For this study, the entire body was scanned to calculate BMC of each pig (see figure 7).

The accuracy of DXA scans has been questioned, but sufficient research exists to prove that it is in fact a reliable measure of BMC. The BMC for three selected scan modes (small animal, standard adult, thick adult) were compared with bone ash obtained via dissection (Schneider & Crenshaw, 2005). The values for each of these scan modes are relative duplicates of the actual bone ash obtained from dissection, with an R^2 value of 0.999 (see figure 8). Hence, the use of DXA eliminates the tedious and time-consuming methods of manual dissection of the entire skeleton.

To determine calcium efficiency from the BMC values obtained via DXA, ash gain and calcium gain are calculated. The following schematic aids in visualizing the mathematical processes involved in determining calcium efficiency:



An assumption that was made in determining calcium gain is that it is approximately 39% of the ash content (Crenshaw, 2001). Analysis of the calcium contents of the diets was carried out to assure the pigs were being fed proper amounts of calcium and phosphorus (see figure 9). The calcium content was 0.525% for treatments 1-2, 0.540% for treatment 3, and 1.05% for treatments 4-6. Treatment 3 had elevated calcium content to allow for increased phosphorus (120%) in the diet.

The calcium content of each diet is multiplied by the feed intake to obtain calcium intake over the 27 day trial period (see table 10). Pigs fed treatments 1-3 consumed 133, 155, and 166g of calcium over the 27 day trial respectively. Pigs fed treatments 4-6 consumed 263, 327, 311g of calcium over the 27 day trial respectively. In order to obtain the calcium efficiencies, calcium gain is divided by these calcium intake values. The calculated calcium efficiencies for the treatments are as follows (see figure 11):

TRT #	Ca Efficiency	SD
1	-0.095	0.069
2	0.406	0.049
3	0.502	0.040
4	-0.071	0.026
5	0.126	0.024
6	0.475	0.043

Pigs fed treatments 1 (75% Ca, 70% P) and 4 (150% Ca, 70% P) resulted in negative calcium efficiency, indicating that these pigs did not gain any bone mass over the 27 day trial period, regardless of the calcium level. Note that these two treatments supplied only 70% of the phosphorus requirement. Pigs fed treatments 2 and 5 showed positive variable results for calcium efficiency; treatment 2 (75% Ca, 95% P) had a greater efficiency than treatment 5 (150% Ca, 95%). The results indicate that pigs fed diets with 95% of the phosphorus requirement have a greater efficiency of calcium retention if calcium was limited (75%), rather than if calcium was in excess (150%). Lastly, pigs fed treatments 3 (75% Ca, 120% P) and 6 (150% Ca, 120% P) had positive, but similar results. Therefore, there was no significant difference detected in calcium efficiency for pigs fed diets with excess phosphorus (120%).

From these results, one can deduce that limiting dietary phosphorus directly reduces the efficiency of calcium in *both* calcium-deficient and calcium-excess diets. In fact, pigs fed diets with limited phosphorus exhibited signs of lameness and were hesitant to stand. Excess dietary phosphorus had no significant effects on calcium efficiency. However, dietary phosphorus concentrations near the requirements improved efficiency of calcium retention in young pigs fed diets with marginal deficiencies of calcium. As a result, the hypothesis can be accepted. The conclusions made in the preliminary study are questionable due to phosphorus limiting the retention of calcium. Further studies are underway to confirm the data from this investigation and to expand our knowledge about nutrient efficiencies. These studies include bone mineral density calculations and breaking strengths of the bones harvested from the pigs.

An understanding of nutrient efficiencies is vital for managing economics, health problems, and environmental pollution. Economically, dietary calcium supplements are supplied via limestone, a relatively cheap ingredient source. The consideration of calcium availability, therefore, is modest (Crenshaw, 2001). Farmers will supplement calcium freely without paying attention to the efficiency of the mineral. Even more problematic is the phosphorus expense, which is of no benefit to the animal if it is deficient or in excess. Animal owners can improve their cost-benefit ratios by having a better understanding of nutrient efficiencies.

