UTILIZATION OF PHOSPHORUS AND OTHER MINERALS
FROM BROILER LITTER AND SWINE WASTE

by

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The kindness of such friends as   ,

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INTRODUCTION

Utilization of nitrogen and energy from animal wastes has been extensively studied over the last 20 yr, and reviews by Bhattacharya and Taylor (1975), Smith and Wheeler (1979) and Fontenot and Jurubescu (1980) have examined these data. Ruminants have been found to efficiently utilize wastes, due to the ability of their resident microbial population to convert the high nonprotein nitrogen and fiber content of wastes into metabolically useful nutrients for the host animal.

However, relatively little research has been done to determine utilization of the minerals contained in waste by ruminants. Reported values for absorption of P from different forms of waste have been extremely variable, partly due to use of different experimental protocols. Only limited work has been conducted to estimate efficiency of utilization by sheep of Ca and Mg from waste. Considering the demonstrated value of animal waste as a protein source for ruminants, more research is vitally needed to determine the potential of this material as a mineral source as well.

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It has been suggested that P supply to the rumen may, in some cases, limit cellulose digestion or cellulytic bacterial populations. Increased cellulose digestion with increased P concentration of media has been demonstrated in vitro. Extensive P recycling into the rumen via saliva acts to maintain ruminal P concentrations even during periods of P deprivation in vivo. However, an extended time on a deficient diet might cause rumen P concentration to drop below the level required for efficient microbial cellulose digestion to occur.

Our objectives in this study were to measure the efficiency of utilization of P, Ca, Mg and certain other minerals from swine waste and broiler litter, using animals which had been equalized in respect to P status, and to determine the effect of the level of P supplied and the source of the P on the number of cellulytic bacteria in sheep fed different P supplements.

CHAPTER I

REVIEW OF LITERATURE

Animal Wastes As Feedstuffs.

   Fifty-two million tons of collectible animal waste were
produced in 1974, including 6.5 million tons of poultry
excreta and 5.5 million tons of swine waste (VanDyne and
Gilbertson, 1978), which have been shown to be valuble
sources of protein and energy for ruminants (Bhattacharya
and Taylor, 1975). Due to the ability of the symbiotic
rumen microorganisms to convert nonprotein nitrogen and
fiber (Smith and Wheeler, 1979; Fontenot and Jurubescu,
1980) found in waste into nutrients available to the host
animal, this material is more efficiently utilized by
ruminants than by nonruminants such as poultry and swine
(Bhattacharya and Taylor, 1975), It has been calculated that
animal wastes have higher value for ruminants as protein
than as energy sources (Smith and Wheeler, 1979). The
relatively high levels of crude protein and minerals suggest
that wastes could supply significant amounts of nutrients at
relatively low dietary levels. Ensiling of wastes shows

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potential as a cost effective method of treatment to eliminate pathogenic bacteria and increase palatability to livestock (Fontenot and Jurubescu, 1980).

Poultry Litter. Material upon which poultry are kept, voided excreta, feathers and wasted feed together comprise poultry litter (Fontenot and Jurubescu, 1980). Broiler litter has a crude protein content of 31.3 + 2.9 %, an ash content of 15 + 3.2 % (Bhattacharya and Taylor, 1975), and total digestible nutrients (TDN) for sheep of 58.1 %. Approximately half the crude protein is composed of the nonprotein compounds, uric acid and ammonia. Virtually all the uric acid and ammonia have been shown to be excreted via the urine in poultry (Krogdahl and Dalsgard, 1981), but due to a single cloacal exit from the body urine is mixed with feces in recovered poultry waste. Ruminants utilize uric acid more efficiently than urea, due to a slower conversion to ammonia by rumen microorganisms (Oltjen et al., 1968).

In a summary of several studies, Smith and Wheeler (1979) reported that approximately 10% more feed was required per unit of gain by cattle fed poultry litter than by cattle fed conventional diets. Average daily gain was .05 kg lower for cattle fed diets containing broiler litter. In at least some of the experiments, available energy was not equalized, which may explain differences in performance.

Caged Layer Waste. This product is composed of excreta, wasted feed, and feathers (Fontenot and Jurubescu, 1980). Calculated TDN averages 52.3 % for sheep (Bhattacharya and Taylor, 1975), slightly less than for poultry litter. The ash content averages 28 + 1.5 %, with a Ca and P content of 8.8 + 1.1 % and 2.5 + .6 %, respectively. The crude protein content is 28 + 3.2 %, which suggests that this substance could be a valuble protein source for ruminants.

Performance of cattle fed caged layer waste as a protein supplement is similar to that of cattle fed conventional diets (Smith and Wheeler, 1979). Sheep tended to reduce intake when fed caged layer waste, probably due to palatability problems. However, when fed the caged layer waste, lambs had a lower feed to gain ratio, suggesting that the waste was utilized very efficiently.

Milk production decreased slightly in cattle fed caged layer waste, and milk fat concentration rose slightly, probably due to the smaller volume of milk produced (NRC, 1983). However, when lactating cows were fed Urimix, a combination of 90% dried layer waste, 5% fat, and 5% molasses, milk production did not decrease (NRC,. 1983). It was postulated that added energy from the fat compensated for lower energy available from the waste.

Swine Waste. Despite its high nutrient content. relatively little research has been conducted with swine waste This material contains 23.5% crude protein which includes a substantially higher true protein content than poultry excreta (Kornegay et al., 1977). Swine waste contains 2.72% Ca and 2.13% P, dry basis, and substantial amounts of Mg, K, Zn, and Cu.

Sheep and cattle in Australia fed 45% pelleted swine waste demonstrated a marked depression of dry matter digestibility (Fontenot and Jurubescu, 1980). This was ascribed to high levels of fiber and ash in the wastes, which may reflect feeding practices in Australia. When fed to sheep, dry matter digestibility of swine waste ensiled with orchardgrass hay was higher than hay alone. Ensiling swine waste with corn grain produced unpleasant smelling silage which was well digested but not well accepted by sheep. Feeding trials with cattle have shown acceptable rates of gain by animals fed swine waste (Smith and Wheeler, 1979).

Cattle Waste. Cattle waste has the lowest nutrient value of the wastes examined (Bhattacharya and Taylor, 1975). The crude protein content for steer manure was 20.33 +.7% and for dairy cow manure 12.7 +.9%, dry basis. The TDN for sheep and the ash contents were 48 % and 15 % for steer

manure, and 45 % and 16.1 % for dairy cows, respectively. Calcium and P contents were .87 and 1.6 % in the two substances under study.

Acceptible rates of gain and feed conversion have been measured with finishing cattle fed diets containing cattle excreta (Smith and Wheeler, 1979). The plane of nutrition of animals from which waste is obtained is important. Cattle fed diets containing a high proportion of concentrate produce waste which is more efficiently digested when fed to sheep (Fontenot and Jurubescu, 1980). Treatment of manure with NaOH or ensiling cattle manure with grass hay or crop residues has been shown to improve its value (Fontenot and Jurubescu, 1980).

