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Bioavailability of calcium in alfalfa hay for gestating swine

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BIOAVAILABILITY OF CALCIUM IN ALFALFA HAY FOR GESTATING SWINE

by

Gerald L. Walker

A DISSERTATION

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BIOAVAILABILITY OF CALCIUM IN ALFALFA HAY FOR GESTATING SWINE

Gerald L. Walker, Ph.D.

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Advisor: D. Murray Danielson

The research objectives were to determine the bioavailability of Ca in alfalfa hay (AH), the response relationship of several bones to Ca source (CaS) and the effect of AH and dietary Ca level (DCL) on reproductive performance and fetal composition. Two CaS (AH and $CaCO_3$), each formulated into four DCL (50, 75, 100, 125% of NRC, 1988) and two gestation stages (GS; 55 and 105 d) were used in a 2 x 4 x 2 factorial arrangement. Response criteria were plasma [alkaline phosphatase (AKP), Ca and P]; bone [shear stress (SS), ash (BA), density (BD) and ash density (AD) from the metacarpal (MC), metatarsal (MT), rib (RB), thoracic vertebrae (TV) and coccygeal vertebrae (CC)]; gilt performance; litter size (LS); litter weight (LW) and fetal composition (DM, N, ash, Ca and P). Shear stress, BA, BD, AD and plasma variables were similar between CaS. Gilts fed 50% of NRC for Ca showed a linear increase in plasma AKP as gestation progressed from 0-105 d. There was little difference in GS for the variables measured. As DCL increased, SS increased in the MC, MT, TV and CC. Most bones collected were correlated in their SS and BA response. Litter size and LW tended to increase in the gilts fed AH as compared to those fed $CaCO_3$. A decline in LS occurred with an increase in DCL from 100 to 125%. Gilts fed at 75% of NRC for Ca were lower in BD and AD in the MC, MT and RB and lower in RB ash, gilt gain, carcass weight,

final weight and BF. Weight gains were higher in the AH fed gilts. Fetal composition did not vary with CaS. The CC bone appears to be most responsive to DCL. These results show alfalfa hay to be an excellent source of Ca when fed to gestating gilts.

TITLE

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BY

GERALD LYNN WALKER

APPROVED	DATE
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LITERATURE REVIEW

I. Introduction

Alfalfa (Medicago sativa L.) originated from the Near East and Central Asia, and is generally assumed to have come from Iran. The high producing crop is found growing from China to Spain. Alfalfa was brought to North and South America by the Portuguese and to Mexico and Peru by the Spaniards in the 16th century. By 1836, it had made its way to the United States and was grown in several areas of the Southwest and quickly became a popular crop (Bolton et al., 1972). Since that time, there have been vast quantities of research conducted on the crop improving its quality and developing different varieties. Today its popularity has progressed to the point of being included in the diet of most farm livestock. Alfalfa is highly productive and palatable. It is an excellent source of nutrients.

For many years, prior to the time that vitamin-trace mineral premixes were available, producers included alfalfa in their swine diets to ensure the presence of certain vitamins and trace minerals. This practice is still common today. Many benefits of feeding alfalfa to swine have been realized and are evident in the literature. Cunha et al. (1944) showed that factors supplied by alfalfa improved reproductive performance of sows fed in drylot by feeding diets with 10% alfalfa meal. Teague (1955) fed gestation diets containing 18% sun-cured alfalfa hay to bred gilts. He found an improvement in litter size from 8.64 to 9.83 over corn-soybean meal diets. Fairbanks et al. (1945) fed diets with 12% alfalfa meal and reported similar results; number of pigs per litter increased from 6.2 to 10. Seerley and Wahlstrom (1963) showed an

improvement in pig survival rate when 10% alfalfa meal was fed. In 1975, Danielson and Noonan fed diets containing up to 96.75% sun-cured alfalfa meal through three parities with acceptable reproductive performance. Reese and Danielson (1988) suggested that alfalfa (sun-cured or meal) may be fed up to 10% of the diet to maintain maximum energy utilization, while levels up to 66% may be included without decreasing reproductive performance. They reported that producers who grind their own feed usually limit the quantity included in the diet to 20-25% due to labor cost of handling. Danielson (1982) suggested that growing-finishing swine diets may contain 20% alfalfa hay without severely affecting performance.

Since alfalfa hay is used substantially in swine diets, attention to its nutritional quality is warranted. Alfalfa hay is generally found to be high in calcium (Ca) content (ranging 1-3% and averaging 1.4%; Bickoff et al., 1972). When included in swine gestation diets at elevated levels (10-50%), alfalfa becomes a major contributor of Ca to the diet. In order to develop a satisfactory calcium:phosphorus (Ca:P) ratio expensive P supplementation is required. However, a portion of alfalfa Ca may not be available for absorption and utilization by swine. If not, then the quantity of P supplementation would need to be adjusted for optimum metabolism and for economical diets. The basic objective of this study was to determine the bioavailability of Ca in alfalfa when fed to gestating gilts during two stages of gestation.

