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The Nutritional Effects of Feeding Zeolite A or Clinoptilolite to Growing Swine

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THE NUTRITIONAL EFFECTS OF FEEDING ZEOLITE A AND CLINOPTILOLITE TO GROWING SWINE

By

Gerald Carlyle Shurson

A THESIS

Submitted to Michigan State University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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ABSTRACT

THE NUTRITIONAL EFFECTS OF FEEDING ZEOLITE A AND CLINOPTILOLITE TO GROWING SWINE

By

Gerald Carlyle Shurson

Incorporation of zeolites into diets for growing swine has resulted in variable responses in growth performance of swine. Detailed evaluation of the efficacy of feeding zeolites is important in assessing the usefulness of zeolites in practical swine feeding systems. Performance trials, nutrient balance trials, blood component determinations, and rate of passage trials were conducted to evaluate the nutritional effects of zeolite supplementation in practical swine diets. Zeolite A appears to be ineffective in improving growth performance due to its depressing effect on energy, nitrogen, and mineral absorption. Urinary p-cresol was not affected by zeolite A diets and passage rate was increased. Clinoptilolite appears to be less detrimental than zeolite A on energy and nitrogen absorption while improving sodium, iron, and zinc absorption. Clinoptilolite is effective in reducing plasma ammonia and urinary p-cresol production. Rate of passage of clinoptilolite supplemented diets was increased.

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INTRODUCTION

The use of feed additives in swine diets is a widely accepted practice in modern swine production and has been an effective means of improving animal performance. However, feed additives vary in their ability to promote growth as well as in their cost of improving growth.

Zeolites may be a less expensive and more effective means of improving growth performance in swine. If zeolites are effective in improving growth performance, they probably mimic antibiotic action by preventing ammonia absorption in the gastrointestinal tract (Francois and Michel, 1968; Visek, 1978; Pond <u>et al</u>., 1981). Positive growth responses due to zeolite supplementation in swine diets have been observed (Kondo and Wagai, 1968; Cool and Willard, 1982; Pond, 1980) along with negative or no growth response (England, 1975; Milne and Froseth, 1981; Thielemans and Bodart, 1982). Effectiveness of zeolites in promoting growth is probably due to the species of zeolite as well as the level of zeolite added to the diet. This study was designed to examine the nutritional effects of adding various levels of a natural zeolite (clinoptilolite) or a synthetic zeolite (zeolite A) to practical swine diets to determine their effectiveness in improving growth performance.

REVIEW OF LITERATURE

I. MOLECULAR CHARACTERIZATION OF ZEOLITE

1. Introduction to Zeolites

Zeolites are crystalline, hydrated aluminosilicate molecules composed of alkali and alkaline earth cations along with small amounts of various other elements. These zeolite molecules are arranged in infinite, three-dimensional structures which create interconnecting channels and voids that can trap certain molecules analogous to a sieve. Hence, zeolites have been called "molecular sieves". Zeolite molecules can also bind and selectively release large quantities of specific molecules by adsorption or ion exchange (Business Week, 1981). Water can be gained or lost reversibly and cations can be exchanged with no major structural change to the molecule (Mumpton and Fishman, 1977). Furthermore, zeolites can also act as catalysts in various chemical reactions (Business Week, 1981).

2. Natural and Synthetic Zeolites

Zeolites are classified as being of either natural or synthetic origin. Natural zeolites were formed over the earth's surface and sea bottom many years ago and are typically found in cavities of basaltic, volcanic, and very fine-grained sedimentary rocks (Kirk-Othmer, 1981). Most of the natural zeolites were formed by natural alteration of volcanic ash present in alkaline environments (Kirk-Othmer, 1981). In most natural zeolite deposits, a high grade zeolite ore is mined.

There are approximately 40 known natural zeolite minerals, and of those, only chabazite, erionite, mordenite, and clinoptilolite are present in large quantities of high purity to be conducive for commercial use (Kirk-Othmer, 1981).

Synthetic zeolites are manufactured commercially under hydrothermal conditions involving zeolite crystallization from aqueous solutions containing various types of reactants (Kirk-Othmer, 1981). See Figure l for a schematic representation of the formation of zeolite crystal nuclei in a hydrous gel. Synthetic zeolites are more purified (and many synthetics have greater ion exchange capacity) than natural zeolites. Common synthetic zeolites that are currently used in commercial applications include zeolites A, X, Y, and Zeolon H (a synthetic form of mordenite). Many of the commercially synthesized zeolites have no natural zeolite counterpart (Kirk-Othmer, 1981). A list of common natural and synthetic zeolites is shown in Table 1.

3. Crystalline Structure and Mechanism of Adsorption and Ion Exchange In order to appreciate the unique physical and chemical properties of zeolites, the arrangement of the crystalline structure along with the mechanism of adsorption and ion exchange should be understood.

The three-dimensional structure of the zeolite molecule can be considered by examining three structural aspects. These aspects include: (A) the basic arrangement of the framework of the molecule; (B) the location and species of the exchangeable metal cations; (C) the dimensions and characteristics of the internal pores and channels of the molecule.

A. Basic Arrangement of Structural Framework

The fundamental arrangement of the individual units that comprise the structural framework of the zeolite molecule are four-membered rings



CHARACTERISTICS OF COMMON NATURAL AND SYNTHETIC ZEOLITES TABLE 1.

Zeolite Species	Composition ^a , ^b	Si/Al ratio ^a	Ion Exchange ^a Capacity (meq g)	Aperture ^b Size (Å)	Void ^{b,C} Volume (cm ³ /g)
Natural Zeolites Chabazite	ca ₂ [(Al0 ₂) ₄ (Si0 ₂) ₈].13H ₂ 0	2.00	3.81-5.00	3.0x4.3	.29
Clinoptilolite	(Na ₄ ,K ₄)[(A10 ₂) ₈ (Si0 ₂) ₄₀]·24H ₂ 0	4.14	2.54-2.60	3.5	.18
Erionite	(Ca.Mg.Na ₂ .K ₂) _{4.5} [(AlO ₂) ₉ (SiO ₂) ₂₇]·27H ₂ O	3.00	3.12-3.80	3.6x5.2	.21
Mordenite	Na ₈ [(A10 ₂) ₈ (Si0 ₂) ₄₀]·24H ₂ 0	5.00	2.29-2.60	4.0	.15
Phillipsite	(K,Na) ₅ [(A10 ₂) ₅ (SiO ₂) ₁₁].10H ₂ 0	2.20	3.87	4.0	.22
Synthetic Zeolites Zeolite A	Na [(Alt.) (Sin.)].27H.D		5 48-7 00	4 2	30
Zeolite X	Na ₈₆ [(A10 ₂) ₈₆ (Si0 ₂) ₁₀₆]·264H ₂ 0	1.23	4.73-6.40	8.0	.36
Zeolite Y	Na ₅₆ [(A10 ₂) ₅₆ (Si0 ₂) ₁₃₆].250H ₂ 0	2.43	5.00	8.0	.35
Synthetic mordenite	Na ₈ [(A10 ₂) ₈ (Si0 ₂) ₄₀]·24H ₂ 0	5.00	2.29-2.60	6.6	.14
^a Taken from Kirk-Otl	hmer, 1981 or Mumpton and Fishman, 1977.				

b Taken from Breck, 1964.

^CBased on the amount of water contained per gram of dehydrated zeolite.



FIGURE 2. Methods of representing SiO₄ and AlO₄ tetrahedra by (A) ball and stick model, (B) solid tetrahedron, (C) skeletal tetrahedron, (D) spacefilling of packed spheres, (E,F) linking of four tetrahedra in a four membered ring, (G) secondary building unit called the truncated octahedron represented by a solid model, (H) fundamental structural unit of the zeolite molecule. Taken from Kirk-Othmer (1982). of SiO₄ and AlO₄ (Kirk-Othmer, 1981). Oxygen ions are located in each of the four corners of each tetrahedron unit and are shared with adjacent tetrahedron units. A schematic representation of the structural arrangement of the ions comprising a zeolite molecule is shown in Figure 2. The silicon and aluminum ions are referred to as "structural ions" since they, along with oxygen ions make up the structural framework of the molecule (Mumpton and Fishman, 1977). The ratio of trivalent aluminum ions to the quadravalent silicon ions determines the positive electrical charges needed to make the zeolite molecule electrically neutral (Mumpton and Fishman, 1977).

B. Exchangeable Metal Cations

The deficiency of positive charges resulting from the ratio of structural ions in the zeolite molecular framework is relieved by the attachment of mono or divalent cations such as Na^+ , K^+ , and Ca^{++} . These cations are referred to as "exchangeable cations" since they are loosely bound to the structural framework and are easily removed or exchanged with other cations when zeolites are added to cation containing media. Crystalline zeolites are powerful ion-exchangers with common exchange capacities of 3-4 meq/g of zeolite. Ion-exchange capacity is dependent upon the degree of substitution of aluminum for silicon (structural cations). As the ratio of Al:Si increases, there is a greater positive charge deficiency due to the aluminum ions, thus creating the need for charge balancing which is obtained from exchange-able cations. Elemental composition of common natural and synthetic zeolites is shown in Table 1.

Ion-exchange capacity depends not only on Al:Si ratio but on other factors as well. Cations can be trapped in structural positions that

are inaccessible in some types of zeolites. This reduces the effective exchange capacity of that zeolite for that ion (Mumpton and Fishman, 1977). Ion sieving may also occur if the size of the exchanging cation is too large to pass through the entry channels into the central cavities of the zeolite structure (Mumpton and Fishman, 1977). The framework of zeolites determines its selectivity toward competing ions. The hydration spheres in the zeolite molecule have high field-strength ions to prevent close proximity of an exchangeable ion to the seat of the charge in the zeolite framework (Mumpton and Fishman, 1977). Thus, ions with low field strength are more tightly bound and selectively exchanged with the zeolite molecule than other ions (Mumpton and Fishman, 1977). To illustrate, consider two zeolites, clinoptilolite (natural) and zeolite A (synthetic). The lesser amount of aluminum relative to silicon in clinoptilolite results in a relatively low ion-exchange capacity (2.54 meq/g). Conversely, zeolite A (5.48 meq/g) has a larger Al:Si ratio which gives its structure greater ion exchange capacity than clinoptilolite. Cation selectivity for clinoptilolite is considered to be Cs>Rb>K>NH_A>Ba>Sr>Na>Ca>Fe>A1>Mg>Li (Ames, 1960; Mercer <u>et al</u>., 1970). Clinoptilolite has a greater affinity for the larger cations, NH_A specifically, which creates interest for agricultural use. Cation selectivity has not been determined for Zeolite A making it difficult to apply it as a molecular sieve. Relative ion-exchange capacities of common natural and synthetic zeolites are presented in Table 1.

C. Dimensions and Characteristics of Internal Pores and Channels

Along with the exchangeable cations, the dimensions and characteristics of the internal pores and channels of the structure are essential for the zeolite molecular sieve properties. Molecular water is loosely

bound to the zeolite structure. Upon dehydration of the molecule, the internal pore system composed of interconnecting cage-like voids and uniform internal channels (which may be two or three dimensional) can be used for adsorption of gases, liquids, salts, elements, and many other substances (Kirk-Othmer, 1981). However, if these molecular substances do not have the proper dimensions to fit into these voids or channels, they will be excluded from the internal cavities of the zeolite molecule. Void volumes in some zeolites may be as large as 50% of the total volume of the molecule (Mumpton and Fishman, 1977). The surface area of one gram of certain zeolites may be as great as several hundred square meters (Mumpton and Fishman, 1977). Aperture size and void volume of common natural and synthetic zeolites is shown in Table 1.

Zeolites not only adsorb or reject molecules based on size and shape, but also on the basis of polarity. When a diffusing molecule approaches a zeolite channel, interaction energy becomes a dominant force (Kirk-Othmer, 1981). If the dimensions of the zeolite channel are small relative to the size dimensions of the diffusing molecule, then a repulsive interaction occurs preventing the diffusing molecule from passing into the zeolite channel (Kirk-Othmer, 1981). This repulsion can be overcome with specific activation energy to enhance the passage of the diffusing molecule (Kirk-Othmer, 1981). The unusual charge distribution within the central cavities of the zeolite molecule allows many diffusing molecules with permanent dipole moments to be selectively adsorbed unlike other sorbents (Mumpton and Fishman, 1977).

II. CURRENT USES OF ZEOLITES

Zeolites are currently used mainly in petroleum refining where additions of small amounts of hydrothermally stable acidic zeolites to conventional cracking catalyst formulations results in greater yields and higher quality products (Kirk-Othmer, 1981). Acidic, hydrothermally stable, large-pore zeolites are also used as catalysts in hydrocracking for fuel production. Other chemical uses of zeolites in the petroleum industry include: a) conversion of methanol to gasoline; b) conversion of most hydrocarbon feed stocks into gasoline; c) conversion of natural gas to gasoline (Business Week, 1982).

Zeolites are also replacing phosphates in detergents to remove "hard" minerals from wash water while reducing pollution problems associated with phosphates. Radioactive waste materials are effectively removed from natural water bodies by zeolites. And, use of the internal pore structure of zeolites to attract and hold large quantities of liquid are currently being developed for applications in solar refrigeration systems (Business Week, 1982).

Direct agricultural applications of zeolite, until recently, have not been researched. Agronomic uses currently being studies are: a) holding NH_4 in the soil for longer periods of time; b) acting as carriers of pesticides and hopefully extending the time of effectiveness in the soil; c) improving the sweetness of sugar beets (Business Week, 1982). In the area of livestock production, Pfizer Incorporated has received permission from the FDA to use a synthetic zeolite as a diluent for adding antibiotics to livestock feed (Business Week, 1982).

Zeolites have been suggested to improve the utilization of feed

nitrogen in animal nutrition, reduce intestinal diseases in young swine and ruminants, and control moisture and ammonia content in animal manure. However, due to limited research, conclusive results of zeolite supplementation in livestock diets have not been established. Japanese researchers were the first to explore the use of zeolites in animal nutrition and observed improvements in feed efficiency and average daily gain, with no adverse effects on health status, in all livestock species fed zeolites. This Japanese work, along with the enthusiasm of synthetic zeolite manufacturers and natural zeolite firms for expanding the uses of zeolites, has prompted some U.S. researchers to investigate the use of zeolites in animal nutrition.