Safety Considerations. Possible safety considerations concerning the use of animal wastes include medicinal drugs, pesticides, minerals, and pathogenic bacteria and fungi.

Arsenic compounds are fed to poultry and swine, and the possibility of tissue contamination in animals fed waste has been raised. However, after a moderate withdrawal period,r arsenic levels in tissue and milk of ruminants fed varying levels of poultry excreta are within FDA tolerance limits (McCasky and Anthony, 1979). Selenium, Cd, and Pb have not been found to be elevated in the tissues and milk of cattle fed waste.

Copper toxicity has been reported in sheep fed high Cu broiler litter (Fontenot et al., 1972). Sixty-four percent of ewes fed 50% broiler litter and 55% of those fed 25 % broiler litter died by 254 d on trial. Ewes showed marked accumulation of Cu in the livers. Olson et al. (1984) found that sheep absorbed Cu from broiler litter efficiently and did not excrete it, although evidence for redistribution of body stores was found.

However, cattle are more tolerant of high Cu levels (McCasky and Anthony, 1979). Cattle fed varying proportions of broiler litter in their diets had elevated liver Cu levels, but muscle Cu levels were not above control, and no overt toxicity symptoms were observed.

The possibility that toxins may be ingested from waste must be considered. Angus et al. (1978) suggested that pathologic symptoms of lambs fed dried battery waste may have been due to toxins contained in the excreta, but they made no effort to isolate the postulated toxins. Kinzell et al. (1983) studied the question of unknown xenobiotics or toxins in poultry waste. They fed 34.8 % dried poultry manure to steers for 180 d, then looked at the activities of the liver drug metabolizing system. They found no evidence of hypoalbunemia. Urine parameters, including protein, glucose, ketones, leucocytes, epithelial cells, bilirubin

and urobilinogen levels were normal, indicating normal liver and kidney functions. Activity levels of the liver microsomal cytochrome 450 dependent drug metabolizing enzymes were normal, suggesting that there were no unknown xenobiotics or toxins to which the steers were susceptible in the waste.

Although there are pathogenic bacteria and toxigenic fungi in waste, appropriate processing is effective in destroying them. Dry heat, autoclaving, paraformaldehyde, ethylene oxide fumigation, and ensiling have been found to be effective in destroying objectionable organisms; ensiling appears to be the most cost-effective method (NRC, 1983).

Phosphorus and Calcium Utilization.

Extensive research has been conducted over the past 15 yr to determine the mechanisms of control for Ca absorption and body Ca stores. Considerably less research has been done with P, possibly due to an early assumption that P mimicked Ca in absorption and control mechanisms. It is more likely that, although P interacts with Ca and is influenced by some of the same control factors, P has seperate mechanisms of absorption, control and excretion.

P and Ca Homeostasis. The small intestine is considered to be the major site for absorption of both P and Ca (Wasserman and Taylor, 1966; Ben-Ghedalia et al., 1975; Braithwaite, 1976; Poppi and Termouth, 1979; Scott and McLean, 1981; Ben-Ghedalia et al., 1982). Rate of absorption is primarily, although not solely, controlled by the amount of 25-hydroxycholecalciferol which is converted into 1,25-dihydroxycholecalciferol by the kidneys (Canalis, 1983). Baseline level of production of the dihydroxy metabolite will be affected by the existence of internal sinks for Ca such as a growing fetus or developing bones, and has been shown to be affected by growth hormone (Spencer and Tobiassen, 1981), prolactin (Bickle et al., 1980), and estrogen (Canalis, 1983). Fluctuations of serum levels of 1,25-dihydroxycholecalciferol around baseline levels act to maintain concentration of serum ionized Ca within a narrow range. A drop in serum Ca stimulates release of parathyroid hormone from the parathyroid glands. Modeling studies of parathyroid secretion suggest that the parathyroid gland is more sensitive to falling than rising serum ionized Ca (Jung et al., 1982), a reasonable response for a hormone which acts to increase serum Ca levels.

Secreted parathyroid hormone acts to stimulate conversion of $25-(OH)_2$colecalciferol to $1,25-(OH)_2$cholecalciferol by

the kidneys, an effect which is associated with a decrease in production of $24,25-(OH)_2$cholecalciferol. The $1,25-(OH)_2$cholecalciferol acts on the intestine to increase absorption of Ca and P. Increased intestinal absorption has been postulated to be due to both membraneophilic actions altering membrane permeability, and to induction of a binding protein by a steroid hormone receptor mediated response (Norman and Ross, 1979). Rapid enhancement of intestinal absorption has been postulated to be due to a direct effect of 1,25-dihydroxycholecalciferol on intestinal membrane fluidity and permeability (O'Doherty, 1979; MacLaughlin et al., 1980; Matsumoto et al., 1980). Maximum production of Ca binding protein tends to occur after maximal Ca absorption in response to administration of 1,25-dihydroxycholecalciferol (Spencer et al., 1976), which obscures its function in Ca transport. Calcium binding protein may serve to fine tune Ca absorption after most of the needed Ca has been absorbed. At the bone, circulating levels of 1,25-cholecalciferol act in concert with parathyroid hormone to enhance bone resorption, releasing Ca and P into the bloodstream. Chronic hypocalcemia increases production of PTH, not by transcriptional or translational control but primarily by hyperplasia (Habener, 1981).

As serum Ca levels rise, less parathyroid hormone is secreted, which reduces the production of 1,25-dihydroxycholecalciferol. In normal or hypercalcemic conditions, 24,25-dihydroxycholecalciferol is the primary vitamin D metabolite found in serum (Koshy, 1982). This variant can support growth, serum Ca levels and bone mineralization of animals fed a normal Ca, normal P diet, protecting the bone reserves so they can be drawn upon when Ca supply is reduced. If serum Ca levels rise above normal, calcitonin is released from the C cells of the thyroid. Calcitonin suppresses bone resorption and may increase urinary excretion of Ca and P (Koshy, 1982). Hypercalcemia reduces secretion of PTH and increases the rate of its degradation within the parathyroid gland (Habener, 1981).

Absorption of both P and Ca is increased by 1,25-dihydroxycholecalciferol (Chen et al., 1974; Walling and Kimberg, 1974; Rizzoli et al., 1977). This common controller acts to coordinate Ca and P absorption, which assures a proper supply of both minerals during bone accretion and production of hydroxyapatite crystals (Braithwaite, 1976). However, these minerals also have independent actions and mechanisms.

Interactions of Dietary Ca and P. The importance of the interaction of Ca and P in the diet of animals has long been

known. A Ca:P ratio of between 1:1 and 2:1 has been considered to be important for monogastrics (Maynard and Loosli, 1969). A Ca:P ratio below 1 can cause a depression of serum Ca, increased PTH secretion, bone loss (Ten-Lin et al.,1974), and increased fecal Ca (Chicco et al., 1973). The importance of this ratio depends on the adequacy of the dietary levels of these minerals. If either P or Ca is marginally deficient, then an improper ratio is much more likely to cause a deficiency of the limiting element (Young et al. 1966). This has been explained by a excess of one mineral complexing with the marginal element and further reducing its supply.