Health problems, such as osteoporosis, can also be prevented by taking adequate levels of calcium, in combination with vitamin D and phosphorus. However, an individual that does not have background knowledge regarding nutrient efficiencies and nutrient requirements cannot prevent such bone diseases. Also, knowledge about the combination of nutrients and nutrient availability is vital in understanding diet formulations.

A large concern amongst our community currently is the environmental pollution caused by excess phosphorus. Phosphorus that is not utilized by animals is excreted in urine and feces, and a small percentage eventually ends up in the lakes via erosion. The phosphorus that erodes into surface waters contributes to pollution since phosphorus is the rate limiting nutrient for aquatic plant growth. Many lakes, including Lake Mendota and Lake Monona, have excessive algae growth due to high levels of phosphorus (Bennett *et. al.*, 1999). This water pollution can be controlled by reducing inefficiencies of phosphorus retention by optimum formulation of diets. The importance of nutrient efficiencies is continuously being explored and bringing about new solutions.

References

Bennett E.M., Reed-Andersen T., Houser J.N., Gabriel J.R., Carpenter S.R. (1999). "A Phosphorus Budget for the Lake Mendota Watershed." *Ecosystems* **2** (1): 69-75.

Cezar, G. (16 Feb. 2007). "Bone Physiology." School of Veterinary Medicine, University of Wisconsin-Madison.

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

Hall D.D., Cromwell G.L., Stahly T.S. (1991). "Effects of dietary calcium, phosphorus, calcium: phosphorus ratio and vitamin K on performance, bone strength and blood clotting status of pigs." *Journal of Animal Science* **69** (2): 646-655.

Huston J.E., Greene L.W. (1999). "Copper toxicosis in sheep: a review." *Sheep and Goat Research Journal* **15** (3).

National Research Council (1998). Nutrient Requirements of Swine: 10th Revised Edition. National Academy Press, Washington, D.C., pp. 107-109.

Radiological Society of North America (2007). Bone Density Scan. Online. 8 May 2007. <http://www.radiologyinfo.org/en/info.cfm?pg=dexa&bhcp=1>

Schneider D.K., Crenshaw T.D. (2005). "GE Lunar Prodigy DXA accurately predicts skeleton ash but not fat and lean composition in growing (1 to 60 kg) pigs." *Journal of Bone and Mineral Research* **20**:S332.

Smiltneek A.J., Crenshaw T.D. (2005). "Recovery of skeletal growth and mineralization from dietary calcium deficiency in young pigs." *Journal of Bone and Mineral Research* **20**: S193.

Figures:

Figure 1: Smiltneek's Experiment: Calcium efficiency is less when calcium is marginal in diet.