III. ANIMAL NUTRITION APPLICATIONS

1. Removal of Toxic (Growth Depressant) Compounds

A suggested nutritional mode of action of zeolites, involves adsorption and/or binding of toxic substances. These toxic substances may be either present in the diet or produced by the animal in normal digestive processes which may interfere with digestion and absorption of nutrients or require energy for removal from the animal's body. Specific toxins of interest in animal nutrition are (A) ammonia, (B) p-cresol, and (C) zearalenone. Free ammonia is produced in the gastrointestinal tract due to breakdown of proteins during digestive processes. P-cresol is an anaerobic bacterial metabolite, produced primarily in the lower gastrointestinal tract, from the degradation of tyrosine. Zearalenone is a mycotoxin occasionally found in feed grains.

A. Ammonia

Ammonia is classified as a toxin in higher animals (Visek, 1964; Visek, 1972). Intestinal microflora make significant contributions to the concentration of portal blood ammonia through deamination of ingested protein and urea hydrolysis (Dintzis and Hastings, 1953; Silen et al., 1955). A suggested mode of action of dietary antibiotics in animals involves a reduction in ammonia production in the gastrointestinal lumen (Francois and Michel, 1968; Visek, 1978). Reductions of intestinal free ammonia improved growth rates in chicks and rats (Visek, 1964). Visek (1962) postulated that improvement in performance in rats and chicks immunized with jackbean urease was due to a decrease in ureolytic activity of the gastrointestinal tract. Kornegay et al. (1964) studied the effects of urease immunization of growing pigs upon growth performance along with blood and intestinal ureolysis and observed an improvement in ADG in pigs injected intraperitoneally with 10 units (modified Sumner units per pound of body weight) of urease and a reduction in plasma ammonia levels in pigs injected with .5 units of urease. Urease immunization was also effective in stimulating antibody production as well as lowering intestinal urease activity. Sodium bentonite has also been shown to lower free ammonia in the digestive tract (Martin et al., 1969). Ion-exchange resins (e.g. sodium bentonite) and antibiotics either inhibit bacterial production of or removal of ammonia (Zuidema et al., 1962).

In ruminants, natural and synthetic zeolites were added to the rumen, in an attempt to reduce any toxic effects of high levels of ammonia, and resulted in rapid ion-exchange with ammonium ions formed from breakdown of NPN sources (White and Ohlrogge, 1974). These ammonium ions remained attached to zeolite for several hours until sodium was

introduced into the rumen via saliva during the post-feeding rumination period. Up to 15% of NH_4^+ in the rumen can be bound to zeolite facilitating a gradual release of NH_4^+ from the zeolite. This allows rumen microbes to continuously synthesize microbial protein and make more efficient use of NPN sources (White and Ohlrogge, 1974). Natural chabazite and clinoptilolite are effective in the NH_{Δ}^{+} -zeolite exchange process but zeolite F (a synthetic zeolite) is even more effective (Milton, 1961). Synthetic zeolites F and W have superior cation selectivity and ionexchange capacity for NH_4^+ (Mumpton and Fishman, 1977). However, certain NH_4^+ selective natural zeolites, such as clinoptilolite and phillipsite, may be less expensive and more abundant than the synthetics and may be more practical for future use in ruminant feeds (Mumpton and Fishman, 1977). Rumen NH_{Δ}^{+} was lower in dairy cattle fed 5% clinoptilolite plus 2% urea than in cattle fed a 2% urea diet which contained no clinoptilolite indicating binding of free NH_{Δ}^{+} by zeolite (Hemken <u>et al.</u>, 1982). In a series of in vitro and in vivo studies in cattle using synthetic zeolite type 13X, 75% of absorbed nitrogen was bound to zeolite and after a three-hour incubation in an artificial saliva solution, only 25% of the bound nitrogen was liberated from the zeolite (Sweeney, 1980). In addition, no zeolite effects were shown on microbial protein output indicating complete availability of ammonia even in excess levels. In beef steers, McCollum and Galyean (1983) observed lower rumen ammonia concentration three hours after feeding a 70% sorghum diet containing 5% clinoptilolite. At six and nine hours postfeeding, diets containing either 2.5 or 5% clinoptilolite resulted in lower rumen ammonia concentrations compared to steers not receiving clinoptilolite.

The degree of reduction in the concentration of portal blood

ammonia by clinoptilolite in rats and administered $(NH_4)_2CO_3$ is affected by the clinoptilolite: $(NH_4)CO_3$ ratio. A ratio of 7-10.5 is sufficient to bind NH_4^+ in the gut but binding was not complete since there was a rise in portal blood NH_4^+ (Pond <u>et al.</u>, 1981). Thus, clinoptilolite has the ability to bind free ammonia in the gastrointestinal tract and the degree of binding is related to the ion-exchange capacity for each species of zeolite.

Ammonia toxicity in swine is prevented when clinoptilolite is given simultaneously with toxic levels of ammonia (Pond, 1980). Ammonia concentration in the jejunum of clinoptilolite-fed pigs increased tenfold while there was no change in ammonia concentration in the ileum (Cool and Willard, 1982). Orbital plasma ammonia concentrations were not affected in clinoptilolite fed pigs but there was a decrease in hepatic portal blood ammonia concentrations 45 and 90 minutes after feeding (Froseth, 1982). Both young and mature pigs were fed 5% clinoptilolite and had lower fecal concentrations of all forms of nitrogen compared to pigs not fed clinoptilolite, indicating more efficient conversion of feed nitrogen to animal protein (Kondo and Wagai, 1968). B. P-Cresol

P-cresol (4-methylphenol) is classified as a type-B toxic agent with an oral LD₅₀ of 1.8 g/kg body weight in the rat (Deichmann and Witherup, 1943). Anaerobic bacterial degradation of tyrosine and tryptophan in the intestine of the pig results in production of several volatile and aromatic metabolites of which one is p-cresol (Bakke, 1969; Yokoyama and Carlson, 1974; Scheline, 1968; Yokoyama and Carlson, 1979). Intestinal p-cresol production may be responsible for growth depressing effects in weanling pigs and additions of dietary antibiotics

could promote growth by inhibiting bacterial production of p-cresol (Yokoyama <u>et al.</u>, 1982). Weanling pigs fed a diet containing a combination of antibiotics consisting of chlortetracycline, sulfamethazine, and penicillin excreted less urinary p-cresol than pigs fed diets containing a single antibiotic, lincomycin sulfate, or no antibiotics (Yokoyama <u>et al.</u>, 1982). Regression analysis of percentage body weight gain on urinary p-cresol excretion resulted in a negative correlation coefficient (r = -.73) (Yokoyama <u>et al.</u>, 1982). Zeolites have been implicated as having antibiotic-like properties in animal nutrition (Pond <u>et al.</u>, 1981). Pigs fed supplemental zeolite A (synthetic) or zeolite A and tyrosine, had increased urinary p-cresol excretion indicating that zeolite A is not antibiotic-like in reducing p-cresol production (Shurson et al., 1982).

C. Zearalenone

Zearalenone is a uterotropic mycotoxin synthesized by <u>Fusarium</u> fungi and is occasionally found in feed grains. Several animal species have been reported to be subject to zearalenone toxicosis following ingestion of infested feeds or purified zearalenone. Swine appear to be particularly sensitive to zearalenone which has detrimental effects on female reproduction (Nelson <u>et al</u>., 1973; Kurtz <u>et al</u>., 1969; Miller <u>et al</u>., 1973). Rats and mice are less sensitive to zearalenone toxicosis but uterine enlargement has been observed (Christensen <u>et al</u>., 1965). Zearalenone toxicosis in rats was prevented when a synthetic anion exchange zeolite was fed at 5% of the diet (Smith, 1980). Alleviation of zearalenone toxicosis by zeolite may be a result of increased absorption of nutrients which is in agreement with the reduction of toxicosis

by high dietary protein and lipid levels (Smith, 1980; Smith <u>et al</u>., 1971; Sisk and Carlton, 1972). Zeolite may also promote absorption of water and speed rate of passage analogous to beneficial effects of fiber additions to the diet to reduce zearalenone toxicosis (Smith, 1980; Ershoff, 1977).

2. Zeolite Effects on Passage Rate of Feedstuffs in the Digestive System

A second possible mode of action of zeolites may be their ability to slow rate of passage of nutrients through the digestive tract thus improving nutrient absorption efficiency. Montmorillonite clay has been reported to slow down rate of passage of nutrients in the digestive tract of chickens leading to an improvement in caloric efficiency (Quisenberry, 1968). In chicks fed a low energy diet containing sodium bentonite, a slight delay of feed passage rate was observed along with improvement in growth rate and increased feed intake (Kurnick and Reid, 1960). Young and mature pigs fed diets containing 50% clinoptilolite excreted feces with smaller sized particles than feces from pigs fed diets with no clinoptilolite which possibly may be a result of slower passage rate and a more complete digestion process (Kondo and Wagai, 1968). Alimentary tract transit time was reduced in hand-fed starter pigs and finisher pigs fed diets containing 3% sodium bentonite (Collings et al., 1980).

3. Effects of Zeolites on Nitrogen, Energy, and Mineral Metabolism

A. Nitrogen

In ruminants, protein digestibility was improved by 7% in steers and heifers fed a diet containing 5% clinoptilolite (Sweeney, 1980).

Hemken (1982) fed a diet containing 5% clinoptilolite and 2% supplemental urea to dairy cows with no effect on milk protein but there was an increase in blood urea nitrogen. Apparent digestibility of protein was improved in diets containing high-solubility protein plus 5% clinoptilolite compared to diets containing high-solubility protein without clinoptilolite (Sweeney, 1982). Clinoptilolite tended to improve ruminant digestion of crude protein but intestinal digestion was reduced (McCollum and Galyean, 1983).

Protein utilization was improved 22% in broilers fed zeolites having calcium exchange capacities ranging from 80 to 120 meq/100g of zeolite (Chung and Lee, 1978).

Zeolite substituted for wheat bran in swine diets at levels from 1 to 6% improved the digestibility of crude protein over a 12-week feeding period (Han <u>et al.</u>, 1975; 1976). Supplemental zeolites fed to young and mature pigs had lower concentrations of nitrogen in feces compared to pigs receiving no zeolite, suggesting more efficient conversion of feed nitrogen to animal protein (Kondo and Wagai, 1968). Feeding 3% zeolite A to growing pigs resulted in lower nitrogen digestibility and reduced net protein utilization (Shurson <u>et al.</u>, 1982).

B. Energy

In ruminants, clinoptilolite increased fiber and organic matter utilization (Sweeney, 1980). Digestibility of organic matter and acid detergent fiber increased in cattle fed high soluble protein plus zeolite and caused a beneficial shift in rumen acetate: propionate ratio from 2.75 to 2.96 (Sweeney and Cervantes, 1982). There was no effect of clinoptilolite on milk fat content in dairy cattle (Hemken <u>et al</u>., 1982). Addition of 2.5% clinoptilolite to a 70% sorghum diet resulted

in greater amounts of propionic acid production when fed to beef steers (McCollum and Galyean, 1983). McCollum and Galyean (1983) observed no difference in starch digestion in steers fed diets containing 0, 1.25, or 2.5% clinoptilolite but steers fed 1.25% clinoptilolite digested starch more completely than steers fed 5.0% clinoptilolite.

Carbohydrate utilization was improved by 1% in broilers fed zeolites of various calcium exchange capacities (Chung and Lee, 1978).

Additions of 5% zeolite A to growing swine diets increased fecal energy excretion resulting in a 10% reduction of ME corrected for nitrogen (Shurson et al., 1982).

C. Minerals

In cattle fed three clinoptilolites at 3% of the diet, there was no change in serum Ca, P, Mg, Na, or K levels compared to cattle receiving no clinoptilolite. Zeolite fed at 2% of the diet to dairy cows for 3 lactations increased Ca and Mg levels in the blood (Fukushima, 1979). No effect on serum Ca, Mg, Na, or K was observed in dairy cows fed 5% supplemental clinoptilolite (Hemken, 1982). No significant effects on blood chemistry were observed, except for a reduction of K, in Holstein steers and heifers fed 5% clinoptilolite (Sweeney and Cervantes, 1982).

The coefficient of calcium utilization improved from 33.9 to 86.7% in zeolite-fed roosters with possibly more K in the feces (Hayhurst, 1980).

In swine, feeding a Ca-K clinoptilolite at 5% of the diet increased blood Co concentrations from 1.3 to 3.2 µmole/2 (Vrzgula and Bartko, 1980). Calcium absorption increased 17% in pigs fed clinoptilolite (Cool and Willard, 1982). No difference was observed in apparent digestibility of K but calcium digestibility was increased in clinoptilolite-fed pigs (Cool <u>et al.</u>, 1980) In growing pigs fed 5% supplemental zeolite A, % Ca, P, Na, K retentions were decreased while % iron retention was improved (Shurson <u>et al.</u>, 1982).

4. Effects of Zeolites on Growth Performance

Cattle fed normal grass and hay diets containing 5% zeolite had a 20% improvement in weight gain as well as an improvement in feed efficiency (Kondo et al., 1969). In cattle fed low quality diets containing zeolite, there was a 13% increase observed in average daily gain (Sweeney, 1982). Milk yields increased 31% in dairy cows fed zeolites (Fukushima, 1979). In contrast to these positive responses of zeolite on growth performance, there are also reports of no response to zeolite supplementation. In dairy cows fed diets containing either soybean meal, urea, or urea plus zeolite as a nitrogen source, milk production increased only with the soybean meal diet (Hemken et al., 1982). Six bullocks were fed a diet containing either 2% zeolite or no zeolite for 329 days. There was no observed difference in final weights between these animals but those receiving zeolite had slightly larger body dimensions (Watanabe et al., 1971). In cattle fed one of 3 types of clinoptilolite or no zeolite, there was an improvement in average gains in cattle fed one of the clinoptilolites and the control diet compared to the gain responses from cattle fed either of the other two clinoptilolites. There was no effect on feed efficiency between these treatments (Hutcheson, 1982). McCollum and Galyean (1983) fed sorghum diets containing 0, 1.25, or 2.5% clinoptilolite to beef steers and observed no effect on growth performance among treatments.