Ratios of Ca:P have been found to be less critical for ruminants (Dowe et al., 1957; Luck and Lofgreen,1961; Young et al., 1966). These animals have evolved on forage diets which are often high in Ca but limiting in P, and they have apparently developed mechanisms to minimize the effects of this imbalance. Dowe et al. (1957) found normal growth with ratios up to 4.3:1, and Lamb et al. (1934) found efficient absorption of P in dairy heifers with ratios up to 6.5:1. Ratios below 1:1 have been associated with increased incidence of urinary calculi in sheep (Emerick and Embry, 1963).

Dietary Levels of Ca and P for Nonruminants. Diets high in P with adequate Ca are associated with problems such as secondary hyperparathyroidism in several species (Ten-Lin et al., 1974), and big head disease in horses (Evans et al., 1977). Bell et al. (1977) found that a diet containing .7g Ca and 2.1 g P, when fed to humans, caused increased serum P, urinary P and urinary hydroxyproline excretion, indicating bone resorption. These researchers also reported reduced serum and urinary Ca. Radioactive tracer studies indicate that there is increased bone resorption and increased Ca loss in the feces when dietary P exceeds dietary Ca (Draper et al., 1972). The increased bone resorption is aparently caused by increased PTH secretion in response to reduced serum Ca (Ten-Lin et al., 1974)

Adequate Ca, low P diets increase the absorption of Ca and P (Tanaka et al., 1973). Low dietary P causes increased production of 1,25-dihydroxycholecalciferol by the kidney, stimulating absorption of Ca and P. However, Ribovich and DeLuca (1975) demonstrated that administration of 1,25-dihydroxycholecalciferol did not completely eliminate the stimulation of Ca uptake in animals fed a low P diet, and they postulated that the P deficiency must have an effect on intestinal transport other than production of 1,25-dihydroxycholecalciferol. Intestinal absorption of P

by thyroparathyroidectomized, vitamin D deficient rats was shown to be enhanced by a diet low in P (Cramer & McMillan, 1980), an effect which is unlikely to be due to 1,25dihydroxycholecalciferol production.

Rats on a low-P diet have been shown to digest total dietary P and phytate P more efficiently compared to those fed a normal P diet (Moore and Veum, 1983). The authors suggested this effect was due to adaptation by the intestinal microflora which may have synthesized more phytase and/or alkaline phosphatase to increase the availability of dietary P.

Renal reabsorption of P also affects P homeostasis. Cheng et al. (1983) showed that a low-P diet increased Na gradient-dependent phosphate uptake by an increase in the Vmax of transport. This is one explanation for the ability of animals on a low-P diet to more efficiently reabsorb P (Troehler et al., 1976) without involvement of PTH or vitamin D (Brazy et al., 1980).

Dietary Levels of Ca and P for Ruminants. The relative insensitivity of ruminants to high Ca:P ratios is probably due to extensive recycling of P into the rumen via saliva (Tomas, 1974; Scott et al., 1984). This may be a practical adaptation for animals which evolved on forages, which tend to contain higher levels of Ca than of P. Approximately 8

to 10 g of P are returned to the rumen by sheep on a P adequate diet (Ben-Ghedalia et al., 1982), which would lower the Ca:P ratio before the small intestine, the primary absorption site, is reached. In contrast to nonruminants, low dietary P reduces Ca absorption, which returns to normal values when dietary P is made adequate (Young et al., 1966). Phosphorus depletion resulted in increased P absorptive efficiency for 11.5 d following return to a P-adequate diet. The authors suggested that the more acidic environment in the small intestine of ruminants reduced precipitation of P as tricalcium P and allowed a greater tolerance to high Ca:P ratios.

Abdel-Hafeez et al. (1981) found that low dietary Ca caused an increase in Ca absorptive efficiency which presumably resulted from an increase in the circulating 1,25-dihydroxycholecalciferol concentrations, a response similar to nonruminants. In agreement with the findings of Young et al (1966) a diet deficient in P resulted in reduced Ca absorption, which the authors ascribed to a reduction in the circulating plasma 1,25-dihydroxycholecalciferol concentrations, in contrast to findings in nonruminant data.

At a dietary range of 40 to 100 mg/day/kg body weight, Braithwaite (1979) found that Ca absorption by mature wethers was regulated at a level just sufficient to replace

losses. He suggested that the theory of Ca absorption proposed by Wasserman and Taylor (1966) also applied to ruminants. They postulated that Ca absorption occurred both by diffusion, which is a nonsaturable, uncontrolled process dependent on intestinal concentration, and by active transport, which is regulated according to body requirements. Braithwaite (1979) suggested that P was absorbed in direct relation to intake and was retained in relation to Ca retention, with the excess being excreted into the intestine. An increase in rate and efficiency of P absorption and a decrease in fecal P in response to an intraveneous infusion of Ca has been shown (Braithwaite, 1984a).

Urinary excretion of P has been shown to be high in a small percentage of sheep. Braithwaite (1984b) suggested that these sheep obligately lost P via urine, and that this loss could be considered part of the maintenance requirement for these animals. He expressed the P requirement as a combination of net production demand and urinary demand. For these sheep to obligately lose P via urine, one would expect a lower renal threshold for P excretion. However, Field and Kamphires (1983), using genetically very similar chimaerically derived triplets, found uniform P excretion pathways within triplets and marked variation between

triplets despite similar renal thresholds for P. They found that the sheep which excreted P in urine were the most efficient at absorbing P, and postulated that P not required by the body is first recycled via saliva. As the salivary secretory capacity is approached, serum inorganic P rises above the renal threshold, resulting in excretion via urine.

Scott et al. (1984) found that ruminal P infusion increased P absorption from the small intestine, plasma P levels, and salivary excretion of P. Absorption in response to increasing intake was curvilinear, indicating a reduced efficiency of absorption at higher levels and thus the ability to control uptake. They suggested that P homeostasis involved both alterations in P recycling via saliva and in absorption rate from the small intestine. Recycling P to a location proximal to the main absorptive site is a very effective method of conserving P.

Sites of Absorption for Ca and P. The small intstine is considered to be the major site for absorption of Ca and P in both nonruminants and ruminants (Braithwaite, 1976; Dillon & Scott, 1979; Poppi & Termouth,1979; Yano et al., 1979). Variable absorption and secretion of Ca have been shown in the preintestinal area of ruminants (Grace et al., 1974; Dillon and Scott, 1979; Green et al., 1981; Ben-Ghedalia et al., 1982). Concentration of

ultrafiltratible P falls drastically along the small intestine, but the concentration of P assoacited with nucleic acid does not, indicating that ultrafiltratible P is the major form absorbed (Poppi & Termouth, 1979). Calcium tends to be absorbed in the proximal small intestine and secreted in the distal small intestine (Ben-Ghedalia et al., 1975; Yano et al., 1979), but P appears to be more effectively absorbed in the middle and lower small intestine (Thewis et al., 1978; Yano et al.,1979; Poppi and Termouth, 1979). This partial separation supports theories that suggest independent absorptive mechanisms for Ca and P, although absorption of both minerals are influenced by similar factors.