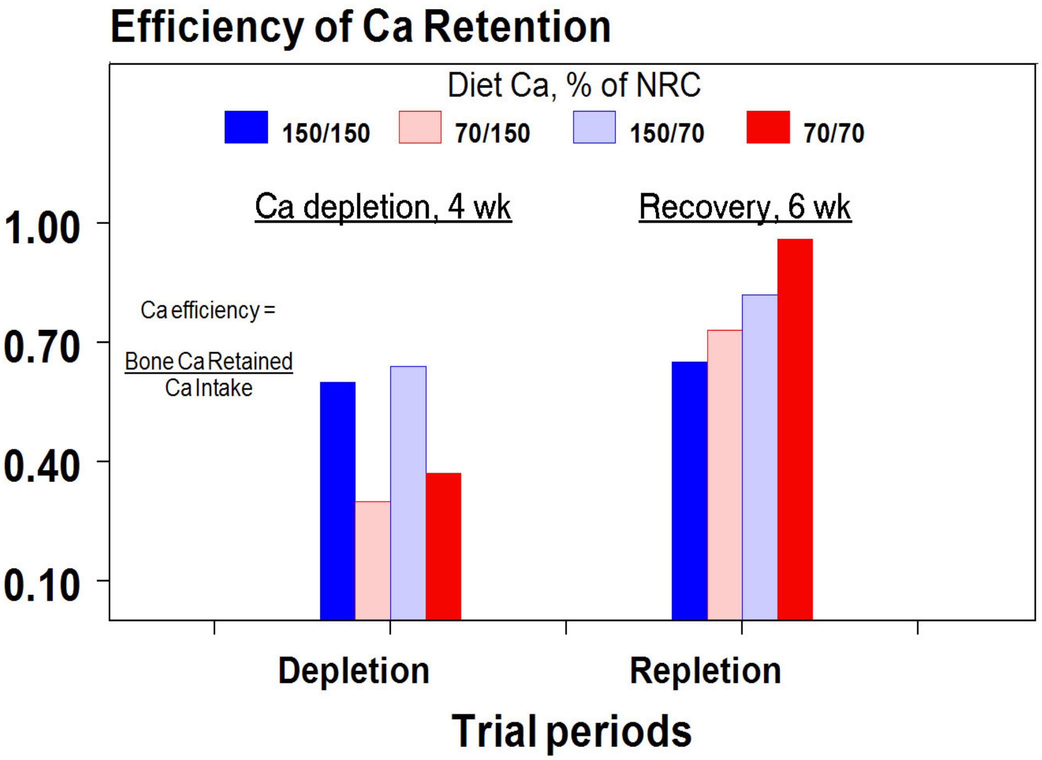


Figure 2: Weight gain per treatment over 27 day trial.

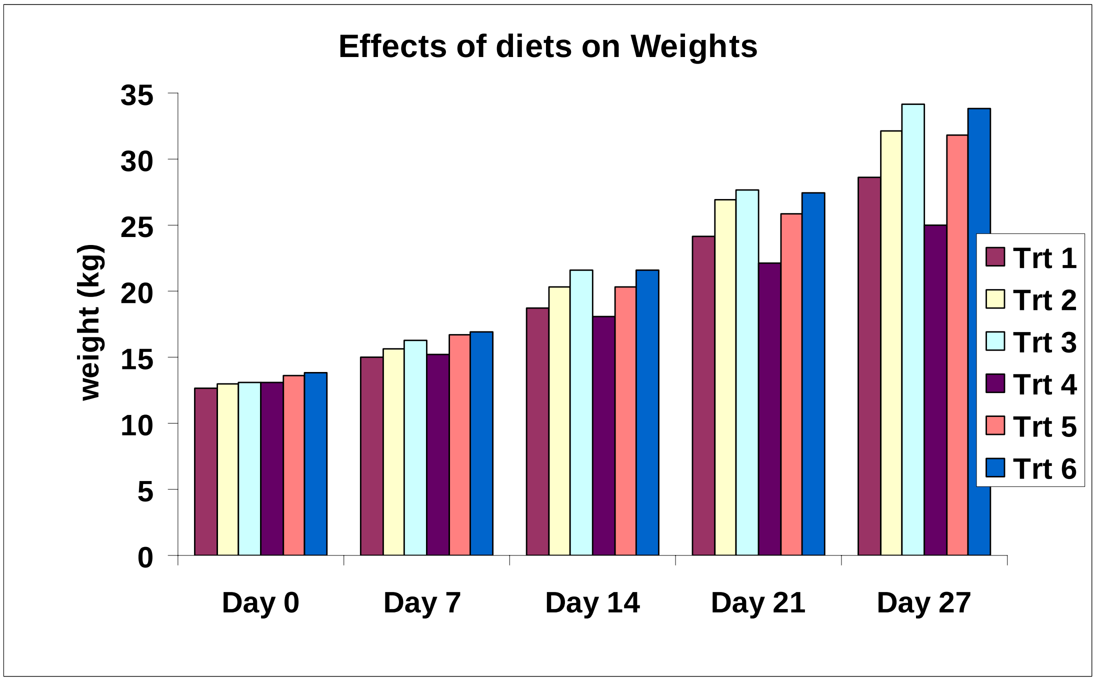


Figure 3: General trend in BMC per treatment over 27 day trial.