Less feed and water were needed in chickens fed clinoptilolite . to get the same weight gain as that of chickens receiving no clinoptilolite in their diet. Feed efficiency ratios were higher at all levels of zeolite substitution and in diets containing 10% clinoptilolite. feed efficiency was improved by 20% (Onagi, 1966). Broiler chickens fed a diet of 5% clinoptilolite gained less weight over a two month period than birds receiving a normal diet, but average feed efficiency values improved 4 - 5% (Arscott, 1975; 1976). Both male and female broilers had faster weight gains when fed diets of 2% clinoptilolite but only male broilers showed an improvement in feed efficiency (Vest and Schutze, 1982). Diets containing 7.5% clinoptilolite fed to day-old Leghorn roosters increased weight gains 10% during the first 10 days of the test and maintained that weight gain for the duration of the 7 week trial while consuming 10% less feed (Hayhurst, 1980). Four different clinoptilolites and one type of mordenite were studied in broiler and turkey rations. Improvements in feed efficiency and weight gain were observed in birds fed one of the clinoptilolites (Bunger, 1982). In another study, there was no advantage of feeding 5 or 10% clinoptilolite diets to male broiler chicks on rate or efficiency of gain (Dion and Carew, 1982).

Addition of 5% clinoptilolite to diets for young and finishing swine resulted in a 26% improvement in weight gain and a 35% improvement in feed efficiency in young pigs while weight gain improved 29% with feed efficiency improving only 6% in finishing swine (Kondo and Wagai, 1968). Zeolite-fed pigs ate 42% less feed for the same weight gain containing 10% clinoptilolite improved weight gain; feed ratio by 34% (Cool and Willard, 1982). Also, in early weaned pigs fed diets

containing 10% Idaho clinoptilolite, a 5% increase in feed conversion efficiency was observed (Pond, 1980). However, in young pigs fed zeolite and zeolite-antibiotic supplements, no large effect on feed efficiency was noted (England and George, 1975). Likewise, clinoptilolite supplementation of corn-soybean meal diets, with or without raw pinto beans, produced no beneficial effect on growth performance in growing pigs (Milne and Froseth, 1981). Thielemans and Bodart (1982) observed no difference in average daily gain of growing-finishing pigs fed zeolite but there was an improvement in feed consumption and feed conversion when zeolite as added to diets containing less that 3250 kcal of digestible energy per kg of diet. Work with pregnant sows fed 400 grams of clinoptilolite per day through gestation and lactation indicated improvement in litter weaning weights by as much as 85% over litters from sows not receiving clinoptilolite (Buto and Takahashi, 1967). In addition, sows fed 3 and 5% zeolite beginning at three different periods of gestation resulted in a 30% improvement in litter size when fed beginning 20-30 days after conception while there was only a 7-8% improvement in litter size when zeolite was fed beginning in later stages of gestation. This implies that zeolite may have a beneficial role in early embryonic survival (Chueng-Shyang et al., 1979). in pregnant Landrace sows fed 2.5 or 5% clinoptilolite, ovulation rate was reduced but there was no effect on embryo survival rate (Chueng-Shyang et al., 1982).

5. Effects of Zeolites on Animal Health

Zeolites have been implicated in reducing diarrhea in swine (Morita, 1967); Buto and Takahashi, 1967; England and George, 1975;

Froseth, 1982) and in ruminants (Kondo et al., 1969; Watanabe et al., 1971; Fukushima, 1979). Zeolites lower the moisture content of manure in poultry (Onagi, 1966; Chung and Lee, 1978; Hayhurst, 1980; Bunger, 1982), in swine (Cool et al., 1980; Vrzgula and Bartko, 1982; Cool and Willard, 1982), and in ruminants (Kondo et al., 1969; Fukushima, 1979). No adverse health effects have been observed in livestock and poultry when fed zeolite-containing diets. Reports vary from improvement of liveability in broilers (Arscott, 1975; 1976) to reduction in mortality, gastric ulcers, pneumonia, and heart dilation of pigs over a 12 month period (Torii, 1977). Furthermore, gastrointestinal nematodes in rats are expelled from the intestine faster when fed diets containing 10% clinoptilolite compared to rats not receiving clinoptilolite (Wells, 1982). As much as 40% clay can be added to animal diets with no adverse effects (Ousterhout, 1970). However, Swiss albino mice were injected intraperitoneally with a single dose of 10 or 30 mg of erionite or mordenite suspended in 1 ml saline solution. Six of ten mice injected developed malignant peritoneal tumors between 8 and 22 months after the single administration. Fibrotic lesions caused by these mineral fibers were found in the primary site of the induced peritoneal tumors. Erionite was more fibrogenic than mordenite. From these results, it was determined that the carcinogenic and fibrogenic effects of fibrous erionite are similar to those of asbestos (Suzuki, 1982).

MATERIALS AND METHODS

The nutritional effects of zeolite A and clinoptilolite supplementation in swine diets reported here is based on eight trials. Trials 1 and 2 were performance trials, Trials 3 and 4 were balance trials, Trials 5 and 6 were evaluations of serum and plasma components, and Trials 7 and 8 were passage rate studies.

1. Performance Trials

Trials 1 and 2 utilized the same group of pigs and had the same experimental design so they will be discussed together. In Trial 1, fifty four growing pigs averaging about 25 kg in body weight were randomly assigned from litters to nine pens of six pigs each for a six-week growing phase trial. Trial 2 utilized the same fifty four pigs averaging about 65 kg in body weight in a continuation of Trial 1 for an eight-week period to examine finishing phase performance.

After allowing five days for the pigs to adjust to new pen mates, Trial 1 was started. Three pens were randomly assigned to each of the three experimental diets containing either no zeolite, .3% zeolite A, or .5% clinoptilolite shown in Table 2. Additions of zeolite A or clinoptilolite were made at the expense of corn. Typical properties and analyses of zeolite A and clinoptilolite used in all studies are shown in Tables 3 and 4, respectively. Table 5 lists the levels of nutrients supplied by the vitamin-trace mineral premix used in all trials. Calculated nutrient or element density of the diets used in Trial 1 is presented in Table 6. In Trial 2, three pens were again

			Dietary	Treatments
Ingredient	Int. Ref. No.	Control	.3% Zeolite A	.5% Clinop- tilolite
Corn, ground-shelled	4-02-931	785	782	780
Dehulled soybean meal (48.5%	CP)4-04-612	180	180	180
Mono-dicalcium phosphate	6-01-080	12	12	12
Calcium carbonate	6-01-632	10	10	10
Sodium chloride		2.5	2.5	2.5
Vitamin-trace mineral premix ^a		10	10	10
Antibiotic premix ^b		0.5	0.5	0.5
Zeolite A		0	3	0
Clinoptilolite		0	0	5
		1000.0	1000.0	1000.0

TABLE 2. DIET COMPOSITION (Trial 1)

a See Table 5 for nutrient levels supplied to all diets. ^bSupplying 55 mg of chlortetracycline per kg diet.

Typical Properties Form White crystalline Formula $Na_6[(A10_2Si0_2)_{12}] \cdot 27H_20$ Molecular weight 2053 95 + % Crystallinity 4 Å Nominal pore diameter 18 - 22% Moisture Specific gravity 1.99 Particle size 2.7 - 2.9 μ pH (1% dispersion) 10.6 Symmetry Cubic

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	Typical Analysis
Aluminum	15.1%
Silicon	13.9%
Sodium	12.9%
Magnesium	29 ppm
Calcium	272 ppm
Potassium	381 ppm
Lead	10 ppm
Arsenic	3 ppm

Molecules Absorbed

 $${\rm H_2S}$$$SO_2$$$NH_3$$Others with dimensions less than <math display="inline">4{\rm \AA}$

Molecules Excluded

Molecules with an effective diameter greater than 4Å
Typical Properties					
Form	Lt. tan crystalline powder				
Formula	Na ₆ [(A10 ₂) ₆ (Si0 ₂) ₃₀]·24H ₂ 0				
Molecular weight	2727				
Purity	85 <u>+</u> 5%				
Impurities	Feldspar, quartz, clay				
Nominal pore diameter	. 3.5 Å				
Ion exchange capacity	2.54 meg/g				
Symmetry	Monoclinic				
Typical	Analysis (%)				
Si0 ₂	63.35				
A1203	10.55				
CaO	2.16				
MgO	.62				
Ti0 ₂	.20				
Na ₂ 0	1.49				
κ ₂ 0	2.95				
Fe ₂ 0 ₃	1.18				
	04				

TABLE 4. TYPICAL PROPERTIES AND ANALYSIS OF CLINOPTILOLITE

 $K^{+}, Cs^{+}, NH^{+}_{4}, Na^{+}, Cd^{+2}, Pb^{+2}, Zn^{+2}, Cu^{+2}, Ca^{+2}, Hg^{+2}, Mg^{+2}, Fe^{+3}, Co^{+3}, A1^{+3}, Cr^{+3}$

 $\frac{Absorbing Gases}{CO_2,SO_2,H_2S,NH_3,O_2,N_2,H_2O,H_2,CH_3OH}$

Nutrient	Amount supplied per kg diet
Vitamin A	3300 IU
Vitamin D	660 IU
Vitamin E	16 IU
Menadione	2 mg
Riboflavin	3.3 mg
Niacin	18 mg
d-Pantothenic acid	13 mg
Choline	110 mg
Vitamin B ₁₂	20 µg
Zinc	75 mg
Iron	60 mg
Manganese	37 mg
Copper	10 mg
Iodine	.2 mg
Selenium	.1 mg

TABLE 5. NUTRIENTS SUPPLIED BY VITAMIN-TRACE MINERAL PREMIX TO ALL DIETS

C. CL. R. L. L.

		Dieta	ary Treatme	ents
Nutrient or Element	NRC	Control	.3% Zeolite A	.5% Clinop- tilolite
Metabolizable energy, kcal/kg	3175	3200	3100	3040
Crude protein, %	0.61	15.6	15.6	15.5
Lysine, %	.70	.77	.77	.77
Calcium, %	.60	.66	.66	. 66
Phosphorus, %	.50	.58	.58	.58
Sodium, %	.10	.14	.18	.15
Aluminum, %	-	trace	.04	.03
Silicon, %	-	trace	.04	.16

TABLE 6. CALCULATED NUTRIENT OR ELEMENT DENSITY (Trial 1)

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randomly assigned to each of the three experimental diets containing either no zeolite, 1% zeolite A, or 5% clinoptilolite shown in Table 7. Calculated nutrient or element density for diets used in Trial 2 is shown in Table 8.

Pigs in Trials 1 and 2 were reared in a modern, total confinement, environmentally controlled growing-finishing building at the Michigan State University Swine Research Farm. Each pen containing six pigs measured 1.37 m x 4.27 m (allowing 1.0 square meter of floor space per pig) with totally slotted floors (15.24 cm concrete slats with 2.54 cm slots) and had one two-hole self feeder and one automatic watering cup.

Each pig was weighed biweekly throughout the six-week trial for Trial 1 and throughout the eight-week trial for Trial 2. Feed consumption of each pen was determined at these same intervals by recording feed added to self feeders (from paper bags containing 22.7 kg of feed) and weighing each feeder after individual pig weights were obtained in order to calculate average daily feed intake for each pen during each period. Data were recorded at the farm in the field data book and then transferred to the permanent record book at the office.

The triplicate pen mean values of average daily gain (ADG), average daily feed intake (ADFI), feed required per unit gain (F/G) and energy utilization efficiency (kcal/g of gain) were statistically analyzed in a completely randomized design, one-way analysis of variance.

2. Balance Trials

Trials 3 and 4 were balance trials utilizing the same experimental design except that different groups of pigs were used in each trial. Because of the similarity of experimental design used in Trials 3 and 4,

		Dietary Treatments			
Ingredient	Int. Ref. No.	Control	1% Zeolite A	5% Clinop- tilolite	
Corn, ground-shelled	4-02-931	840	830	790	
Dehulled soybean meal (48.5%)	CP) 4-04-612	125	125	125	
Mono-dicalcium phosphate	6-01-080	12	12	12	
Calcium carbonate	6-01-632	10	10	10	
Sodium chloride		3	3	3	
Vitamin-trace mineral premix		10	10	10	
Zeolite A		0	10	0	
Clinoptilolite		0	0	50	
		1000	1000	1000	

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TABLE 7. DIET COMPOSITION (Trial 2)

		Die	Dietary Treatments				
Nutrient or Element	NRC	Control	1% Zeolite A	5% Clinop- tilolite			
Metabolizable energy, kcal/kg	3195	3230	3195	3060			
Crude protein, %	13.00	13.50	13.40	13.00			
Lysine, %	.57	.60	.60	.59			
Calcium, %	.50	.67	.67	.67			
Phosphorus, %	.40	.56	.56				
Sodium, %	.10	.18	.32	.26			
Aluminum, %	-	trace	.15	.28			
Silicon, %	-	trace	.13	1.17			

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 TABLE 8.
 CALCULATED NUTRIENT OR ELEMENT DENSITY (Trial 2)

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they will be discussed together.

Sixteen crossbred pigs from two litters (4 barrows and 4 gilts per litter) with average initial body weight of 7.5 kg in Trial 3 and 7.0 kg in Trial 4 were assigned to the four experimental diets in each trial. One barrow and one gilt from each litter were assigned to each of the four dietary treatments containing 0, 1, 2, or 3% zeolite A in Trial 3 or 0, 2.5, 5.0, or 7.5% clinoptilolite in Trial 4. Diet composition for Trials 3 and 4 is shown in Tables 9 and 10, respectively. Calculated nutrient or element density for Trials 3 and 4 is shown in Tables 11 and 12, respectively.

In each balance trial, the pigs were reared in individual stainless steel collection cages (55 x 70 x 76 cm) located in the west room of the nutrition laboratory at the MSU Swine Research Farm. They were removed twice daily from these collection cages and fed in individual stainless steel feeding cages ($45 \times 90 \times 76$ cm) with feed cups containing an amount of finely ground feed equivalent to 2% of each pig's initial body weight. The feed was mixed with an equal amount of water to form a slurry which the pigs consumed within 5 to 10 minutes. Additional water was added to the feed cups to allow the pigs to clean the feed cup thoroughly. The pigs were then quickly removed from the feeding cages and placed in collection cages preventing urination and defecation in the feeding cages. This method was used to prevent feed and water contamination of the feces and urine being collected which provided for accurate nutrient balance. Room temperature was held constant at 27° C.