Effect of Age on Ca and P Absorption. Calcium absorption varies with age in monogastrics. Absorption from the small intestine of suckling rats had a linear relationship to luminal Ca concentration (Gishan et al., 1980). Ca was absorbed when the intestinal concentration was higher than the concentration of serum ionized Ca, and was secreted when the luminal concentration was below the serum level, suggesting uptake by diffusion. Absorption of Ca by weanling rats showed a curvilinear relationship with luminal Ca concentration in all parts of the small intestine, indicating control of absorption. In adolescent rats the

relationship was curvilinear in the proximal small intestine but linear in the distal small intestine and large intestine. Maturation appeared to be accompanied by a change to a carrier mediated mechanism which allowed a more exact matching of need and supply.

Calcium transport appears to decline after adolescence. At 3 mo, active transport in everted gut sacs from Sprague-Dawley rats could not be demonstrated (Armbrecht et al., 1979). By 12 mo of age, the rats showed only marginal adaptation to a low Ca diet. Calcium binding protein content paralleled the decrease in absorptive capacity but alkaline phosphatase activity increased.

In contrast, rats retain the ability to regulate urinary P excretion to maintain a non-negative P status as adults (Armbrecht et al., 1980). Both young and old rats on varying combinations of Ca and P dietary levels remained in positive P status by balancing absorption and urinary excretion (Armbrecht et al., 1980). Phosphorus absorption appears to be less affected by age than Ca absorption.

Hansard (1954) reported that cattle on an adequate Ca diet did not reach a negative Ca status until the relatively advanced age of 12 years. The ability to increase absorption in response to a low Ca or P supply has been shown to occur in adult sheep (Young et al., 1966;

Braithwaite, 1979; Abdel-Hafeez et al., 1982; Field et al., 1982). Dillon and Scott (1979) calculated that Ca absorption in lambs, although gradually declining with age, consistently matched requirement. They felt that lowered percent absorption in older lambs may actually have been due to excess supply. Field et al. (1982) found no significant difference in fractional absorption of P between 3.5 mo old lambs and 2 yr old ewes. The ability to adapt to a diet low in Ca or P appears to be retained longer in ruminants than nonruminants.,

Relative Value of Mineral Sources. Hemingway and McLaughlin (1979) found availabilities of P from magnesium phosphate, monocalcium phosphate or dicalcium phosphate to be approximately equal for growing wethers. Webb et al. (1975) found a tendency for gain to be lower for animals supplemented with a mixture of 87% mono- and 13% di- calcium phosphate, compared to steers supplemented with deflourinated phosphate, Mexican rock phosphate, sodium tripoly phosphate, and a mixture of deflourinated phosphate and sodium tripoly phosphate. Conversely, Ammerman et al. (1957) reported good utilization of dicalcium phosphate, deflourinated phosphate, bone meal and Curaco Island phosphate. Ruminants appear to be capable of efficiently using a wide variety of P supplements.

Phosphorus Utilization by Rumen Bacteria.

Any mineral requirement for a ruminant can be subdivided into the requirement of the animal and the requirement of the rumen microorganisms. Minerals are available for uptake by rumen microbes before they are available to the host. While it is probable that ruminants and bacteria have co-evolved so that a sufficient level for one will supply the needs of the other, very little research has been done on this.

Minerals affect the osmotic pressure, buffering capacity, redox potential, and dilution rate in the rumen (Durand and Kawashima, 1980). Phosphorus is a component of the buffering system in the rumen and phosphate supplements have been suggested for use as rumen buffering agents (Murphy et al., 1983).

Phosphorus serves in both structural and enzymatic capacities in miocroorganisms. Phospholipids are structural elements of plasma and outer membranes. High energy phosphate bonds serve in energy transfer for reactions involving nucleotide phosphates, and inorganic polyphosphates can serve as an energy reserve (Durand and Kawashima, 1980). Nucleotides, and many coenzymes such as flavin phosphates, ·pyridoxal phosphate and thiamine pyrophosphate, contain P.

A P concentration of 100 mg/l in rumen fluid or 4 g/kg digestible organic matter has been estimated from in vitro experiments to be adequate for bacterial growth and cellulytic activity (Durand and Kawashima, 1980). In vitro cellulose digestion was studied when 20 to 60 mcg/ml P was added to media innoculated by washed rumen bacteria (Hall et al., 1961). At 20 and 40 mcg/ml concentrations, cellulose digestion was stimulated less by Ca phytate than by vitreous sodium metaphosphate, sodium and potassium phosphate, monosodium orthophosphate and acid sodium pyrophosphate but at 60 mcg/ml these differences disappeared. The authors emphasized that some forms of P, such as vitreous sodium metaphosphate, supported cellulytic activity but were poorly utilized by the animal, making them unsuitable choices for supplementation despite effective use by bacteria.

Ruminants conserve dietary P by recycling absorbed P through saliva (Tomas, 1974; Scott et al., 1984). Sheep fed from .7-1.7 g/kg dry matter P, which provided less than the animal's requirement, had ruminal P concentrations of 300 to 460 mg/l (Durand and Kawashima, 1980). In some cases the P requirement may be overestimated. Poppi and Termouth (1979) have suggested that, although maintenance requirements for wether sheep in their study was about 2 g/day, 1.56 g/day may have supplied an adequate amount of

this mineral. Liveweights and costal bone P (g/kg fresh bone) did not differ between animals fed diets supplying 1.56g to 3.88g of P per day. However, increases in Ca and Mg concentrations tended to increase the P requirement of cellulose digesting rumen bacteria. Therefore, a dietary level of these minerals could affect P availability, in spite of the amount of P supplied by the saliva. One important consideration is the length of time the animal is fed the atypical diet. Efficient recycling of P will probably enable ruminants to tolerate short term imbalances or P deficient diets.

Insoluble P, such as dicalcium phosphate, has been suggested to have limited availability to rumen microflora (Durand and Kawashima, 1980). Soluble phosphates are suggested to be highly available, but if in the acid form, they may disrupt the rumen buffering system.

Microbes have been shown to be able to adapt to low P concentrations in media by increasing alkaline phosphatase concentrations (McComb et al., 1979). This could be another way for rumen bacteria to maximize use of P in aninals on a low P diet.

Mineral Utilization from Waste

Nitrogen and energy utilization by cattle and sheep from different types of animal wastes have been extensively studied (Bhattacharya and Taylor, 1976), but relatively little research has been done to determine mineral utilization. Forages often contain adequate Ca for ruminants but may be deficient in P and Mg. Substantial amounts of P and Mg are contained in both poultry and swine excreta, which may prove to be effective supplements for animals on forage diets.

Bull and Reid (1971) examined the value of air dried chicken manure as a N, Ca, and P source for cattle. They fed 0 to 45 % waste with a hay, corn meal and corn sugar basal diet. Steers fed the 12.3 % waste diet absorbed 30.2 % P and 28.9 % Ca from the diet, which would indicate good availability. Few animals were used in this study, particularly at the higher waste levels, so statistical analysis was impossible, and absorption was not compared to a standard source such as dicalcium phosphate. However, cattle did appear to make effective use of the Ca and P in the supplemented diets.