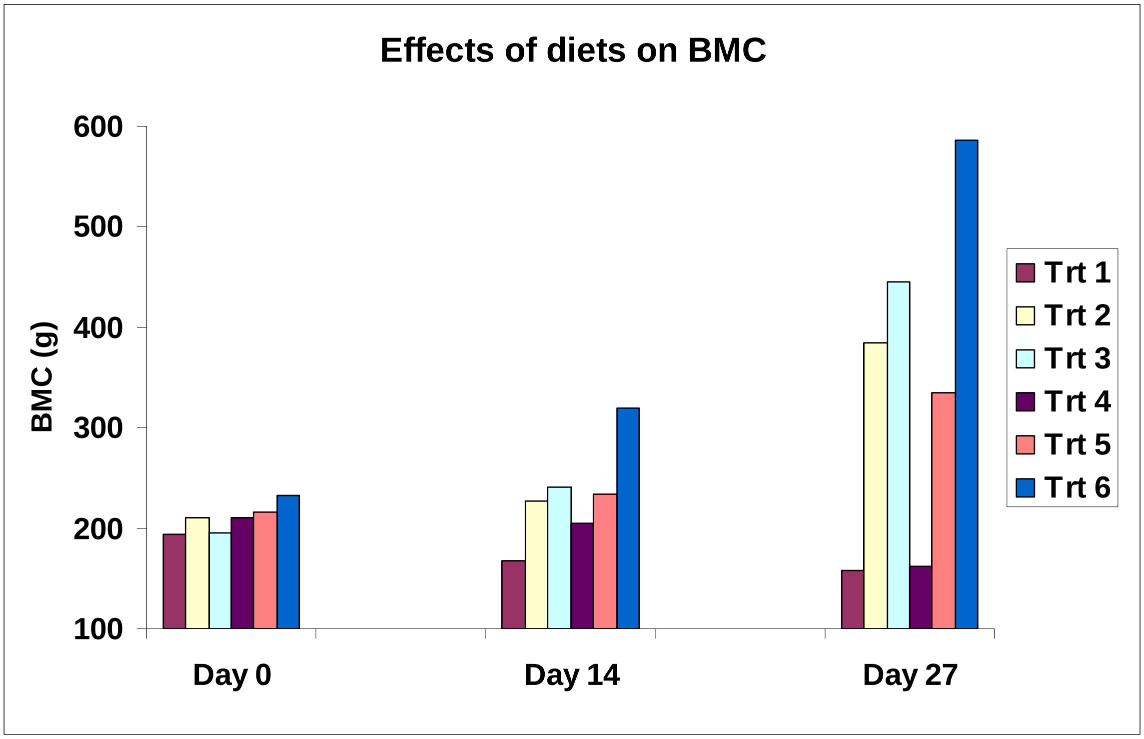


Figure 4: Effects of diets on BMC (Day 0, with error bars).