Prior to the five day collection period, the pigs were given a seven day adjustment period to allow them to adapt to the feeding

		Lev	els of	Zeolite	A (%)	
Ingredient	Int. Ref. No.	0	1	2	3	
Corn starch		3.0	2.0	1.0	0.0	
Corn, ground shelled	4-02-931	68.2	68.2	68.2	68.2	
Dehulled soybean meal (48.5%CF	9) 5-04-612	25.0	25.0	25.0	25.0	
Mono-dicalcium phosphate	6-01-080	1.5	1.5	1.5	1.5	
Calcium carbonate	6-01-632	1.0	1.0	1.0	1.0	
Sodium chloride		0.3	0.3	0.3	0.3	
Vitamin-trace mineral premix		1.0	1.0	1.0	1.0	
Zeolite A		0.0	1.0	2.0	3.0	
		100.0	100.0	100.0	100.0	

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TABLE 9. DIET COMPOSITION USING SUPPLEMENTAL ZEOLITE A (Trials 3, 5, and 7)

Levels of Clinoptilolit				ite (%
Int. Ref. No.	0	2.5	5.0	7.5
	7.5	5.0	2.5	0.0
4-02-931	63.7	63.2	63.2	62.7
P) 5-04-612	25.0	25.5	25.5	26.0
6-01-080	1.5	1.5	1.5	1.5
6-01-632	1.0	1.0	1.0	1.0
	0.3	0.3	0.3	0.3
	1.0	1.0	1.0	1.0
	0.0	2.5	5.0	7.5
	100.0	100.0	100.0	100.0
	Int. Ref. No. 4-02-931 P) 5-04-612 6-01-080 6-01-632	Int. Ref. No. 0 7.5 4-02-931 63.7 P) 5-04-612 25.0 6-01-080 1.5 6-01-632 1.0 0.3 1.0 <u>0.0</u> 100.0	Int. Ref. No. 0 2.5 7.5 5.0 4-02-931 63.7 63.2 P) 5-04-612 25.0 25.5 6-01-080 1.5 1.5 6-01-632 1.0 1.0 0.3 0.3 1.0 1.0 0.0 2.5 100.0 100.0	Int. Ref. No.0 2.5 5.0 7.5 5.0 2.5 5.0 4-02-931 63.7 63.2 63.2 P) 5-04-612 25.0 25.5 25.5 $6-01-080$ 1.5 1.5 1.5 $6-01-632$ 1.0 1.0 1.0 0.3 0.3 0.3 0.3 1.0 1.0 1.0 1.0 0.0 2.5 5.0 100.0 100.0 100.0

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TABLE 10.DIET COMPOSITION USING SUPPLEMENTAL CLINOPTILOLITE
(Trials 4, 6, and 8)

	Levels of Zeolite A (%)			
NRC	0	1	2	3
3160	3239	3205	3172	31 39
18.00	18.00	18.00	18.00	18.00
.79	.97	. 97	.97	.97
.65	.75	.75	.75	.76
.55	.66	.66	.66	.66
.10	.14	.27	.40	.53
-	trace	.15	.30	.45
-	trace	.14	.28	.42
	NRC 3160 18.00 .79 .65 .55 .10 - -	Leve NRC 0 3160 3239 18.00 18.00 .79 .97 .65 .75 .55 .66 .10 .14 - trace - trace	Levels of NRC 0 1 3160 3239 3205 18.00 18.00 18.00 .79 .97 .97 .65 .75 .75 .55 .66 .66 .10 .14 .27 - trace .15 - trace .14	Levels of Zeolite NRC 0 1 2 3160 3239 3205 3172 18.00 18.00 18.00 18.00 .79 .97 .97 .97 .65 .75 .75 .75 .55 .66 .66 .66 .10 .14 .27 .40 - trace .15 .30 - trace .14 .28

TABLE 11.CALCULATED NUTRIENT OR ELEMENT DENSITY IN DIETS CONTAINING
ZEOLITE A (Trials 3, 5, and 7)

		Levels of Clinoptilolite (%)			
Nutrient or Element	NRC	0.0	2.5	5.0	7.5
Metabolizable energy, kcal/kg	3160	3239	3156	3073	2991
Crude protein, %	18.00	18.40	18.40	18.10	18.10
Lysine, %	.79	.97	.98	. 97	. 98
Calcium, %	.65	.75	.75	.75	.75
Phosphorus, %	.55	.67	.66	.66	.65
Sodium, %	.10	.23	.33	.42	.52
Aluminum, %	-	trace	e .15	.30	.45
Silicon, %	-	trace	e .77	1.54	2.32

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TABLE 12.CALCULATED NUTRIENT OR ELEMENT DENSITY IN DIETS CONTAINING
CLINOPTILOLITE (Trials 4, 6, and 8)

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schedule and adjust to the experimental diets. After the adjustment period, the collection cages were cleaned and prepared for a five day collection period. Ten ml of 1% thimersol solution was added to the urine collection containers to prevent microbial degradation of urinary compounds. Feces were collected on a fine wire screen while urine was collected on a tray located beneath the wire screen and directed to the urine collection containers located under the tray. Urine volumes were measured, recorded and a 150 ml sample from each pig was retained at the end of the collection period. Feces were collected and dried in an oven at 70° C. Total dried feces collected from each pig were weighed, recorded, and finely ground for laboratory analyses.

Samples of the fecal matter and urine from each pig were analyzed in duplicate for gross energy (total caloric value), N, Ca, P, Mg, Na, K, Fe, and Zn. Only urine samples were used in p-cresol determinations. Gross energy values of feed, feces, and urine were determined using an adiabatic bomb calorimeter¹ standardized with benzoic acid. Urine gross energy was determined after freeze-drying .5 ml of urine on cotton balls and subtracting the caloric contribution of the cotton ball. The nitrogen content of feed, feces, and urine was determined by a semi-micro Kjeldahl method (A.O.A.C., 1980). Calcium, magnesium, iron, and zinc concentrations were determined by atomic absorption spectrophotometry², while sodium and potassium concentrations were determined by atomic emission spectrophotometry². Phosphorus determinations were conducted using colorimetric spectrophotometry (Gomori, 1942). Gas chromatography³ was used to determine urinary p-cresol concentrations

¹Parr Instrument Company, Moline, IL.

²Model 951, Instrument Laboratory, Inc., Lexington, MA.

³Model 5840A, Hewlett-Packard Co., Farmington Hills, MI.

as described by Yokoyama <u>et al</u>. (1982). Values of these parameters were then used to calculate daily intake and excretion of energy, nitrogen, minerals, and urinary excretion of p-cresol.

The data were analyzed using linear regression analysis (Gill, 1978) to determine if there was a significant linear response in the nutrient parameters measured for the zeolite A or clinoptilolite levels fed.

3. Evaluation of Serum and Plasma Components

In Trials 5 and 6, the concentrations of plasma ammonia and glucose along with serum concentrations of Ca, P, Na, and K were determined at 30, 60, 90, and 120 minutes after feeding. The same sixteen pigs used in Trial 4 were used in Trials 5 and 6. Trials 5 and 6 had the same experimental design and will be discussed together. These pigs, averaging 11.5 kg in initial body weight in Trial 5 and 15.6 kg in Trial 6 were allotted to the same experimental diets, containing four levels of zeolite A or four levels of clinoptilolite, used in Trials 3 and 4, respectively. Diet composition and calculated nutrient or element density of experimental diets used in Trials 5 and 6 are shown in Tables 9, 10,11, and 12. Pigs were reared and managed under the same conditions described for Trials 3 and 4 and Trial 6 began immediately following the completion of Trial 5. In each of these blood sampling trials, the pigs were allowed a five day adjustment period for acclimation to their respective experimental diets. After the five day adjustment period, the pigs were fed an amount equivalent to 2% of their initial body weights at 7 a.m. on day 6, and time of complete ingestion of the meal was recorded. Pigs were then removed from feeding cages and returned to their original collection cages from which

they were removed for blood sampling at 30, 60, 90 and 120 minutes after feeding. Each pig was bled in the same order that it was fed.

Blood samples were taken by placing each pig on its back and while one person held the pig to restrain movement, a second person removed 10 ml of blood by venipuncture of the anterior vena cava using a 3.81 cm. 16 gauge needle attached to a 10 ml glass syringe. The needle was then removed from the syringe (to prevent hemolysis) and 5 ml of blood was expelled into each of two 15 ml.labeled plastic test tubes Tubes were labeled prior to bleeding to identify pig number, time of bleeding, and whether the sample was for serum or plasma determinations. Tubes containing 50 μ l of sodium heparin allowed extraction of plasma while tubes containing no sodium heparin yielded serum samples. The heparinized blood samples were immediately centrifuged at 2000 rpm for 10-15 minutes allowing plasma to be extracted and placed in 5 ml labeled plastic storage tubes located in a cooler with an ice bath. This methodology was used to decrease enzyme activity and prevent ammonia loss. Non-heparinized blood samples were allowed to clot for 15 minutes, rimmed (separation of the clot from the inside test tube wall) to provide better serum yield, and centrifuged at 2000 rpm for 10-15 minutes. Serum was then extracted and put into labeled 5 ml plastic storage tubes located in the ice bath. This process continued until all blood samples were processed and stored in the ice bath. Plasma and serum samples were taken to the laboratory where plasma samples were immediately analyzed in duplicate for plasma ammonia and glucose concentrations. These determinations were conducted spectrophotometrically⁴ using ammonia and glucose assay kits (obtained from

⁴Beckman-Gilford Spectrophotometer, Model 2400, Beckman Instruments, Inc., Fullerton, CA and Gilford Instrument Laboratories, Inc., Oberlin, OH.

Sigma Chemical Company, St. Louis, MO). Serum samples were frozen until convenient analysis of calcium, phosphorus, sodium, and potassium was possible. For the serum calcium and phosphorus determinations, 1 ml of 12.5% TCA was added to 1 ml of serum to precipitate serum proteins. Addition of TCA releases calcium and phosphorus bound to the serum proteins providing for determination of total serum calcium and inorganic phosphorus. These solutions were mixed and centrifuged for 15 minutes at 2500 rpm and then decanted into 5 ml plastic storage tubes. Calcium concentrations were determined by atomic absorption spectrophotometry⁵ while phosphorus determinations were conducted using a colorimetric spectrophotometry assay (Gomori, 1942). Serum samples, which were not precipitated with TCA, were analyzed for sodium and potassium concentrations by atomic emmission spectrophotometry⁵. Since serum sodium and potassium are present in free form, it was unnecessary to precipitate serum proteins with TCA. Plasma and serum concentrations were then calculated for each parameter and analyzed statistically using a split plot design (Gill, 1978). Correlations were also examined to try to identify relationships between dietary intake of nutrients and plasma and serum concentrations.

4. Passage Rate Trials

In Trials 7 and 8, passage rate was determined by feeding the same pigs utilized in Trials 5 and 6, diets containing the same four levels of zeolite A and clinoptilolite. Diet composition and calculated nutrient or element density of the diets used in Trials 7 and 8 are shown in Tables 9, 10, 11, and 12. Trials 7 and 8 were conducted in conjunction with Trials 5 and 6 and thus pigs were reared and managed under the same conditions described for Trials 3, 4, 5, and 6. The sixteen pigs in Trials 7 and 8

⁵Model 951, Instrument Laboratory, Inc., Lexington, MA

were allowed a five day adjustment period at the start of each trial for adaptation to their respective experimental diets. At the end of the adjustment period, each pig was weighed and fed an amount of experimental diet, containing .5% chromic oxide, equivalent to 2% of its' initial body weight. Time of feeding was recorded and all feces were collected every four hours after the single chromic oxide dosing. Pigs were fed twice daily at 8 am and 5 pm and received the same amounts of feed during the entire collection period. Each fecal collection was placed in aluminum pans identified with pig number, time of collection, and experimental diet number. Feces were collected until all pigs were excreting feces containing no visible chromic oxide which was approximately 84 hours after the initial chromic oxide dosing. Each trial was duplicated for accurate determination of rate of passage. After the first chromic oxide dosing and 84 hour collection, the pigs were given their second single chromic oxide dose in one of the meal feedings and time of feeding was recorded. Diets, feeding schedule, and fecal collection intervals were identical to the first collection. After two collections were made for Trial 7 (zeolite A diets), the pigs were weighed and adjusted to diets containing the four levels of clinoptilolite for five days prior to the start of Trial 8. Each duplicate collection period for the clinoptilolite fed pigs in Trial 8 was handled identically to the methods described in Trial 7. At the end of each collection period, the fecal samples were dried in an oven at 70° C, finely ground, and stored in small individual plastic bags which were labeled to identify pig number, time of collection, and dietary treatment received. Individual fecal samples were taken to the laboratory and analyzed in duplicate for chromic oxide concentration (mg) per gram of air dry feces at each collection time and was used to calculate a mean retention

time (θ) using the formula:

$$\theta = \frac{\prod_{i=1}^{n} C_{i} t_{i}}{\prod_{\substack{i=1\\j=1}^{\Sigma} C_{i}}}$$

where C_i is the marker concentration at time t_i after dosing (McDonald and Warner, 1974). Mean retention time values (hours) were analyzed statistically using a factorial analysis (considering the four factors of treatment levels, sexes, litters, and collection duplicates) to determine significant differences in mean retention time between the four dietary levels of zeolite A or clinoptilolite fed (Gill, 1978). Linear regression analysis was also used to determine if there was a linear relationship in mean retention time between the four dietary levels of clinoptilolite (Gill, 1978).

RESULTS AND DISCUSSION

I. Performance Trials

In Trial 1, low levels of zeolite A and clinoptilolite fed during the growing phase of production resulted in no significant effect on average daily gain (ADG), average daily feed intake (ADFI), or feed efficiency (F/G). Metabolizable energy utilization efficiency was improved (P<.06) in pigs fed either zeolite A or clinoptilolite. There was a non-significant trend for more efficient gains (lower F/G) by feeding either zeolite diet. A comparison of performance parameters of pigs fed .3% zeolite A, .5% clinoptilolite, or no zeolite is summarized in Table 13.