II. The Importance of Calcium to the Animal

Calcium, one of the most important minerals in the body, is essential for skeletal system rigidity where 99% of the total body Ca is found in the bones and teeth (Church and Pond, 1982). Together with

phosphorus (P) it makes up bone in the form of hydroxyapatite crystals in a 2:1 ratio. The remaining 1% is distributed throughout the tissue, blood and other organs. Along with its cellular receptor protein, calmodulin, it regulates intracellular Ca levels, enzyme activation, and control of the activity of cellular filamentous organelles. Many hormones require the presence of Ca for activation (Hadley, 1984). Calcium is found in almost every cell in the body, and is important for the integrity of cell membranes and cellular communication. Serum levels range between 9-11 mg/dl with about 50% ionized and 50% bound to albumin (Sanford, 1982). The free Ca (ionized form) found in body fluids is utilized for blood coagulation, normal cardic and skeletal muscle contraction and nerve function.

A. Calcium regulation and absorption.

Plasma homeostasis is controlled within very narrow limits by the endocrine system through three main messengers. These are the parathyroid hormone (PTH), 1,25-dihydroxycholecalciferol (1,25-OH_2D_3) and calcitonin. When the levels in the plasma decline sensor mechanisms stimulate the secretion of PTH into the blood stream. Parathyroid hormone may then act in different ways: one acts directly on bone tissue to cause resorption of Ca from the bone moving it into the blood. Another is to increase Ca absorption by activating 1-α-hydroxylase (1α-OH'ase) enzyme in the kidney to energize the vitamin D metabolite and calcium binding protein (CaBP; Wassermann and Taylor, 1972). Since the discovery of vitamin D in animal fats in 1920 and its naming in 1925 by McCollum and his associates, a large number of studies have shown that the vitamin is involved in and required for Ca absorption (Scott et al., 1971).

A series of studies by Wassermann (1962), Wassermann and Taylor (1966), Schachter et al. (1961), Taylor and Wassermann (1967) and Wassermann and Taylor (1972), resulted in a general consensus that Ca absorption occurs by two methods: (1) by simple diffusion it passes freely from high concentrations down a concentration gradient through the intestinal wall, and (2) by an active transport mechanism requiring a carrier mechanism (CaBP) to overcome the electronegative gradient. The rate of Ca absorption was found to be directly proportional to the CaBP present in the mucosal tissue.

Ultraviolet light from the sun acts on 7-dehydrocholesterol present in the skin to produce vitamin D_3 which is transported by a protein carrier to the liver. In the liver it is converted to 25-hydroxycholecalciferol (25-OH-D_3). The PTH activated 1-α-OH'ase enzyme converts 25-OH-D_3 to 1,25-$(OH)_2D_3$ (Haddad et al., 1983). This vitamin D metabolite acts on the nuclei of the epithelial cell of the small intestine to initiate the synthesis of mRNA, which by translation dictates information at the site of the endoplasmic reticulum for synthesis of CaBP (Sanford, 1982). Calcium binding protein is then secreted into the lumen of the small intestine and is responsible along with ATP'ase and alkaline phosphatase for transporting Ca into the cell and subsequently into the bloodstream.

The efficiency of Ca absorption is affected by the intraluminal presence of dietary components, by the Ca and vitamin D status of the body, and by the physiological state such as growth, age, pregnancy and lactation. Most of the Ca in feed ingredients is in the form of complexes with other dietary constituents. These complexes must be broken down and

the Ca released in a soluble and probably ionized form before it can be absorbed (Schacter et al., 1960). The gastric acid in the stomach is thought to increase solubility of these Ca complexes (Mahoney et al., 1975). Most of the digestive enzymes which release Ca from dietary components are pH dependent. The approximate pH of the intestinal contents postprandial is 6.0 (3.5-6.7) in the proximal jejunum and 7.6 beyond the mid-small intestine (Fordtran and Locklear, 1966). Calcium tends to precipitate from solution when the pH is greater than 6.1 (Albright and Rerfenstein, 1948), so that dietary Ca is in a more absorbable form in the duodenum and the proximal jejunum. Calcium binding protein is found mainly in the duodenum and relatively small amounts in the proximal jejunum. Distal to this point little is found (Gleason et al., 1979). The combination of pH and CaBP in the duodenum and upper jejunum may explain why Ca absorption occurs in this segment at greatest efficiency. Another factor influencing absorption is residence time in this segment. This may change depending on dietary fiber content.