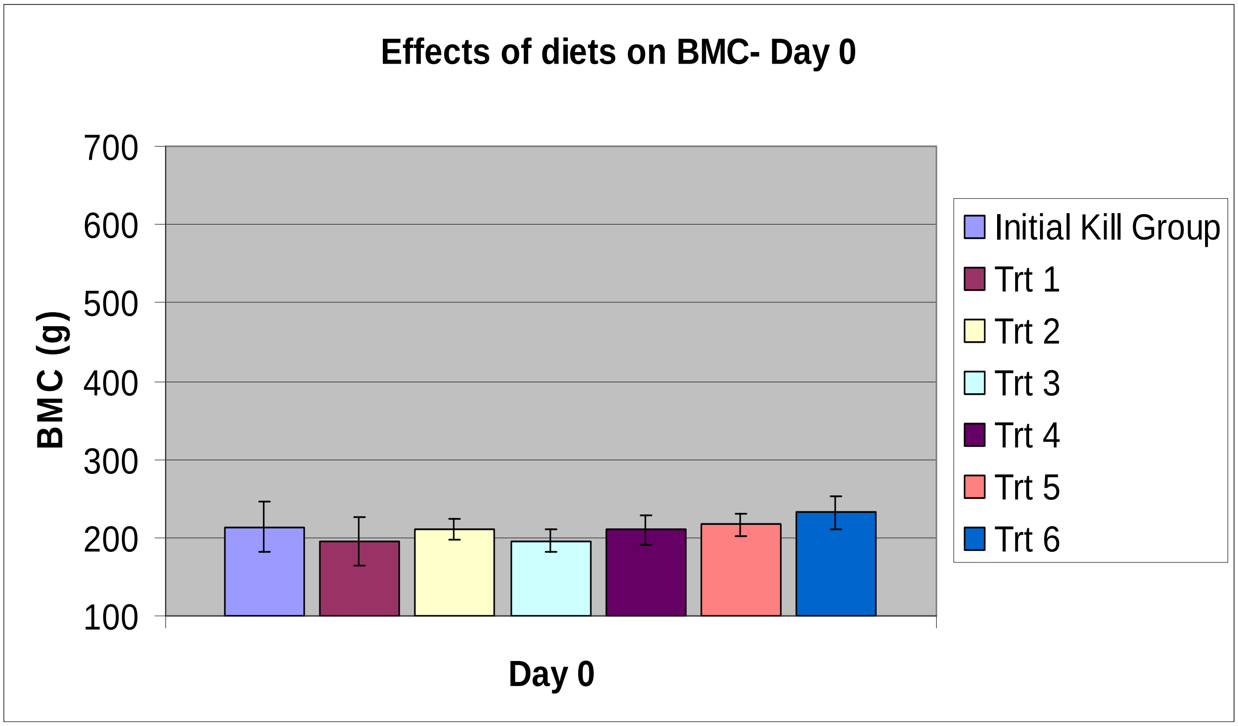


Figure 5: Effects of diets on BMC (Day 14, with error bars).