Tagari et al. (1981) calculated the availability of P from heat sterilized poultry litter for 40 kg Awassi lambs. They fed a basal diet supplemented with dicalcium phosphate

for 20 d, then fed one of the following experimental diets for 20 d : basal, basal plus .176 % dicalcium phosphate, basal plus .35 % dicalcium phosphate, basal plus 2.01 % poultry litter and basal plus 3.94 % poultry litter. Calcium was equalized at .88 %, and the two levels of supplements contributed to a total of either .143 % or .182 % P in the experimental diets. Excreta was collected for the last 10 days of the second feeding period.

Using a slope method of analysis, they calculated the net P absorption of dicalcium phosphate to be 63.7 %, while that of poultry litter was 34.9 %. Standard calculations for apparent absorption would give values of 10 % to 15 % for poultry litter and 16 % to 25 % for dicalcium phosphate. These values seem low for lambs of this age fed below requirement. Phosphorus levels in the feed had to be kept low to obtain a linear response to P supplementation, which is one of the assumptions of the calculations in the slope method. Phosphorus absorption from diets containing low concentrations of this mineral may be obscured by endogeneous secretion. However, low P intake may have reduced the ability of sheep to absorb P relative to that of animals fed a replete diet. A low dietary P level of .07 % has been shown to reduce the rate of both Ca and P absorption (Young et al., 1966) possibly by a reduction in

the circulating serum 1,25-dihydroxycholecalciferol (Abdel-Hafeez et al., 1982). A P concentration of .24 % caused a moderate decline in the unidirectional fractional absorption of Ca in adult sheep (Abdel-Hafeez et al., 1982). If P absorption were equally sensitive, then the P levels in the diets could have limited absorption. Tagari et al. suggested that heat sterilization of the litter reduced P availability. The ortho form of P, which is well utilized by ruminants, can be converted to pyro and meta forms by heating; pyro and meta forms are not as available as ortho forms to ruminants (Ammerman et al., 1957; Chicco et al., 1965).

Despite differences in availability, serum P levels were more affected by the level of intake than by the source of P in the diet (Tagari et al., 1981). Sheep fed equal amounts of P had similar serum inorganic P levels regardless of the P source.

Ben-Ghedalia et al. (1982) studied absorption of Ca, P, and Mg along the intestinal tract of animals fed diets supplemented with either soybean meal or ensiled poultry litter. Supplements were added in amounts sufficient to bring the dietary nitrogen level to 12%. Substantially more P, Ca and Mg was supplied by the poultry litter diet, due to higher mineral content and level of dietary inclusion of the

waste. Forty kg Awassi lambs were cannulated in the proximal duodenum and in the distal ileum to partition absorption. Animals were adapted to the experimental diets for 45 d, then fecal collection was done for 10 d, including 4 d of intestinal sampling.

The researchers found the small intestine to be the major site for Ca and P absorption, and the stomach for Mg absorption. There was a large net secretion of P into the stomach, which has been shown to be due to recycling of P via saliva (Tomas, 1974; Scott et al., 1984). Net absorption of P was about 35% on both diets, but more P in g/d was absorbed by animals fed the waste diet. Expressed as percent, less Ca was absorbed from the poultry litter diet but expressed as g/d, similar amounts of Ca were absorbed from both diets. Sheep can regulate Ca absorption to meet requirements in a range of 40 to 100 mg/dietary P/d (Braithwaite, 1979). These animals were apparently adjusting their Ca absorption from both experimental diets to meet their needs.

Availability of Mg from the waste diet was 41.6% versus 51.5% for the soybean meal diet, although more g/d of Mg were absorbed by sheep fed the waste diet. The marked difference in mineral intake between the 2 diets makes direct comparisons of utilization difficult. In addition,

intestinal samples were only collected during the day. If diurnal variations in absorption occurred, these samples would not be represenative.

Conclusions by the authors were the following: 1.) poultry litter could serve as a good source of P, 2.) for levels up to 25% of the diet, a low rate of absorption of Ca from waste would tend to preclude calcinosis or parturient pariesis problems.

Field et al. (1977) examined utilization of P by 8 wk old Scottish Blackface lambs from broiler waste and from battery waste supplemented diets. Lambs were fed barley supplemented with 15 to 60 percent of one of the waste supplements. Absorption was corrected for endogeneous excretion by use of radioactive P.

Sheep ingested from 4.64 to 15.98 g of P per d. True absorption ranged from 49% to 90% and was inversely related to P intake. Lambs on 15% and 30% waste diets tended to be osteoporotic, which was postulated to be due to either reduced bone mineralization or increased rate of matrix synthesis. Regardless of intake, all lambs were in positive P balance. Partitioning of P excretion between urine and feces was extremely variable between animals. The authors concluded that poultry wastes were a good source of P for ruminants.

Keys and Smith (1981) conducted a growth and digestion trial using dairy heifers in which poultry excreta (PE) was used as a nitrogen, Ca and P source for corn silage diets. The corn silage was made from either corn stover and ear corn or whole corn plant. Gains were not significantly different between the two diets despite the higher Ca and P content of the waste supplemented diet. However, N retention tended to be higher for the soybean meal diet.

Results from these limited numbers of studies indicate that poultry waste can be a good source of P and Mg. Ca is less efficiently utilized, but the high levels of Ca in poultry waste make efficient use unnecessary.

CHAPTER II

JOURNAL ARTICLE 1

UTILIZATION OF PHOSPHORUS AND RELATED MINERALS

FROM SWINE WASTE AND BROILER LITTER

Summary

Two trials were conducted with 15 wethers surgically equipped with duodenal and ileal cannulas to study the absorption of P and certain other minerals from swine waste and broiler litter. Animals were fed a low P basal diet until serum inorganic P averaged 5.5 mg/dl, then were randomly assigned to one of the following diets: low P basal, basal + swine waste, basal + broiler litter, basal + dicalcium phosphate, basal + soybean meal. Each diet was ensiled at 50 percent dry matter in 210 liter metal drums, double lined with polyethylene. Each trial consisted of a 7-d preliminary period, a 7-d collection of feces and urine, and a 6-d sampling of duodenal and ileal digesta and feces. Sheep fed the waste supplemented diets absorbed P at least as well as

sheep fed the conventionally supplemented diets. Phosphorus was secreted into the stomach, absorbed from the small intestine, and both absorbed and secreted in the large intestine of sheep fed the different diets. Sheep fed the swine waste diet tended to absorb the greatest amount of P and have the highest serum inorganic P. Phosphorus absorption calculated by difference tended to be higher from the waste supplements than from dicalcium phosphate and soybean meal (P< .1). Less Ca was absorbed from the waste diets than from the conventional diets (P< .05). Expressed as g/d, there was no difference in Mg absorption between the waste diets and the conventional diets. More Cu (g/d) was absorbed from the waste diets (P< .05), but no difference was found in Fe absorption values across diets. Expressed as percent of intake, animals ingesting the waste diets tended to be in less negative Zn status than animals fed the conventionally supplemented diets (P< .1). Both broiler litter and swine waste appear to be good sources of P and Mg for ruminants.