Due to improved energy utilization and a non-significant trend for better F/G using low levels of zeolite A and clinoptilolite, Trial 2 was designed to determine if the use of higher levels of zeolite A and clinoptilolite would improve the growth performance of finishing pigs. Incorporation of 1% zeolite A in the finishing diet for pigs appears to have no effect on ADG, ADFI, or F/G, while supplementing the diet with 5% clinoptilolite increased F/G but had no effect on ADG or ADFI. When metabolizable energy utilization efficiency was calculated, the performance from feeding the three experimental diets was very similar. Table 14 shows the comparison of performance parameters of finisher pigs fed diets containing 1% zeolite A, 5% clinoptilolite, or no zeolite.

The results of these two performance trials appear to indicate that if zeolite A or clinoptilolite promote growth performance in growing-

	Die	Significance ^a			
Ingredients	Control	.3% Zeo A	.5% Clinc	MSE	Ρ
Number of replicate pens	3	3	3		
Number of pigs	18	18	18		
Average initial weight, kg	26.8	27.3	24.7	3.37	NS
Average daily gain, g					
Replicate 1	799	863	826		
Replicate 2	867	754	776		
Replicate 3	822	903	890		
Average	831	840	831	3470	NS
Average daily feed intake,	9				
Replicate 1	2311	2325	2198		
Replicate 2	2288	2084	2175		
Replicate 3	2374	2456	2388		
Average	2325	2288	2238	15,100	NS
Feed/Gain					
Replicate 1	2.89	2.70	2.66		
Replicate 2	2.64	2.76	2.80		
Replicate 3	2.90	2.73	2.63		
Average	2.81	2.73	2.70	.01	NS
Metabolizable Energy Utiliza	ation Effi	ciency (kcal/	'g of gain)	-	
Replicate 1	9.26	8.35	8.09		
Replicate 2	8.44	8.57	8.52		
Replicate 3	9.24	8.43	7.99		
Average	8.98 ^b	8.45 ^C	8.20 ^d	.10	.06

TABLE 13.COMPARISON OF PERFORMANCE PARAMETERS OF PIGS FED CONTROL, .3%
ZEOLITE A OR .5% CLINOPTILOLITE DIETS DURING THE GROWING PHASE

^aNS=Not significantly different (P>.10).

b,c,d_{Means} within treatment having different superscript letters differ significantly.

	Di	Signifi	cancea		
Ingredients	Control	1% Zeo A	5% Clino	MSE	Ρ
Number of replicate pens	3	3	3		
Number of pigs	18	18	18		
Average initial weight, kg	64.9	65.2	65.0	7.08	NS
Average daily gain, g					
Replicate 1	799	758	649		
Replicate 2	708	645	636		
Replicate 3	<u>726</u>	<u>686</u>	<u>690</u>		
Average	745	696	658	2130	NS
Average daily feed intake,	9				
Replicate 1	2974	2892	2574		
Replicate 2	2592	2379	2461		
Replicate 3	2724	2561	2769		
Average	2765	2611	2601	43,200	NS
<u>Feed/Gain</u>					
Replicate l	3.73	3.82	3.96		
Replicate 2	3.66	3.69	3.86		
Replicate 3	3.82	3.73	4.01		
Average	3.74 ^b	3.75 ^b	3.94 ^C	.01	.02
Metabolizable Energy Utiliza	ation Effi	ciency (kcal	/g of gain)		
Replicate 1	12.02	12.19	12.14		
Replicate 2	11.83	11.78	11.84		
Replicate 3	12.12	11.93	12.28		
Average	11.99	11.97	12.09	.04	NS

TABLE 14.COMPARISON OF PERFORMANCE PARAMETERS OF FINISHER PIGS FED DIETS
CONTAINING 1% ZEOLITE A, 5% CLINOPTILOLITE, OR NO ZEOLITE

^aNS=Not significantly different (P>.10).

^{b,C}Means within treatment having different superscript letters differ significantly.

finishing pigs, then they must be fed at a level between the low and high levels of each zeolite used in these two trials.

II. Nutrient Balance Trials

Two balance trials were conducted to more completely examine the effects of a linear increase in dietary levels of each zeolite, with the intention of determining an optimum level of zeolite supplementation based on energy, nitrogen, and mineral balance, along with urinary p-cresolexcretion.

1. Effects of Zeolite A Supplementation on Nutrient Balance

A. Energy balance

In Trial 3, there was no significant linear effect on daily gross energy (GE) intake but daily fecal energy excretion was increased linearly (P<.01) with the addition of increasing levels of 0, 1, 2, or 3% zeolite A to the diet. Increases in daily fecal energy excretion resulted in a linear reduction of digestible energy (DE), metabolizable energy (ME), ME corrected for nitrogen balance (ME_N), and ME corrected for nitrogen balance and the energy dilution effect of zeolite A supplementation (ME_{NZ}) (P<.01). Of particular interest is the linear reduction in ME_{NZ} which indicates that even when ME is corrected for nitrogen balance and energy dilution by zeolite A, there is still a negative effect of zeolite A on energy utilization. Table 15 shows the effects of adding 0, 1, 2 or 3% zeolite A on energy balance.

Zeolite A in an aqueous suspension, has a basic pH due to limited hydrolysis. If the pH of this aqueous suspension is reduced by addition of an aqueous acid to a pH below 5 (normal hydrochloric acid secretion

	Lev	el of Zeo	Significance			
Energy Parameter ¹	0	1	2	3	MSE	P value
GE ² , kcal/g	3.93	3.88	3.84	3.79	-	-
Daily feed intake, g	268	288	308	306	-	-
Daily GE intake, g	1053	1118	1182	1151	37,424	NS
Daily fecal GE, kcal	103	117	127	145	354	.01
DE ³ , kcal/g	3.54	3.47	3.43	3.31	.00	.01
DE/GE x 100%	90.2	89.3	89.3	87.4	1.26	.01
Daily urine GE, kcal	24.4	25.9	31.3	22.5	43.6	NS
ME ⁴ ,kcal/g	3.45	3.37	3.32	3.24	.00	.01
Daily N balance ⁵ ,g	4.71	5.77	5.86	6.02	1.64	NS
Caloric correction ⁶ , kcal	31.9	39.1	39.7	40.8	75.0	NS
ME _N ⁷ , kcal/g	3.33	3.24	3.19	3.10	.00	.01
ME _N /GE x 100%	84.9	83.4	83.2	81.8	1.4	.01
ME ⁸ _{N7} ,kca1/g	3.33	3.28	3.27	3.23	.00	.01
$ME_{NZ}^{NZ}/GE_{Z}^{9} \times 100\%$	84.9	83.6	83.6	82.5	1.3	.05

TABLE 15. EFFECTS OF ADDING 0, 1, 2, and 3% ZEOLITE A ON ENERGY BALANCE

¹All values presented are mean values from four pigs assigned to each treatment group. ²GE = gross energy ³DE = digestible energy ⁴ME = metabolizable energy ⁵Daily N balance from Table 16. ⁶Caloric correction = N balance (g) x 6.77 kcal/gN (Diggs <u>et al.</u>, 1965). ⁷Nitrogen-corrected ME ⁸ME corrected for nitrogen balance and zeolite A dilution ⁹GE corrected for zeolite A dilution lowers the pH to the pig's stomach to a pH of 2), aluminum ions are removed from the framework of the zeolite A molecule and the molecular structure is destroyed (Breck, 1964). Zeolite A can exchange up to 35% of the sodium ions with acid before destruction of the molecule occurs (Breck, 1964). The detrimental effect of increasing levels of zeolite A supplementation on energy absorption, may be related to the dissociation of the zeolite A molecules by the acid environment of the stomach.

B. Nitrogen balance

Addition of linearly increasing levels of zeolite A had no significant linear effect on daily nitrogen intake but increased daily fecal nitrogen excretion (P<.01). As a result, apparent digestibility of nitrogen was reduced linearly (P<.01). Although not statistically significant, daily urinary nitrogen excretion tended to be reduced with higher levels of zeolite A supplementation. If zeolite A is effective in binding ammonia produced in the gastrointestinal tract by deamination of proteins during digestive processes and preventing its absorption and removal through the urea cycle, then fecal nitrogen would be expected to increase and urinary nitrogen excretion would be expected to decrease when zeolite A is added to the diet. Presence of this relationship is shown in Table 16 indicating that even if zeolite A is destroyed under the acid environment of the stomach some zeolite may bypass degradation (due to small particle size and frequent emptying of the stomach) and remain intact to carry out an ion exchange, ammonia binding function. As a direct indication of ammonia-zeolite binding, there was a significant linear improvement (P<.05) in the biological value of protein with additions of increasing levels of zeolite A. There was no linear effect on net protein utilization. Table 16 shows the effects of adding 0, 1, 2, or

	Leve	l of Zeo	Significance			
Nitrogen Parameter ¹	0	1	2	3	MSE	P value
Daily N intake, g	8.00	9.09	9.27	9.47	2.50	NS
Daily fecal N, g	1.02	1.25	1.31	1.73	.04	.01
Daily absorbed N, g	6.98	7.84	7.96	7.74	2.14	NS
Apparent dig. of N, %	87.3	85.9	85.8	81.6	4.3	.01
Daily urine N, g	2.27	2.06	2.12	1.73	2.01	NS
Daily N balance, g	4.71	5.77	5.84	6.02	1.64	NS
Net Protein Util. ² ,%	58.7	63.3	63.0	62.7	20.5	NS
Biol. Value of Protein ³	67.3	73.7	73.5	76.8	20.0	.05

TABLE 16. EFFECTS OF ADDING 0, 1, 2, and 3% ZEOLITE A ON NITROGEN BALANCE

¹All values presented are mean values from four pigs assigned to each treatment group.

²Net protein utilization, % = 100 (N balance + N intake).

³Biological value of protein = 100 (N balance ÷ N absorbed).

3% zeolite on nitrogen balance.

C. Mineral balance

Daily calcium intake and daily fecal calcium excretion were not linearly affected by increasing levels of zeolite A while daily urinary calcium increased linearly (P<.01). The non-significant tendency for a reduction in daily calcium balance produced a significant linear reduction in calcium retention (P<.01). Refer to Table 17 for comparison of daily intake, fecal and urinary excretion, balance, and retention for calcium, phosphorus, magnesium, sodium, potassium, iron, and zinc in pigs fed 0, 1, 2, or 3% zeolite A.

Linear increases in fecal excretion of phosphorus (P<.05) resulted in a linear reduction in phosphorus retention (P<.01) while daily phosphorus intake and excretion were unaffected by additions of higher levels of zeolite A in the diet.

When zeolite A is exposed to an acid environment with pH below 5, aluminum ions are removed from the framework of the zeolite A molecule and the structure is destroyed. Supplemental aluminum interferes with phosphorus absorption (Cox <u>et al.</u>, 1931; Deobald and Elvehjem, 1935; Jones, 1938; Street, 1942; Storer and Nelson, 1968; Valdivia <u>et al.</u>, 1982) and to a lesser extent calcium absorption (Valdivia <u>et al.</u>, 1982). Since zeolite A is probably dissociated at pH 2 in the pig's stomach, free aluminum released would interfere with calcium and phosphorus absorption producing the linear reductions observed in calcium and phosphorus retention.

Although daily magnesium intake, fecal and urinary excretion were not linearly affected by increasing levels of zeolite A in the diet, there was a significant linear reduction in daily magnesium balance (P<.05) and

Mineral ¹	Lev	Significance				
Calcium	0	1	2	3	MSE	P value
Daily intake, g	2.83	3.03	2.99	3.24	.27	NS
Daily fecal, g	0.75	0.66	0.60	1.04	.09	NS
Daily urine, g	0.05	0.24	0.49	0.33	.02	.01
Daily balance, g	2.03	2.13	1.91	1.88	.12	NS
% Retention	/2.3	70.2	63.8	58.4	36.8	.01
Phosphorus						
Daily intake, g	1.82	1.95	2.06	2.04	.11	NS
Daily fecal, g	.67	.84	. 92	1.13	.07	.05
Daily urine, g	.04	.01	.02	.01	.00	NS
Daily balance, g	1.11	1.10	1.12	.90	.03	NS
% Retention	61.7	55.3	55.2	44.0	24.6	.01
Magnésium						
Daily intake, g	0.37	0.41	0.44	0.40	.00	NS
Daily fecal, g	0.18	0.23	0.20	0.25	.00	NS
Daily urine, g	0.03	0.05	0.08	0.05	.00	NS
Daily balance, g	0.16	0.13	0.16	0.09	.00	.05
% Retention	43.8	31.2	36.7	23.5	66.5	.01
Sodium						
Daily intake, g	0.43	1.01	1.71	2.23	.07	.01
Daily fecal, g	0.08	0.20	0.21	0.39	.01	.01
Daily urine, g	0.05	0.22	0.58	0.73	.01	.01
Daily balance, g	0.30	0.59	0.91	1.11	.02	.01
% Retention	69.1	58.1	53.9	50.0	87.5	.05
Potassium						
Daily intake, g	2.54	2.79	2.69	2.42	.22	NS
Daily fecal, g	0.55	0.57	0.55	0.67	.01	NS
Daily urine, g	0.61	0.75	0.86	0.65	.05	NS
Daily balance, g	1.38	1.47	1.28	1.10	.08	NS
% Retention	54.4	52.3	47.7	45.0	9.6	.01
Iron						
Daily intake, mg	79.4	84.1	75.2	74.8	189.8	NS
Daily fecal, mg	34.0	44.1	36.7	41.8	87.5	NS
Daily urine, mg	0.18	0.50	0.67	0.47	.07	NS
Daily balance, mg	45.2	39.5	37.8	32.6	57.9	.05
% Retention	56.8	46.2	48.7	43.3	42.9	.05
Zinc						
Daily intake. mg	25.4	24.1	29.1	30.3	22.9	NS
Daily fecal. mg	16.6	16.1	16.8	20.3	14.5	NS
Daily urine. mg	2.53	1.11	1.74	1.13	.44	.05
Daily balance, mg	6.29	6.96	10.57	8.87	5.68	NS
% Retention	25.0	28.3	36.8	29.4	21.8	NS

TABLE 17. EFFECTS OF ADDING 0, 1, 2, and 3% ZEOLITE A ON MINERAL BALANCE.