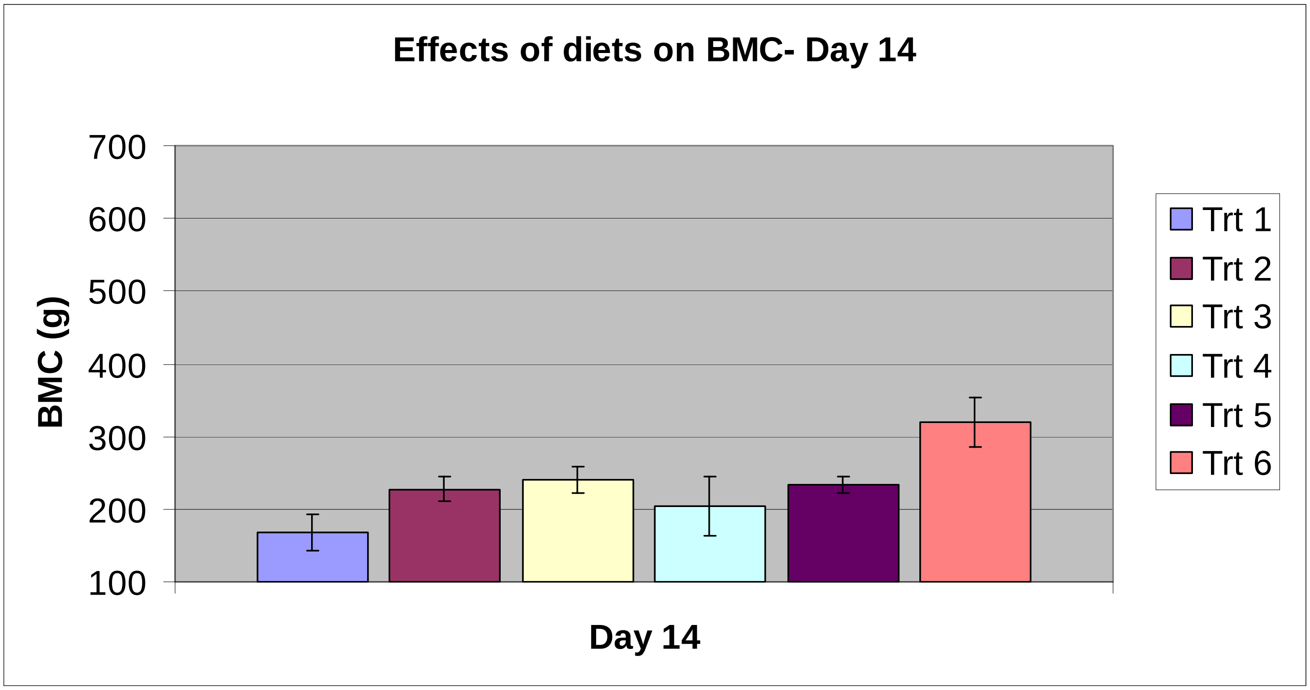


Figure 6: Effects of diets on BMC (Day 21, with error bars).