Key words. Phosphorus, Waste, Feces, Swine Waste, Broiler Litter, Sheep.

Introduction

Animal wastes have been shown to be valuble sources of protein and energy for ruminants (Bhattacharya and Taylor, 1975; NRC, 1983). The high content of certain minerals in animal wastes, particularly P, indicate they might be valuble mineral sources as well.

Sheep (Field et al., 1977; Ben-Ghedalia et al., 1982) and steers (Bull and Reid, 1972) have been shown to absorb P effectively from poultry manure, although limited absorption from heat sterilized poultry manure has been reported (Tagari et al., 1981). The authors suggested that heat treatment might have rendered the P unavailable.

Calcium absorption by sheep from a poultry litter supplemented diet was found to be markedly lower than that from a soybean meal supplemented diet (Ben-Ghedalia et al., 1982). It was concluded that the low net absorption would prevent problems , such as parturient paresis, in ruminants fed up to 25% of the diet as waste. Steers have shown good utilization of Ca from air dried poultry manure (Bull and Reid, 1972).

Magnesium from a poultry manure supplemented diet was well utilized by sheep (Ben-Ghedalia et al., 1982), and it was suggested that poultry manure represents a highly available source of both P and Mg for ruminants. However,

unequal intakes of these two elements made direct comparisons of mineral utilization difficult.

The objectives of this study were to assess utilization of P, primarily, but also of Ca, Mg, Cu, Fe and Zn from two waste sources, broiler litter and swine waste, compared to diets supplemented with dicalcium phosphate or soybean meal; and to partition the absorption and secretion of these minerals along the intestinal tract.

Experimental Procedure

Fifteen 2 yr old crossbred wethers weighing 45 to 50 kg were used in two trials. Animals were surgically equipped with duodenal cannulas approximately 3 cm distal to the pyloric valve, and with ileal cannulas approximately 5 cm proximal to the ileo-cecal junction in order to partition absorption and secretion into the stomach, small intestine and large intestine.

Sheep were allotted a minimum of 4 wk to recover from surgery, during which they were fed 1100 g/d of a diet consisting of 60% ground orchard grass hay(IFN 1-03-438), 30% ground corn (IFN 4-02-935) and 10% soybean meal (IFN 5-04-604), supplying .36% P (dry basis). Prior to the second trial, they were placed in individual pens and fed

this diet for 2 wk to allow recovery from the stress of the first trial and to allow animals fed the low P diet to replenish body stores of P. Animals were fed twice per day and had constant access to water and trace mineralized salt.

After recovery from surgery animals were given 1000 g/d of a low P basal diet supplying .15% P in two daily feedings until serum inorganic P averaged 5.5 mg/dl. During the depletion period, jugular serum samples were taken every 10 d before the morning feeding. Animals were placed in metabolism crates allowing seperate fecal and urine collection at the beginning of the depletion period before the first trial and during the third week of the depletion period before the second trial. It was necessary to put sheep in metabolism stalls to remove them from from peanut hull bedded pens in order to lower the serum inorganic P below 6 mg/dl.

Upon achieving P depletion for each trial, sheep were blocked by breed and randomly allotted to one of five ensiled experimental diets: basal , basal + swine waste , basal + broiler litter, basal + dicalcium phosphate and basal + soybean meal (table 1). For trial 2, incomplete randomization was used, with the restriction that no animal would receive the same diet as in trial 1. Mineral content of these diets is given in table 1. Ingredients were ground

TABLE 1.   INGREDIENT AND MINERAL COMPOSITION
OF EXPERIMENTAL SILAGES[a,b]

Item	Supplement				
	None	Swine waste	Broiler litter	Dical phos	Soybean meal
Ingredient %					
Grass hay IFN1-03-438	9.1	8.5	8.4	9.0	7.2
Corn IFN4-02-935	13.9	13.0	12.8	13.8	10.9
Corn cobs	43.8	41.0	40.4	43.5	34.7
Beet pulp IFN4-00-669	4.5	4.2	4.2	4.5	3.6
Corn starch	18.1	16.9	16.7	18.0	14.3
Molasses IFN4-04-695	5.8	5.4	5.4	5.8	4.6
Soy protein	3.0	2.8	2.8	3.0	2.4
Urea IFN5-05-070	1.5	1.4	1.4	1.5	1.2
Limestone IFN6-02-632	.2	.2	.2	.2	.2
Trace mineral	.1	.1	.1	.1	.1
Waste	–	6.5	7.7	.7	20.9
Mineral:					
Phosphorus, %	.15	.27	.27	.30	.31
Calcium, %	.32	.38	.38	.40	.38
Magnesium, %	.11	.17	.16	.14	.17
Copper, ppm	12	15	20	15	14
Iron, ppm	437	530	447	482	446
Zinc, ppm	39	54	53	39	37

[a]Dry matter basis
[b]1,000,000 IU vit A and 150,000 vit D were injected i.m. every 60 d

through a 2.5 cm screen and ensiled at 50% dry matter for at least 7 wk before feeding. Chromic oxide was incorporated into the silage by hand mixing at the time of feeding at the rate of .25%.