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All values presented are mean values from four pigs assigned to each treatment group.

magnesium retention (P<.01). In sheep fed supplemental aluminum, there was no effect on magnesium absorption <u>per se</u> but there was a reduction in plasma magnesium which might suggest that there must be a reduction in magnesium absorption from high dietary aluminum levels even though no significant differences in absorption were observed (Valdivia <u>et al.</u>, 1982). Thus, free aluminum (or silicon) may bind magnesium, reducing magnesium absorption by forming unabsorbable aluminates (and silicates).

Due to the high sodium content of zeolite A, linearly increasing amounts of sodium intake (P<.01) resulted in a linear increase in daily fecal (P<.01) and daily urinary sodium excretion (P<.01) when increasing amounts of zeolite A were fed. Daily sodium balance increased linearly (P<.01) with each higher level of zeolite A fed but in proportion to daily intake. sodium retention was reduced in a linear fashion (P<.05). Since zeolite A molecules are probably dissociated by the low pH of the stomach, free sodium is available for absorption in excess of the sodium requirement resulting in increasing amounts of urinary sodium excreted with higher amounts of zeolite A fed in order to maintain osmotic balance in the body. The physiological role of sodium, potassium, and chloride in the regulation of osmotic pressure and acid-base balance in the animal has been well established (Best and Taylor, 1961; Shohl and Sato, 1923; Chan, 1974; Mongin, 1981). The acid-base balance of an animal is determined by the difference between total cation and anion intake and total cation and anion excretion, taking into account the hydrogen ions generated from organic constituents (i.e. proteins) during normal metabolic processes (Shohl and Sato, 1923, Chan, 1974; Mongin, 1981; Lennon et al., 1966). Excess sodium may also play a role in

reducing growth performance as observed in chicks fed high amounts of sodium relative to potassium (Nesheim <u>et al.</u>, 1964). Thus, high amounts of sodium may be one of the reasons for the linear reduction in ME and nitrogen absorption as discussed previously.

Daily potassium intake, daily fecal and urinary excretion, and daily potassium balance were unaffected linearly as the amount of zeolite A was increased in the diet. However, potassium retention was linearly reduced (P<.01) with increasing diet level of zeolite A. The proportion of sodium relative to potassium is a closely regulated mechanism. There is a sparing action regarding sodium levels in the diet relative to the amount of potassium required for maximum growth in chicks (Burns et al., 1953). Burns et al. (1953) observed that .1% sodium and 1% potassium in the diets for chicks produced maximum growth rate whereas when the sodium level was increased to .18%, the potassium requirement was reduced to .33% to obtain the same growth response. There is a slight sparing effect of the sodium requirement by dietary levels of potassium while there is a larger sparing effect of the potassium requirement when high levels of sodium are fed (Grunertet al., 1950). Thus, excess sodium supplied by zeolite A reduces the potassium requirement as more sodium is absorbed resulting in the linear reduction in potassium retention which is needed to maintain osmotic balance. Sodium and potassium may also form aluminates and silicates with the components of dissociated zeolite A, also affecting sodium and potassium absorption.

Daily iron intake and daily fecal and urinary iron excretion were unaffected by increasing levels of zeolite A in the diet. Iron excretion relative to intake linearly reduced daily iron balance (P<.05) resulting in a linear reduction in iron retention (P<.05). Helbock and Saltman

(1967), Forth and Rummel (1973), Terato <u>et al</u>. (1974), and Linder <u>et al</u>. (1975) have shown the efficacy of chelating agents in iron transport processes of mucosal cells. Martinez-Torres <u>et al</u>. (1981) observed a two-fold increase in iron absorption when cysteine was added to the diet in humans and postulated that proteins and cysteine share the same mechanism (forming ligands with iron for absorption) for inducing an increase in iron absorption. Since zeolite A appears to impair nitrogen (amino acid and protein) absorption, then it may be expected that iron absorption would be impaired as evidenced by the non-significant linear trend for increasing fecal iron excretion as the levels of zeolite A are increased in the diet. Thus, a linear reduction in iron retention may be a result of poor iron absorption due to reductions in amino acid absorption or by interfering with chelates need for optimum absorption.

Daily urinary zinc excretion was linearly reduced (P<.05) while increasing levels of zeolite A did not linearly affect daily zinc intake, fecal excretion, balance, or retention. Kratzer <u>et al</u>. (1961), studied factors that influence binding of zinc by protein (in vitro) and determined that the amount of zinc bound was influenced by the pH range near the isoelectric point of an insoluble protein. Thus, if zeolite A changes the pH of the intestine, inefficient binding of zinc with proteins may cause a reduction in zinc absorption, producing a need for conservation of zinc in the body by reducing the urinary zinc excretion, as observed when increasing amounts of zeolite A are fed. Reduced calcium absorption may also play a role in forming a Ca-Zn phytate complex and thus reducing zinc absorption.

D. Urinary p-cresol excretion

Daily free or conjugated urinary p-cresol excretion was not linearly affected by increasing levels of zeolite A in the diet due to the variability of the observations within treatment levels. Table 18 shows the treatment means of free and conjugated p-cresol excreted in the urine from pigs fed 0, 1, 2, and 3% zeolite. There was a large amount of variation among observations in each treatment and could be explained in three ways. First, due to the arrangement of the collection cages, feces often sift through the wire screen and settle on the urine collection tray and thus enter the urine collection container by being washed into the container by subsequent urinations. Since p-cresol is present in various concentrations in feces (Spoelstra, 1977; 1978; Anderson, 1975; Yasuhara and Fuwa, 1979) fecal contamination in urine would elevate free urinary p-cresol levels giving inaccurate urinary excretion values depending on the amount of fecal contamination in the urine. Second, there may be glucuronide deconjugation occurring on the urine collection tray or in the collection beaker resulting in the tendency for conjugated p-cresol values to be greater than the free p-cresol values and producing the variability in urinary p-cresol values observed. A bacteriastat is added to prevent the hydrolysis of sulfate or glucuronide conjugates. If urine is trapped on the collection tray by feces or if the Timersol is not mixed well with the urine when entering the collection vessel, variability in urinary p-cresol values could occur. Third, zeolite A may not have any effect on p-cresol excretion although free p-cresol tends to be elevated in urine collected from pigs fed increasing amounts of zeolite A while the amount of conjugated p-cresol tends to decrease. There was a tendency for the conjugated form to be greater

than the free form for each urine sample and may be explained by the inoculation of urine with feces which results in hydrolysis of the conjugated form of p-cresol (Spoelstra, 1977; 1979). Another possibility of the higher free p-cresol excretion compared to the hydrolyzed may be due to acid destruction of some p-cresol during the acid hydrolysis procedure used in determining the amounts of conjugated urinary p-cresol.

2. Effects of Clinoptilolite Supplementation on Nutrient Balance

In Trial 4, diets containing 0, 2.5, 5.0 or 7.5% clinoptilolite were fed to growing pigs to determine if there is an optimum level of clinoptilolite supplementation based on energy, nitrogen, and mineral balance.

A. Energy balance

There was no linear relationship for daily GE intake or daily fecal and urinary energy excretion when increasing amounts of clinoptilolite were fed but there was a linear reduction in DE, ME, ME_N, and ME_{NZ} as clinoptilolite levels were increased in the diet. A comparison of energy balance parameters is shown in Table 19. Even when ME_N was corrected for the energy dilution of the addition of clinoptilolite, there still was a linear reduction in energy utilization when increasing amounts of clinoptilolite were fed. Thus, clinoptilolite (intact or dissociated) must be exerting a detrimental effect on energy absorption.

B. Nitrogen balance

As in Trial 3, daily nitrogen intake was not linearly affected but fecal nitrogen excretion was linearly increased (P<.O1) resulting in a linear reduction in apparent digestibility of nitrogen (P<.O1) even though daily nitrogen absorbed was not linearly affected when increasing

P-cresol parameter ^a	Dietary	level o	Significance ^b			
	0	1	2	3	MSE	P Value
Daily urinary p-cresol excretion (free form), mg.	15.26	17.01	37.11	29.17	250.32	NS
Daily urinary p-cresol excretion (conjugated form), mg	23.81	22.35	25.62	18.33	57.51	NS

TABLE 18.EFFECTS OF FEEDING DIETS CONTAINING 0, 1, 2, and 3% ZEOLITE
A ON DAILY URINARY P-CRESOL EXCRETION.

bNS = Not significant (P>.05)

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amounts of clinoptilolite were fed. Daily urinary nitrogen excretion, daily nitrogen balance, and biological value of protein were not linearly affected by feeding increasing amounts of clinoptilolite. However, net protein utilization was reduced in a linear manner (P<.05) as more clinoptilolite was added to the diets. Table 20 shows the effects of supplementing diets with 0, 2.5, 5.0, and 7.5% clinoptilolite on nitrogen balance. When comparing the levels and responses in nitrogen balance from zeolite A fed pigs (Trial 3) to the levels and responses observed in feeding clinoptilolite (Trial 4), it appears that high levels of clinoptilolite are less effective than the levels of zeolite A fed on improving the biological value of protein. As discussed in the nitrogen balance section in Trial 3, if zeolites are effective in binding ammonia produced by deamination of proteins during digestion and thus reducing urea cycle activity, then it would be expected that urinary nitrogen excretion would be reduced while fecal nitrogen excretion would increase as more clinoptilolite is fed. Despite the non-linearity in urinary nitrogen excretion, there was a non-significant trend for urinary nitrogen excretion to be reduced as more clinoptilolite was fed. The high levels of clinoptilolite fed are probably in excess to obtain maximum ammonia removal resulting in a plateau response of high levels of supplementation. Furthermore, it appears that clinoptilolite may bind ammonia to some degree due to some clinoptilolite by-passing degradation of low pH in the stomach but degradation of most of the clinoptilolite would not be capable of this phenomena and may reduce amino acid absorption as seen by the linear reduction in net protein utilization when increasing amounts of clinoptilolite are fed. Thus, it appears that the 5.0 and 7.5% levels of clinoptilolite used in Trial 4 are far above levels needed

	Level	of Clino	Significance			
Energy Parameter ¹	0	2.5	5.0	7.5	MSE	P value
GE ² ,kcal/g	3.99	3.83	3.68	3.58	-	-
Daily feed intake, g	276	264	269	268	-	-
Daily GE intake, kcal	1098	1012	991	961	8884	NS
Daily fecal GE, kcal	126	121	142	140	341	NS
DE ³ , kcal/g	3.53	3.37	3.15	3.06	.00	.01
DE/GE x 100%	88.6	88.0	85.5	85.4	2.3	.01
Daily urine GE, kcal	30.3	32.4	25.3	29.4	33.7	NS
ME ⁴ , kcal/g	3.42	3.25	3.06	2.95	.00	.01
Daily N balance ⁵ , g	5.36	5.03	5.25	4.77	.26	NS
Caloric correction ⁶ , kcal	36.3	34.0	35.5	32.3	11.8	NS
ME _N ⁷ , kcal/g	3.29	3.12	2.93	2.83	.00	.01
ME _N /GE × 100%	82.5	81.4	79.4	79.0	1.6	.01
ME ⁸ NZ,kcal/g	3.29	3.22	3.12	3.12	.00	.01
ME _{NZ} /GE ⁹ Z × 100%	82.5	81.9	80.5	80.6	1.5	.05

TABLE 19. EFFECTS OF ADDING 0, 2.5, 5.0, and 7.5% CLINOPTILOLITE ON ENERGY BALANCE

All values presented are mean values from four pigs assigned to each treatment group.

²GE = gross energy

 3 DE = digestible energy

⁴ME = metabolizable energy

⁵Daily nitrogen balance from Table 19.

⁶Caloric correction = N balance (g) x 6.77 kcal/g N (Diggs <u>et al.</u>, 1965). ⁷Nitrogen-corrected ME

⁸ME corrected for nitrogen balance and clinoptilolite dilution

⁹GE corrected for clinoptilolite dilution

for optimum response.

C. Mineral balance

Daily calcium intake and daily fecal calcium excretion were not linearly affected by increasing levels of clinoptilolite in the diet while daily urinary calcium increased linearly (P<.01). Table 21 shows the effects of adding 0, 2.5, 5.0, and 7.5% clinoptilolite to the diet on calcium, phosphorus, magnesium, sodium, potassium, iron, and zinc balance. But due to small contribution of daily urinary calcium excretion in daily calcium balance and calcium retention, the parameters were also not linearly affected. Thus, it appears that supplementing clinoptilolite, up to 7.5% of the diet, has no effect on calcium absorption and utilization.

Linear decreases in daily phosphorus balance and phosphorus retention were observed when increasing amounts of clinoptilolite were added to the diet even though daily intake, fecal and urinary excretion of phosphorus was not linearly affected. If clinoptilolite is dissociated into its primary components (sodium, aluminum, and silicon) then the linear reduction observed in daily urinary calcium excretion and phosphorus retention can be attributed to the formation of insoluble phosphorus-calcium-aluminum complexes resulting in poor absorption of calcium and phosphorus.

Daily magnesium intake, daily fecal excretion, and daily balance were linearly increased (P<.O1) with additions of higher levels of clinoptilolite in the diet, while not affecting daily urinary magnesium excretion. Even though magnesium retention was not linearly affected, addition of each higher level of clinoptilolite tended to improve magnesium retention.

Due to the high sodium content of clinoptilolite, linearly increasing

	Level	of Clino	Significance			
Nitrogen Parameter ¹	0	2.5	5.0	7.5	MSE	P value
Daily N intake, g	8.10	7.65	7.96	7.78	.51	NS
Daily fecal N, g	1.14	1.14	1.38	1.59	.03	.01
Daily absorbed N, g	6.96	6.52	6.58	6.19	. 37	NS
Apparent dig, of N, %	86.1	85.2	82.6	79.6	3.1	.01
Daily urine N, g	1.61	1.49	1.35	1.43	.05	NS
Daily N balance, g	5.35	5.03	5.25	4.77	.26	NS
Net Protein Util. ² , %	66.3	65.7	65.8	61.2	6.4	.05
Biol. Value of Protein ³	77.0	77.2	79.7	77.0	7.5	NS

TABLE 20. EFFECTS OF ADDING 0, 2.5, 5.0, and 7.5% CLINOPTILOLITE ON NITROGEN BALANCE.

All values presented are mean values from four pigs assigned to each treatment group.