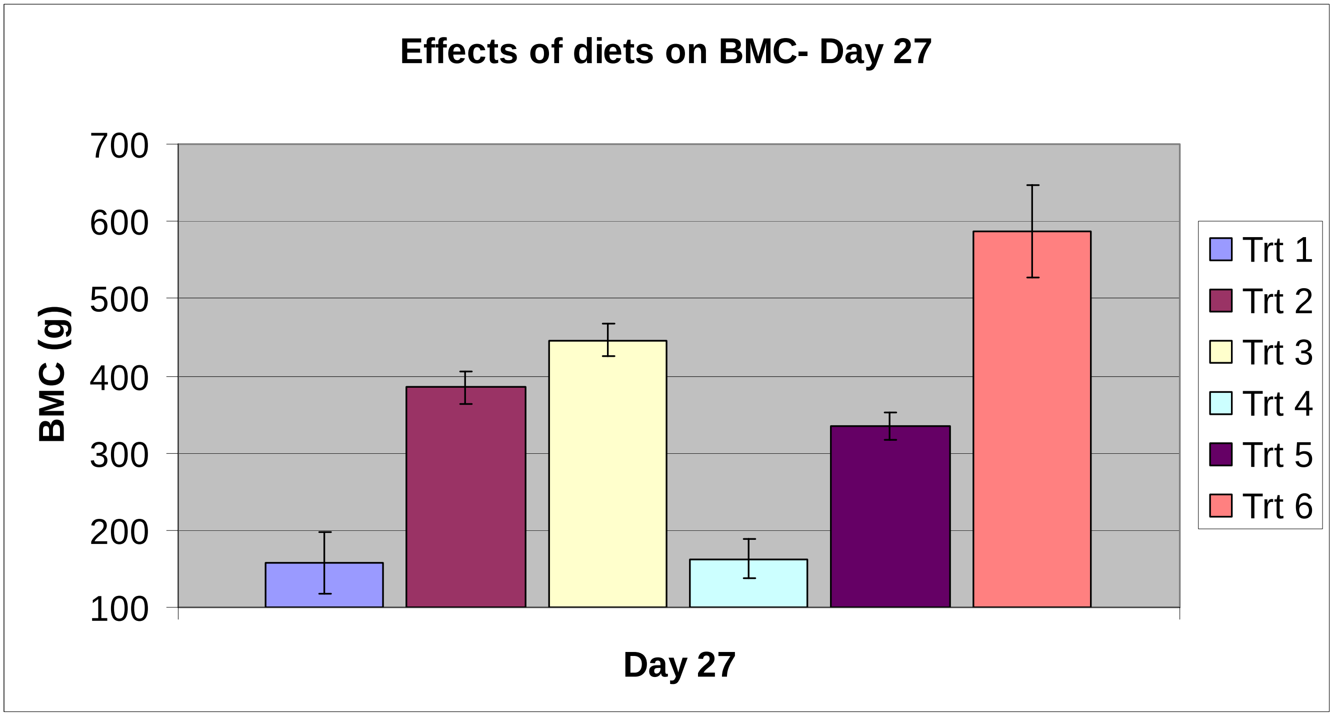


Figure 7: Image of typical DXA Scan.

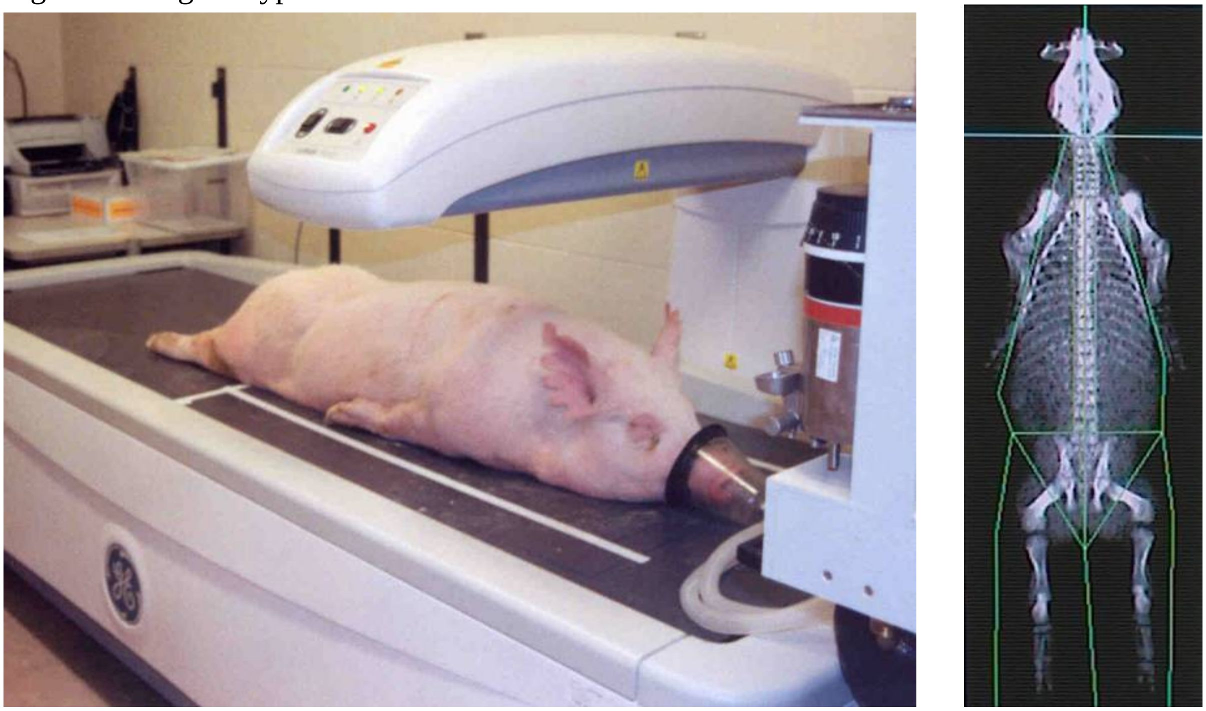


Figure 8: Accuracy of DXA Scan.