A flow chart of the trials would show:

```
depletion     rumen          transition  adaptation    metabolism
              sampling                                  trial

    40 d        1 d             5 d         7 d          7 d
|------------>---------->-------------->------------->---------->

            intestinal      serum       rumen
            sampling        sampling    sampling

              6 d             1 d         1 d
        --------------->----------->------------|
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Sheep were fed 1000 g/d dry matter (DM) of the silages, divided into two equal feedings at 0630 h and at 1730 hr. The experimental diets were fed for a preliminary period of 7-d, followed by a 7-d collection of feed, feces and urine (period 1), a 6-d collection of feed, feces and intestinal digesta (period 2), 1-d for serum sampling and 2-d for sampling of rumen contents.

On the 14th day, blood samples were drawn before the morning feeding. Blood was drawn without anticoagulant for serum analyses, and ethylenediaminetetraacetic acid was used as an anticoagulant for hematocrit determination.

On the last day of the depletion period and on the 16th day of the trial, rumen fluid was taken via stomach tube at 4 p.m.. Samples were pipetted immediately into 1 ml freezer tubes containing 2 drops dimethyl sulfoxide as a cryoprotectant, frozen in liquid nitrogen, then stored at -80 C until used for innoculation.

Feed samples were collected at time of feeding from 2 d prior to the start until 2 d prior to the end of each collection period. Total fecal material was collected daily, dried in a forced air oven for 24 to 48 h at a maximum of 60 C and composited by animal seperately for each period. Prior to collection, 15 ml of a 1:1 (w/w) concentrated sulfuric acid:water solution and 200 ml of water were added to the urine collection container. Daily urine collections were diluted to a constant weight, and a 2% aliquot (by volume) was composited by animal.

Duodenal and ileal samples were collected twice daily at times determined by randomly assigning even hours of the clock to each of 6 d (Potter et al., 1969), then frozen for subsequent analysis. Equal weights (20 g duodenal, 15 g ileal) of samples for each sampling time for duodenal and ileal samples were composited and freeze dried.

Feed, fecal and intestinal samples were dried and ground through a 1 mm screen. Samples for mineral analyses were

wet ashed with nitric and perchloric acid. Urine and dig-
ested feed, fecal, and intestinal samples were analyzed for
Ca, Mg, Cu, Zn, and Fe by atomic absorption spectrophotome-
try (Perken Elmer model 403). Samples analyzed for Ca and
Mg were diluted in a .5% (v/v) solution of lanthanum oxide
in order to reduce interference. Feed , fecal, urine, in-
testinal and serum samples were analyzed for P by the method
of Fiske and Subbarow (1925). Urine was digested with sul-
furic acid and hydrogen peroxide, hydrolyzed by addition of
water, incubated in a boiling water bath for 30 min to dis-
rupt pyrophosphates formed during acid digestion, then neu-
tralized before accurate P readings could be obtained.
Chromic oxide was determined on composited duodenal, ileal
and fecal samples by the Hill and Anderson (1958) procedure.
Fecal and diet samples from the collection were analyzed for
neutral detergent fiber (NDF)(Vansoest, 1963), and fecal,
diet and duodenal samples were analyzed for acid detergent
fiber (ADF), lignin and cellulose content (Goering and Van-
soest, 1970).

Data from one sheep were deleted from the final analysis,
due to very low intakes which grew progressively worse dur-
ing the trials.

Data were analyzed using the general linear models proce-
dure of Statistical Analysis System (SAS, 1979). The sta-

tistical model included effects of treatment, trial, block, and treatment*trial interactions. Block was deleted from the final model because of lack of significance. Contrasts tested were: basal vs supplemented diets, waste supplemented diets vs dicalcium phosphate and soybean meal diets, swine waste vs broiler litter diets, and dicalcium phosphate vs soybean meal.

Results

Sheep remained in apparent good health during both trials, gained weight during the first trial, and maintained that weight during the second trial.

Phosphorus Metabolism. As calculated, the sheep fed the supplemented diets ingested approximately twice as much P as sheep fed the basal dist (table 2). Supplemented diets provided enough P to meet the maintenance requirements for a 50 kg sheep (NRC, 1975).

Fecal excretion of P was higher by sheep fed the supplemented diets (P<.01), a reflection of higher intakes. Animals fed the waste diets excreted less fecal P than animals ingesting the dicalcium phosphate and soybean meal diets (P<.05), due in part to a higher intake by wethers fed the dicalcium phosphate diet. Although not significant, urinary P excretion tended to be higher for sheep fed the supple-

TABLE 2. PHOSPHORUS BALANCE IN WETHERS FED DIFFERENT SUPPLEMENTS

Item	Supplement					SE
	None	Swine waste	Broiler litter	Dical phos	Soybean meal	
Intake, g/d[a,b,c]	1.32	2.59	2.56	2.82	2.72	.03
Excretion, g/d						
Fecal[a,b]	1.07	1.56	1.69	2.14	1.88	.15
Urinary	.06	.34	.17	.24	.08	.17
Total[a,b,c]	1.12	1.90	1.86	2.37	1.96	.08
Absorption						
Grams/d[a]	.25	1.04	.86	.68	.84	.15
% of intake[a]	18.8	39.2	34.0	24.3	30.8	6.3
Retention						
Grams/d[a,c]	.19	.70	.70	.44	.77	.09
% of intake[e,f]	14.4	26.4	27.4	15.8	28.0	4.2
% of abs	86.0	76.0	83.9	81.1	90.5	11.5

[a] Basal vs supplemented (P<.01)
[b] Waste vs conventional supplements (P<.01)
[c] Dicalcium phosphate vs soybean meal (P<.05)
[d] Waste vs conventional supplements (P<.05)
[e] Basal vs supplemented (P<.1)
[f] Dicalcium phosphate vs soybean meal (P<.1)

mented diets. Urinary excretion was very low for sheep fed soybean meal.

Phosphorus absorption was higher by animals fed supplemented diets, whether expressed as g/d or percent of intake (P < .01). Among the sheep fed supplemental P, those fed the swine waste diet tended to have the highest rate of absorption.

All animals were in positive P balance. More P was retained (g/d) by sheep fed the supplemented diets (P < .01) However, less difference was found when retention was expressed as percent of intake (P < .1). The percent of absorbed P which was retained did not differ across diets.

Large amounts of P were secreted into the stomach region of sheep fed all diets (table 3), but more was secreted by sheep fed the supplemented diets (P < .05) . Sheep fed the swine waste diet secreted more P than sheep fed broiler litter (P< .05). Expressed as percent of intake, sheep excreted P into their stomachs at a higher rate when fed the basal diets than when fed the supplemented diets (P < .05). Sheep fed the waste diets also demonstrated a higher rate of ruminal secretion (P < .05), primarily due to a very high rate of recycling by sheep fed the swine waste diet. Recycling was higher for sheep fed the dicalcium phosphate diet than for sheep fed the soybean meal diet (P< .05).

TABLE 3.   PHOSPHORUS INTAKE, FLOW, AND ABSORPTION IN WETHERS
FED DIFFERENT SUPPLEMENTS

Item	Supplement					SE