²Net protein utilization, % = 100 (N balance + N intake)

³Biological value of protein = 100 (N balance \div N absorbed)
amounts of sodium intake (P<.01) produced a linear increase in daily fecal sodium excretion (P<.01) and daily sodium balance (P<.01) as higher levels of clinoptilolite were incorporated into the diet. Increasing levels of clinoptilolite did not linearly affect daily urinary sodium excretion or sodium retention even though retention tended to be reduced.

Daily potassium intake was not affected in a linear manner while daily fecal potassium excretion increased linearly (P<.05) and daily urinary potassium excretion decreased linearly (P<.01) when higher amounts of clinoptilolite were fed. There was no linear relationship on daily potassium balance or potassium retention even though there as a tendency for these parameters to be reduced. The increased fecal excretion and decreased urinary excretion of potassium as more clinoptilolite was added to the diet, may be explained by the sodium-potassium sparing effect (Grunert<u>et al</u>., 1950). When high amounts of sodium are fed, the potassium requirement is reduced. Thus, less potassium is absorbed resulting in higher fecal potassium excretion and less potassium is excreted in the urine to maintain osmotic balance in the body.

Clinoptilolite typically contains 1.18% Fe₂O₃ (see Table 4). As a result, daily iron intake increased linearly (P<.O1) as larger amounts of clinoptilolite were added to the diet. The linear increase in daily fecal iron excretion (P<.O1) was a direct result of high amounts of iron intake when supplementing higher amounts of clinoptilolite in the diet. There was no linear effect of increasing increments of clinoptilolite on daily urinary iron excretion while daily iron balance was improved linearly (P<.O1). There was no linear effect on iron retention when higher levels of clinoptilolite were added to the diet to the diet but there was a

non-significant tendency for iron retention to be improved.

Cox <u>et al</u>. (1931), Brock and Diamond (1934), Rehm and Winters (1940), Jones (1938), Alsmeyer <u>et al</u>. (1963), Furugouri(1972) and O'Donovan <u>et</u> <u>al</u>. (1963) have shown that soluble iron sources in excess levels interfere with phosphorus utilization possibly by precipitating phosphorus as an insoluble phosphate within the intestinal tract. Thus, the reduction in phosphorus retention may be due to high amounts of iron as well as aluminum in the diet and interfering with phosphorus absorption.

Daily zinc intake increased linearly (P<.05) as greater amounts of clinoptilolite were fed which might have been caused by the impurity of clinoptilolite which may include small amounts of zinc. Despite increasing amounts of zinc intake, daily fecal and urinary excretion or zinc retention was not affected in a linear manner as more clinoptilolite was fed. Nevertheless, daily zinc balance was improved linearly (P<.05) as more clinoptilolite was fed.

The linear improvements in iron and zinc balance can be explained simply by the linear increases in intake of these two elements but there may be improved amino acid absorption or a reduction of chelates which are thought to be important in facilitating iron and zinc absorption.

D. Urinary p-cresol excretion

Daily free and conjugated urinary p-cresol excretion was reduced linearly (P<.01) when increasing amounts of clinoptilolite were fed. Table 22 shows the treatment means for free and conjugated urinary p-cresol excretion produced from pigs fed 0, 2.5, 5.0, and 7.5% clinoptilolite. Thus, it appears that clinoptilolite is effective in fecal elimination of p-cresol produced by the breakdown of tyrosine in the lower gastrointestinal tract. Note that the amounts of free p-cresol

Mineral ¹	Level of Clinoptilolite (%)					Significance	
Calcium	0	2.5	5.0	7.5	MSE	P value	
Daily intake, g Daily fecal, g Daily urine, g Daily balance, g % Retention	2.39 0.84 0.05 1.50 63.1	2.28 0.77 0.08 1.42 62.9	2.53 0.94 0.11 1.49 58.4	2.62 0.91 0.13 1.59 60.5	.07 .02 .00 .03 18.4	NS NS . 01 NS NS	
Phosphorus Daily intake, g Daily fecal, g Daily urine, g Daily balance, g % Retention	1.85 .82 .01 1.02 55.7	1.72 .69 .01 1.02 59.3	1.79 .88 .01 .91 50.4	1.74 .90 .01 .83 47.8	.03 .02 .00 .01 26.1	NS NS NS . 05 . 05	
Magnesium Daily intake Daily fecal, g Daily urine, g Daily balance, g % Retention	.39 .26 .05 .08 21.1	.43 .28 .05 .10 22.7	.53 .33 .07 .13 24.0	.59 .37 .07 .16 26.4	.00 .00 .00 .00 14.0	.01 .01 NS .01 NS	
Sodium Daily intake, g Daily fecal, g Daily urine, g Daily balance, g % Retention	.20 .04 .03 .13 64.4	.24 .05 .04 .15 63.2	.31 .08 .06 .17 55.6	.34 .09 .05 .20 59.5	.00 .00 .00 .00 79.4	.01 .01 NS .01 NS	
Potassium Daily intake, g Daily fecal, g Daily urine, g Daily balance, g % Retention	1.02 .15 .40 .48 47.4	1.02 .21 .36 .45 44.0	.96 .22 .28 .47 48.3	.97 .25 .30 .43 43.7	.01 .00 .00 .00 21.9	NS . 05 . 01 NS NS	
Iron Daily intake, mg Daily fecal, mg Daily urine, mg Daily balance, mg % Retention	65.5 45.0 .52 20.0 29.9	96.2 59.9 .64 35.7 36.7	174.0 103.2 .69 70.1 40.2	206.4 130.6 .65 75.2 36.5	197.7 110.7 .04 139.6 47.5	.01 .01 NS .01 NS	
Zinc Daily intake, mg Daily fecal, mg Daily urine, mg Daily balance, mg % Retention	22.8 18.2 1.74 2.92 12.3	24.6 16.9 1.28 6.50 26.5	26.7 20.0 1.29 5.39 19.8	26.7 18.5 1.76 6.41 24.1	4.6 4.5 .30 3.54 51.9	. 05 NS NS . 05 NS	

TABLE 21. EFFECTS OF ADDING 0, 2.5, 5.0, and 7.5% CLINOPTILOLITE ON MINERAL BALANCE (Trial 4).

All values presented are mean values from four pigs assigned to each treatment group.

excretion are much lower than the amounts of conjugated p-cresol. This indicates that little, if any, fecal contamination of urine samples occurred with much less variability and more accurate results than those observed in Trial 3. If p-cresol is a cause in reducing growth performance in pigs (Yokoyama <u>et al</u>., 1982) then it would be expected that if clinoptilolite is effective in the fecal elimination of p-cresol and prevention of its absorption, then this should result in improved growth performance.

III. Evaluation of Plasma and Serum Components

After conducting nutrient balance Trials 3 and 4, it was decided to try to substantiate the effects of zeolite A (Trial 5) and clinoptilolite (Trial 6) on nutrient balance by a blood sampling at 15, 30, 60, and 120 minutes after ingestion of a meal containing the same levels of zeolites used in balance Trials 3 and 4. Plasma ammonia and glucose and serum calcium, phosphorus, sodium, and potassium were determined to more closely evaluate the effects of zeolites in the absorption of ammonia and nutrients.

1. Effect of zeolite A on plasma and serum components

Table 23 shows the effects of 0, 1, 2, and 3% zeolite A diets on plasma ammonia and glucose and serum calcium, phosphorus, sodium, and potassium.

A. Ammonia

There was no effect of levels of zeolite A, time of bleeding, or treatment x time interaction on plasma ammonia concentrations up to two hours after bleeding. Most ammonia production occurs in the large intestine by bacterial deamination of proteins (Michel, 1966; Fauconneau and Michel, 1970). Kvasnitskii (1951) showed that 61% of the dry matter

TABLE 22.	EFFECTS OF FEEDING DIETS CONTAINING 0, 2.5, 5.0, and 7.5	%
	CLINOPTILOLITE ON DAILY URINARY P-CRESOL EXCRETION.	

	Dietary level of clinoptilolite (%)					Significance	
P-cresol parameter ^a	0	2.5	5.0	7.5	MSE	P value	
Daily urinary p-cresol excretion (free form), mg	9.27	3.08	5.17	2.49	6.95	.01	
Daily urinary p-cresol excretion (conjugated form), mg.	. 152.35	132.18	92.98	91.19	685.46	.01	

^aAll values presented are treatment mean values.

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leaving the stomach in 24 hours passed the small intestine in 8 hours after feeding. Kvasnitskii (1951) also observed that there is considerable variation between pigs and the quantity of feed given and that fullness of the stomach and activity of the animal are the two major factors contributing to amount and time of stomach emptying. Neimeier (1940) observed that ingesta passes through the small intestine between 2-6 hours. Based on these references, it is not very likely that the meal fed would reach the large intestine for ammonia production in two hours. However, the previous meal was given 12 hours prior to blood sampling and would be located in the large intestine at the time of blood sampling. Nevertheless, differences were not detected which may be due to the inability of zeolite A to prevent ammonia absorption or perhaps, stress during bleeding producing variable and abnormal values.

B. Glucose

Plasma glucose concentrations were unaffected by levels of zeolite A fed, time of bleeding, or treatment x time interaction. Auffray <u>et</u> <u>al</u>. (1967) observed that after 15 minutes of ingesting a meal, 40% of the stomach contents passed into the duodenum and the volume of digesta emptied was related to the amount ingested. After 15 minutes, little stomach emptying occured until 90 minutes after feeding. Monosaccharides are absorbed from the gut lumen at different rates; glucose and galactose are absorbed rapidly. Thus, glucose absorption should start to occur by 15-30 minutes if the ingesta has entered the small intestine. No significant effect of level of zeolite A or time was observed, indicating that zeolite A has no effect on glucose absorption, ingesta had either not entered the small intestine or was not present for a long enough time

for differences to occur, or the stress of the pig occurring during bleeding may have produced variable and artificial values.

C. Calcium

Serum calcium levels were elevated (P<.001) when higher amounts of zeolite A were fed and decreased at each successive bleeding (P<.01). It appears that zeolite A improves calcium absorption which is contrary to calcium balance determinations in Trial 3. Since serum phosphorus concentrations were reduced by feeding zeolite A, serum calcium concentrations may have been elevated due to lowered phosphorus availability as shown by Hiller <u>et al</u>. (1964; 1966).

D. Phosphorus

Serum phosphorus concentrations were significantly lowered (P<.001) by feeding zeolite A and decreased in each successive bleeding (P<.001). Reductions in serum phosphorus were expected since zeolite A is probably dissociated in the acid environment of the stomach resulting in the reduction in phosphorus absorption by the binding of free aluminum (from zeolite A) with phosphorus forming an insoluble phosphate complex as observed by Valdivia <u>et al</u>. (1982). Time of bleeding may have lowered serum phosphorus because of more time allowed for the formation of the insoluble phosphorus-aluminum complex.

E. Sodium

Serum sodium levels were elevated (P<.001) when zeolite A was fed, due to the high levels of sodium present in the diet from the zeolite A and the rapid absorption of sodium in the duodenum after ingestion. Thus, it appears that zeolite A improves sodium absorption in contrast to the observations of sodium balance in Trial 3.

F. Potassium

Serum potassium concentrations were unaffected by zeolite A or time of bleeding.

2. Effect of clinoptilolite on plasma and serum components.

Table 24 illustrates the effects of 0, 2.5, 5.0, and 7.5% clinoptilolite diets on plasma ammonia and glucose and serum calcium, phosphorus, sodium, and potassium.

A. Ammonia

Plasma ammonia was reduced by adding higher levels of clinoptilolite to the diet (P<.003) and was reduced at each successive bleeding (P<.002). There was also a significant treatment x time interaction (P<.005). Since two hours is not a long enough period of time for a single meal feeding to reach the large intestine, which is the primary site of ammonia production (Michel, 1966; Fauconneau and Michel, 1970), the reductions in plasma ammonia concentrations in clinoptilolite fed pigs is probably due to the binding of ammonia in the large intestine by the meal fed 12 hours prior to blood sampling. Reductions in plasma ammonia at each successive bleeding may be due to continued binding of ammonia by clinoptilolite from a slowing of the bacterial deamination of proteins reaching the large intestine from the previous meal.

B. Glucose

At each successive bleeding times, plasma glucose concentrations increased (P<.001) which is due to rapid absorption of glucose once it enters the small intestine which takes place by two hours after feeding (Auffrey, <u>et al.</u>, 1967). There was no effect of clinoptilolite levels on plasma glucose concentrations.

.		Plasma or Serum Component ^a						
B' Sar	me or lood mpling	Level of Zeolite A	NH4+	Glucose	Ca ^b	Р	Na	к
_(r	min.)	(%)	(µmo1/l)	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)
		0	4υ	90	10.8	8.7	288	25.8
	16	1	29	89	11.7	8.4	274	27.6
	15	2	65	87	13.0	7.2	261	28.2
		3	33	98	14.1	6.8	270	27.7
		0	52	124 ·	10.6	9.2 .	307	26.1
		I	25	105	11.9	8.5	280	28.0
	30	2	34	94	13.3	6.8	261	26.6
		3	26	107	13.6	6.5	269	24.6
		0	37	1 38	10.5	8.8	301	26.5
	60	1	36	80	11.6	8.2	278	26.8
		2	15	96	12.7	5.9	270	29.7
		3 ·	24	105	13.5	5.8	261	25.1
		0	31	108	10.5	8.2	299	25.2
12	20	I	23	107	11.7	8.0	291	29.1
		2	33	100	13.1	5.7	259	28.0
		3	20	116	14.0	5.3	267	27.1
_د ۲,	,d							
I		Trt.	747	1549	32.4	27.4	4303	21.0
GN	MS	Time	754	1009	. 32	3.5	128	1.2
I F		Trt. x Time	e 505	573	.15	.43	159	1.8
I C	·	Trt.	NS	NS	.001	.001	.001	NS
A.	<u>P value</u>	Time	NS	NS	.01	.001	NS	NS
		Trt. x Time	e NS	NS	NS	NS	NS	NS

TABLE 23.EFFECTS OF FEEDING 0, 1, 2, and 3% ZEOLITE A ON PLASMAAMMMONIA AND GLUCOSE AND SERUM Ca, P, Na, and K AT 15,30, 60 and 120 MINUTES AFTER A MEAL FEEDING.

^aAll values presented are treatment means at each time of blood sampling. ^bSignificant sex effect (P<.05)

^CTrt. x Time = treatment level x time interaction

^dNS = not significant (P>.05)

C. Calcium

Clinoptilolite did not affect serum calcium concentrations which is consistent with calcium balance measures observed in Trial 4 (Table 21). There was also no effect on time of blood sampling. Thus, calcium absorption appears to be unaffected by feeding clinoptilolite supplemented diets.