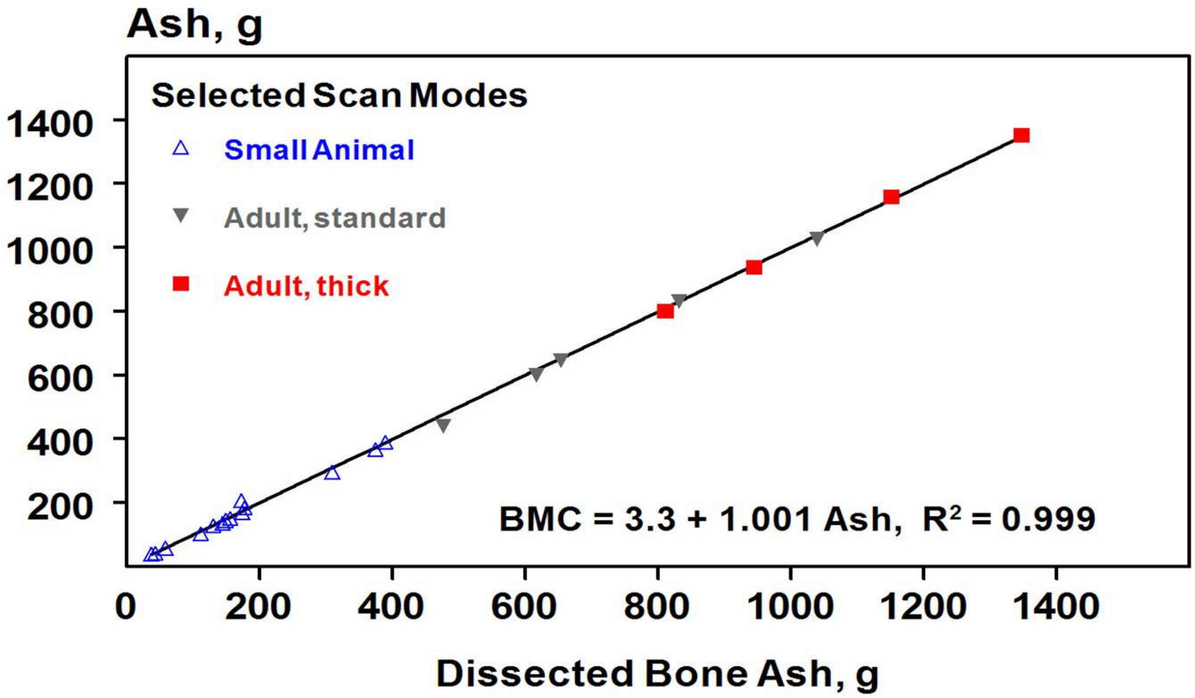


Figure 9: Calculated Analysis of the Diets

Ca Requirement	Diet 1 75% Ca	Diet 2 75% Ca	Diet 3 75% Ca	Diet 4 150% Ca	Diet 5 150% Ca	Diet 6 150% Ca
Lys %	1.20	1.20	1.20	1.20	1.20	1.20
Ca %	0.525	0.525	0.540	1.05	1.05	1.05
P %	0.42	0.57	0.72	0.42	0.57	0.72
Avail P	0.085	0.238	0.39	0.085	0.237	0.389
Ca:tP	1.25	0.92	0.75	2.50	1.84	1.46
Ca:aP	6.15	2.21	1.39	12.36	4.43	2.70

**diet 3 increased Ca to allow 0.72% P
**Avail P required = 0.32%

Figure 10: Calculating Ca intake from feed intake values (averages per treatment).

TRT #	Average Daily Feed Intake (g)	Feed Intake over 27 days (g)	Ca Content of diet (%)	Ca Intake (g)
1	936	25,275	0.525	133
2	1,091	29,458	0.525	155
3	1,136	30,675	0.54	166
4	928	25,067	1.05	263
5	1,153	31,125	1.05	327
6	1,099	29,663	1.05	311

Figure 11: Ca Efficiencies according to the treatment groups after 27 days.