Bile salts have been shown to increase the <u>in vitro</u> solubility of Ca salts and the absorption of Ca (Webling and Holdsworth, 1966; Kies, 1985). Bile salts may even be necessary for solubilization of Ca.

The overall Ca absorption is an exponential function of intake, and is the sum of the saturable (hyperbolic) and nonsaturable (linear) function. Calcium absorption is a complex process involving many factors. The factors with the most control occur in the diet. Research has provided information regarding Ca bioavailability from several supplements and feeds, but there is more to investigate.

B. Calcitonin.

Another hormone involved in Ca homeostasis is calcitonin, which is secreted by the C-cells or parafollicular cells of the thyroid gland. Secretion is stimulated by elevated Ca concentration in the plasma (Ganong, 1983). The main function of the hormone is to lower plasma Ca levels to a normal range by increasing clearance in the urine and by decreasing bone resorption.

III. Techniques for Measuring Bioavailability

When a feed ingredient is analyzed for mineral composition the total quantity of the element is determined. However, this does not indicate the portion actually available to the animal for utilization. Bioavailability of a nutrient is that proportion of the dietary mineral which may be absorbed into the body to satisfy the net requirement (Whittemore, 1972). There are several methods that have been used to estimate the bioavailability of feed ingredients. These include: (1) in vitro analysis, (2) blood variables, (3) balance trials, (4) radiotracer methods, (5) slaughter techniques, and (6) target tissue responses such as bone characteristics. All techniques will be discussed in terms of Ca bioavailability.

1. In vitro analysis. Bioavailability estimation by in vitro techniques are the least expensive, but the results may not reflect the phenomena in the biological system. However, this method is useful for preliminary studies for estimations of dosage, etc. The solubility of Ca is estimated by determining the quantity which goes into solution when added to a solvent. Although it can be assumed that Ca must be soluble in the gut milieu in order to be absorbed other constituents present may

effect absorption, such as pH and dietary components e.g. amino acids, sugar and fiber (Greger, 1988).

2. Blood variables. Blood variables Ca, P and alkaline phosphatases (AKP) are normally used as indices of dietary effects on Ca status of the body. However, the endocrine system is quite specific in maintaining plasma and serum levels of Ca within a narrow range so these indicators usually will not reflect Ca intake, unless the animal is subjected to extremely deficient diets (Greger, 1988). Grandhi and Strain (1983) fed Ca-P levels at 100 and 150% of NRC recommendation during gestation and found no effect on serum Ca levels (10.8 vs 10.6 mg/dl). In 1981, Nimmo et al. reported a small response of plasma Ca to dietary Ca level when diets containing two widely different Ca levels were fed to gilts during growing-finishing, gestation and lactation.

3. Balance trials. Balance trials measure the total intake and excretion of Ca, which is a classic method of monitoring nutrient availability. In this case, Ca absorption is assumed to be Ca intake in the diet minus the Ca found in the feces and urine. The problem with this method is that it assumes that excreted Ca losses are unabsorbed dietary Ca, which is false since appreciable quantities of endogenous Ca from the body are lost via the intestinal route through the biliary tract, through sloughing of intestinal cells and from losses through re-entry from the intestinal wall. Bile salts have been estimated to contribute 100 g of Ca/d to the digesta contents (Kies, 1985). The balance method may underestimate Ca absorption, because it estimates apparent not true absorption.

4. Radiotracer methods. Radiotracer methods involve some type of radioisotope used to separate the endogenous Ca from the Ca in the diet. There are several variations of this technique, but basically all follow the same idea. Bioavailability is determined by using radionuclide recoveries from paired animals. One animal of the pair is dosed orally and the other is injected intramuscularly with an identical quantity of either ^{45}Ca or ^{47}Ca. Recoveries are measured in the whole body or in a responsive tissue such as bone. Usually this procedure is used with small animals such as rats, mice or rabbits; however, it is not uncommon in large animals. The radionuclide quantity is determined, then compared to dosage and bioavailability which is then determined by the following equation:

% bioavailability = (% dose in sample from orally dosed animal x 100)/% dose in sample from injected animal (Whittemore, 1972).

5. Slaughter technique. The slaughter technique is a method which also uses paired animals. Half of the animals are sacrificed at the onset of the study and the other half are fed treatment diets for a trial period and then sacrificed. Whole body Ca is determined in both groups of animals, and percent absorption is calculated by difference. Usually there is one group of animals fed a standard source and one group fed a test source; by comparing the two responses bioavailability may be estimated (Armstrong, 1957).

6. Target tissue response. Bone ash and breaking strength have been used extensively in mineral studies because they are reasonably good indicators of degree of calcification and the general well being of the animal as a result of dietary Ca status (Hayes et al., 1979; Crenshaw et