D. Phosphorus

Clinoptilolite significantly reduced serum phosphorus concentrations (P<.016) which is probably due to dissociation of a portion of clinoptilolite molecules in the acid environment of the stomach causing free aluminum to bind phosphorus forming unabsorbable aluminum-phosphorus complexes as noted by (Valdivia, <u>et al.</u>, 1982). Serum phosphorus was also reduced at each successive bleeding (P<.001) which may be due to a longer time for aluminum to inhibit phosphorus absorption. Furthermore, there was a significant treatment x time interaction. These results are consistent with the reductions in phosphorus retention observed in Trial 4 (Table 21).

E. Sodium

Serum sodium levels were reduced (P<.001) by feeding increasing amounts of clinoptilolite while successive blood samplings seemed to increase serum sodium (P<.001). It appears that clinoptilolite reduces sodium absorption which is contrary to the sodium balances relationship observed in Trial 4 (Table 21). It is expected that more sodium would be absorbed into the plasma at each successive bleeding since sodium is rapidly absorbed in the intestine and more and more ingesta from the stomach enters the duodenum increasing the amount of sodium available

for absorption.

F. Potassium

Serum potassium levels increased (P<.002) as higher levels of clinoptilolite were fed which may be due to the potassium-sodium sparing effect (Burns <u>et al.</u>, 1953, Grunert <u>et al.</u>, 1950). Since sodium absorption is impaired by feeding clinoptilolite, potassium absorption is improved. There was also a significant treatment x time interaction (P<.001).

IV. Passage Rate Trials

After completing the performance, balance, and blood sampling trials, it was decided to examine the effects of feeding 0, 1, 2, and 3% zeolite A or 0, 2.5, 5.0, and 7.5% clinoptilolite diets on rate of passage. Rate of passage of feedstuffs through the gastrointestinal tract is difficult to assess because of the insensitivity and ambiguity of many of the criteria commonly used. Measurement of the initial appearance and disappearance of a marker is unsatisfactory because of the rise and gradual fall of the marker concentration (Kidder and Manners, 1978). Mean retention times (MRT) were the most accurate mode of expression in chromium mordanted wheat bran (Van Soest <u>et al.</u>, 1983). Thus MRT was used to measure rate of passage (average amount of time a meal stays in the digestive tract) based on the concentration of marker in the feces at various points in time after feeding a single dose of marker (McDonald and Warner, 1975) to better understand how zeolites affect nutrient absorption and utilization.

		Plasma or	Serum Co	omponent ^a			
Blood Sampling	Level of Clinop- tilolite	NH4+	Glucose	Ca ^b	Р	Na	K
<u>(min.)</u>	(%)	(µmo1/2)	(mg/dl)	(mg/dl)	(mg/dl) (mg/dl)(mg/dl)
	0.0	130	93	11.7	8.6	265	24.5
15	2.5	100	83	11.7	9.3	269	27.1
15	5.0	59	83	11.4	8.4	229	21.6
	7.5	53	85	12.6	8.2	259	30.8
	0.0	49	87	11.8	8.7	282	26.0
30	2.5	90	94	11.7	8.6	272	25.7
50	5.0	34	92	11.5	8.4	242	23.5
	7.5	55	96	12.1	7.4	257	26.3
	0.0	48	91	11.8	8.2	265	25.0
	2.5	100	89	11.9	8.5	264	26.2
60	5.0	56	8 9	11.6	· 8.6	221	23.5
	7.5	72	84	12.5	7.0	246	25.5
	0.0	52	94	11.7	8.5	276	25.3
120	2.5	76	104	12.2	8.4	267	29.8
120	5.0	45	102	11.7	8.0	227	22.1
s ^{b,c,d}	7.5	49	101	12.1	6.9	251	27.3
I G	Trt.	9.3	3.9	1.7	5.8	5855	79.3
N MS	Time	6.3	9.0	.08	1.5	503	4.1
F I	Trt. x Tim	e 3.3	1.1	.14	. 37	83	12.2
C A	Trt.	.003	NS	NS	.016	.001	.002
N P value	Time	.002	.001	NS	.001	.011	NS
ι Ε	Trt. x Tim	e.005	NS	NS	.019	NS	.001
2							

TABLE 24.	EFFECTS OF FEEDING 0, 2.5, 5.0, and 7.5% CLINOPTILOLITE ON
	PLASMA AMMONIA AND GLUCOSE AND SERUM Ca, P, Na, and K AT
	15, 30, bu and 120 MINULES AFLER A MEAL FEEDING.

^aAll values presented are treatment means at each time of blood sampling ^bThere was a significant sex effect ^CTrt. x Time = treatment level x time interaction

dNS = not significant (P*:05)

A. Mean retention time in zeolite A fed pigs

Table 25 summarizes the MRT values for the four levels of zeolite A in relation to litter, sex, and the two collection periods. MRT was not affected by litter, sex, or collection period, but when increasing levels of zeolite A are fed, MRT declines (P<.02), indicating a faster rate of passage. Furthermore, when MRT values were tested for a linear relationship, MRT was linearly reduced (P<.05) in the first period and linearly reduced (P<.01) in the second period. Castle and Castle (1957) showed that the rate of food passage was inversely related to the amount of bulk in the diet regardless of moisture content of the feed. Thus, additions of zeolite A to the diet may act like bulk in the diet resulting in faster rate of passage. Different sized particles may travel through the digestive tract at different speeds (Argenzio and Southworth, 1974; Van Soest et al., 1983). In this case, the small zeolite A particles may cause more rapid passage through the digestive tract than if diets contained no zeolite. In addition, rate of passage is not influenced by digestibilities of diets (Castle and Castle, 1956; Krüger and Meyer, 1958; Seerley et al., 1962). Decreases in the digestibility of nutrients observed in Trial 3 would not have any effect on rate of passage. However, rate of passage may affect the amount of p-cresol produced in the lower digestive tract, since faster passage rates would reduce the time allowed for microbial breakdown of the aromatic amino acids thus yielding less free p-cresol. Figure 3 shows the average fecal excretion curves for each level of zeolite A in the diet.

B. Mean retention time in clinoptilolite fed pigs

MRT was significantly faster (P<.001) and in a linear manner (P<.01) in each collection period when pigs were fed increasing amounts of

		0% Zeolit	<u>e A</u>		
	Litte	<u>r 1</u>	Litte	<u>er 2</u>	
	M S	F	M Se	F	Collection Mean
Collection 1 (hrs.)	38.21	43,54	42.16	43.59	41.88
Collection 2 (hrs.)	42.05	43.28	42.48	44.84	43.16
Trt. mean		42	2.52		
		1% Zeolit	<u>:e A</u>		
	Litte	er 1	Litte	$\frac{r}{2}$	Collection
	_M =	F	M	F	Mean
Collection 1 (hrs.)	39.20	32.96	39.59	37.82	36.73
Collection 2 (hrs.)	38.27	31.87	35.18	36.27	36.06
		30	0.40		
		2% Zeolii	<u>e A</u>		
	Litte	<u>er 1</u>	Litte	<u>er 2</u>	Collection
	M <u>3</u>	F	M <u>Se</u>	F	Mean
Collection 1 (hrs.)	41.91	31.71	28.99	40.95	35.89
Collection 2 (hrs.)	38.57	35.07	33.45	34.91	35.50
Irt. medn		35	5.70		
		<u>3% Zeolit</u>	<u>te A</u>		
	Litte	<u>er 1</u>	Litte	er 2	Co 11 o o tri o tr
	M <u>2</u>	F	M M	ex F	Mean
Collection 1 (hrs.)	34,67	35,42	34,84	37.30	35.56
Collection 2 (hrs.)	32.28	33.91	34.74	35.69	34.16
Irt. mean		34	.86		
	Stat	istical S	Summary		
		MS		P Value	
Trt. effect		97.56		. 02	
Litter effect		3.05		.69	
Sex effect Period effect		.23		.91	
IEIIVU EIIEUU		./0			
Linearity Period 1		14 82		05	
Period 2		6.75		.03	

TABLE 25. EFFECTS OF FEEDING 0, 1, 2, and 3% ZEOLITE A ON PASSAGE RATE AS MEASURED BY MEAN RETENTION TIME (MRT) IN TRIAL 7.ª

^aAll numerical values refer to mean retention time (MRT).



Fecal excretion curves from pigs fed a chromic oxide marked meal containing 0, 1, 2, and 3% zeolite A.

cr₂0₃ mg/g feces

clinoptilolité. There was no litter or sex effect but MRT values in collection period 1 were lower (faster passage rate) than period 2.

Table 26 shows the MRT values for the four levels of clinoptilolite fed in relation to litter, sex, and collection period (P<.01). Due to the increased amount of bulk in the diet and the addition of smaller particles of clinoptilolite, rate of passage was increased. The significant period effects (period 1 faster passage rate than period 2) may be caused by the time of day in which the marker was fed. Chromic oxide was administered in the evening in period 1 while chromic oxide dosing occurred in the morning in period 2. Krüger and Meyer (1958) showed that when pigs were fed in the morning (period 2), the ingesta remained in the tract for a shorter time than if the pigs were fed in the afternoon (period 1). This faster rate of passage could explain the linear reductions in free and conjugated p-cresol as greater amounts of clinoptilolite are fed since there would be less time allowed for microbial action of the ingesta.

It is interesting to note that when more zeolite is fed (regardless of type) passage rate is increased. Passage rate values for zeolite A fed up to 3% of the diet were slower than MRT values for 5.0 and 7.5% clinoptilolite diets suggesting that the primary difference in MRT of different levels of zeolite, is purely due to the bulk and particle size effect. Figure 4 shows the average fecal excretion curves in pigs fed 0, 2.5, 5.0, and 7.5% clinoptilolite diets. The biphasal excretion patterns observed in these graphs are due to the pulsative emptying of the cecum (Van Soest <u>et al.</u>, 1983; Ehle, 1980; Luckey <u>et al.</u>, 1979) which leads to difficult interpretation of passage rate or MRT.

	0%	Clinoptil	olite		
	Litte S M	<u>r 1</u> ex F	<u>Litte</u> Se M	e <mark>r 2</mark> Ex	Collection <u>Mean</u>
Collection 1 (hrs.) Collection 2 (hrs.) Trt. mean	38.21 39.84	42.69 43.03 41	41.31 42.21 .37	39.13 44.56	40.34 42.41
	2.5%	Clinopti	<u>lolite</u>		
	<u>Litte</u> S M	<u>r 1</u> ex F	<u>Litte</u> Se M	<u>r 2</u> <u>x</u> F	Collection Mean
Collection 1 (hrs.) Collection 2 (hrs.) Trt. mean	39.13 38.64	40.94 39.02 39	38.25 36.11 .46	42.45 41.15	38.61 40.32
	5.0%	Clinopti	<u>lolite</u>		
	<u>Litte</u> M	r 1 ex F	<u>Litte</u> Se M	er 2 2x F	Collection Mean
Collection l (hrs.) Collection 2 (hrs.) Trt. mean	34.66 42.19	36.92 37.24 36	37.45 36.67 .84	32.56 36.99	35.40 38.27
	7.5%	Clinopti	lolite		
	Litte S M	<u>r 1</u> ex F	Litte Se M	er 2 Ex F	Collection Mean
Collection l (hrs.) Collection 2 (hrs.) Trt. mean	35.01 38.16	33.77 32.63 34	31.82 36.40 .29	32.25 34.28	33.21 35.37
	Statisti	cal Summa	iry		
		MS		P Value	
Trt. effect Litter effect		76.34		.001	
Sex effect Period effect		2.60 38.83		.46 .01	
Linearity Period 1 Period 2		3.40 4.31		.01 .01	

TABLE 26. EFFECTS OF FEEDING 0, 2.5, 5.0, and 7.5% CLINOPTILOLITE ON PASSAGE RATE AS MEASURED BY MEAN RETENTION TIME (MRT) IN TRIAL 8.

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^aAll numerical values refer to mean retention time (MRT).



CONCLUSIONS

1) Feeding trials indicate that additions of .3% zeolite A and .5% clinoptilolite to swine diets have no effect on average daily gain while tending to improve feed/gain and the efficiency of energy utilization. Additions of 1% zeolite A and 5% clinoptilolite tended to reduce average daily gain while feed efficiency (gain/feed) was reduced when growing pigs were fed 5% clinoptilolite.

2) Additions of up to 3% zeolite A in corn-soy diets resulted in reductions in energy absorption and utilization. Biological value of protein was improved even though apparent digestibility of nitrogen was reduced indicating poor absorption of amino acids or effective binding of ammonia produced from deamination of proteins. Mineral absorption and % mineral retention was reduced when increasing amounts of zeolite A were added to the diet suggesting dissociation of zeolite A in the acid environment of the stomach preventing absorption of minerals.

3) Additions of up to 7.5% clinoptilolite decreased energy absorption and utilization. Apparent digestibility of nitrogen and net protein utilization were reduced which may suggest that clinoptilolite does bind free ammonia in the gastrointestinal tract. Phosphorus absorption was reduced while sodium, iron, and zinc absorption was improved indicating some destruction and release of free aluminum from clinoptilolite which decreases phosphorus availability.

4) Plasma ammonia and glucose were not affected by zeolite A up to two hours after a meal feeding. Serum calcium was elevated by feeding

zeolite A which may have been a response to the reduction of available phosphorus when zeolite A was present in the diet. Serum sodium levels were higher in zeolite A fed pigs due to the high sodium content of zeolite A.

5) Plasma ammonia concentrations were reduced by feeding clinoptilolite while plasma glucose concentrations were not affected by clinoptilolite but plasma glucose concentrations did increase up to two hours after feeding. Serum calcium was not affected by feeding clinoptilolite even though serum phosphorus was reduced from some dissociation of the clinoptilolite molecules. Serum sodium levels were reduced while serum potassium levels increased when diets were supplemented with clinoptilolite. This sodium-potassium relationship illustrates the sodium-potassium sparing effect of each mineral's requirement.

6) Rate of passage of zeolite A or clinoptilolite supplemented diets was increased due to the addition of bulk and the small particle size contributed from these two zeolites.

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