	None	Swine waste	Broiler litter	Dical phos	Soybean meal	
Intake, mg/d[a,b,c,d]	1.35	2.92	2.79	3.23	3.02	.03
Flow, g/d						
Stomach[a,c,d]	8.47	13.72	10.35	13.47	9.38	.70
Small int	1.88	2.14	1.95	1.52	1.71	.26
Large int[a,e]	1.20	1.78	2.01	2.40	1.90	.20
Absorption, g/d						
Stomach[c,d,f]	-7.12	-10.80	-7.56	-10.24	-6.36	.69
Small int[a,c,d]	6.59	11.58	8.40	10.78	7.68	.65
Large int[a,b,d]	.68	.36	-.06	-1.21	-.19	.29
Total[a]	.15	1.14	.78	.84	1.12	.20
Absorption, % of intake						
Stomach[a,b,c,d]	-526.2	-370.9	-271.8	-314.9	-208.6	26.6
Small int[a,c,e,g]	483.8	397.3	302.0	330.8	252.5	28.5
Large int[a]	53.3	12.8	-1.9	-37.2	-6.5	16.8
Total[a]	10.9	39.1	28.4	25.9	37.0	7.0

[a] Basal vs supplemented (P<.05)
[b] Waste vs conventional supplements (P<.05)
[c] Swine waste vs broiler litter (P<.05)
[d] Dicalcium phosphate vs soybean meal (P<.05)
[e] Dicalcium phosphate vs soybean meal (P<.1)
[f] Basal vs supplemented (P<.1)
[g] Waste vs supplemented (P<.1)

Absorption from the small intestine followed the same trends. When expressed as g/d, P absorption from the small intestine was higher for sheep given the supplemental P (P < .05) and was higher for sheep fed the swine waste diet (P < .05) than for those fed broiler litter. Sheep fed the dicalcium phosphate diet absorbed more P from this area than sheep fed the soybean meal diet (P< .05). However, expressed as percent of intake, sheep fed the basal diet absorbed more P from the small intestine than those given supplemental P (P< .05).

There was variable secretion and absorption from the large intestine. Sheep fed all diets except the basal diet and the swine waste diet showed a negative P absorption from the large intestine. As a result, more P was absorbed in g/d from the large intestine by sheep fed the basal diet (P < .05) and by sheep fed the waste diets (P < .05). Expressed as percent of intake, more P was absorbed from the large intestine of sheep fed the basal diet than of sheep fed the supplemented diets (P< .05). Total apparent P absorption was similar across supplemented diets and was higher for sheep fed the supplemented diets than for those fed the basal diet (P < .05).

Phosphorus absorption calculated by difference tended to be higher for sheep fed the waste (table 4) than for sheep

TABLE 4.   MINERAL ABSORPTION CALCULATED BY DIFFERENCE

| Mineral | Supplement | | | | SE |
	Swine waste	Broiler litter	Dical phos	Soybean meal	
	------------------%----------------				
Phosphorus[a]	63.9	54.7	30.2	43.4	12.6
Calcium[b,c]	-49.8	6.6	-7.1	173.9	21.5
Magnesium[a,c]	49.1	48.9	50.5	49.1	.3

[a]Waste vs conventional supplements (P<.1)
[b]Waste vs conventional supplements (P<.05)
[c]Dicalcium phosphate vs soybean meal (P<.05)

fed conventional supplements. The values were 63.9 and 54.7% for swine waste and broiler litter, compared to 30.2 and 43.4% for dicalcium phosphate and soybean meal, respectively. The highest rate of P absorption was associated with the swine waste supplement. Serum inorganic P concentrations were higher (P< .05) in sheep fed the supplemented diets than in sheep fed the basal diet (table 5), and tended to be highest from sheep fed ·the swine waste diet.

Calcium Metabolism. Calcium intakes were similar from all supplemented diets (table 6). More Ca was excreted in the feces of sheep fed supplemented diets than those fed the basal diet (P< .05), reflecting greater Ca intake. Sheep fed waste diets excreted more Ca via feces than sheep fed the dicalcium phosphate and soybean meal diets (P< .05), primarily due to a markedly reduced fecal Ca excretion by sheep fed the soybean meal diet. Sheep fed the waste diets excreted less Ca in urine than sheep fed the conventionally supplemented diets (P< .05), although animals fed the soybean meal diet excreted less Ca via urine than those fed the dicalcium phosphate diet (P< .05).

Apparent Ca absorption, expressed as g/d, was higher in sheep fed the supplemented diets (P< .05), and lower in sheep fed the waste diets (P< .05), primarily due to accelerated Ca absorption by sheep fed the soybean meal diet.

TABLE 5.   SERUM MINERAL LEVELS OF WETHERS

FED DIFFERENT SUPPLEMENTS

Item	Supplement					SE
	None	Swine waste	Broiler litter	Dical phos	Soybean meal	
	----------------mg/dl-------------------					
Inorganic P[a]	4.6	6.9	6.1	5.6	6.3	.6
Calcium[b]	8.8	8.6	9.4	9.7	9.0	.3
Magnesium	1.9	2.0	2.1	1.9	2.1	.1

[a]Basal vs supplemented (P<.05)
[b]Swine waste vs broiler litter (P<.05)

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TABLE 6.   CALCIUM BALANCE IN WETHERS FED DIFFERENT SUPPLEMENTS

Item	None	Swine waste	Broiler litter	Dical phos	Soybean meal	SE
Intake, g/d[a,b]	2.76	3.59	3.61	3.94	3.64	.02
Excretion, g/d						
Fecal[c,d,e]	2.10	3.13	2.83	3.33	1.70	.13
Urinary[d,e]	.21	.07	.19	.36	.15	.06
Total[c,d,e]	2.3	3.2	3.0	3.7	1.8	.16
Absorption						
Grams/d[c,d,e,f]	.66	.46	.78	.61	1.94	.11
% of intake[d,e]	23.1	12.8	21.6	15.7	53.8	3.3
Retention						
Grams/d[d,e,g]	.45	.34	.61	.25	1.80	.13
% of intake[d,e]	15.5	9.5	16.8	6.6	49.8	4.3
% of abs	50.6	79.9	71.4	27.6	92.6	19.0

[a]Waste vs conventional supplements(P<.05)
[b]Dicalcium phosphate vs soybean meal (P<.05)
[c]Basal vs supplemented (P<.05)
[d]Waste vs conventional supplements (P<.05)
[e]Dicalcium phosphate vs soybean meal (P<.05)
[f]Swine waste vs broiler litter (P<.1)
[g]Basal vs supplemented (P<.1)

However, animals fed the basal diet absorbed as much Ca as those fed any diet except soybean meal. Expressed as percent of intake, absorption was higher for wethers fed the conventional supplements than those fed the waste diets (P<.05). Calcium absorption was markedly higher by sheep fed the soybean meal diet than by those fed the dicalcium phosphate diet (P< .05). Less Ca was retained by sheep fed the dicalcium phosphate diet than by sheep fed the soybean meal diet (P< .05).

More Ca was absorbed from the stomach of sheep fed the basal diet than sheep fed the supplemented diets (P< .05) (table 7), which tended to secrete Ca into this region. However, expressed both as g/d and percent of intake, sheep fed the supplemented diets absorbed more Ca from the small intestine than sheep fed the basal diet (P< .05). Less Ca was absorbed from the small intestine of sheep fed the waste diets compared to sheep fed the conventionally supplemented diets (P< .05), due to a very high absorption by sheep fed the dicalcium phosphate diet. Animals fed the dicalcium phosphate diet absorbed more Ca from this area than those fed the soybean meal diet (P< .05).

Only sheep fed the swine waste diet absorbed Ca from the large intestine. Sheep fed the dicalcium P diet tended to secrete more Ca into this region than animals fed any other

TABLE 7.   CALCIUM FLOW AND ABSORPTION IN WETHERS
FED DIFFERENT SUPPLEMENTS

Item	Supplement					SE
	None	Swine waste	Broiler litter	Dical phos	Soybean meal	
Intake, g/d[a,b,c]	3.19	4.12	3.82	4.10	3.72	.28
Flow, g/d						
Stomach[a,b]	2.63	5.12	4.50	6.38	3.46	.28
Small int[c,d]	2.02	3.76	2.59	2.49	1.78	.37
Large int[a,b,d]	2.37	3.56	3.17	3.72	1.79	.20
Absorption, g/d						
Stomach[a,b]	.56	−1.0	−.68	−2.27	.26	.27
Small int[a,b,d]	.62	1.37	1.91	3.89	1.68	.43
Large int[e]	−.35	.20	−.48	−1.23	0	.48
Total[b,d]	.83	.56	.65	.38	1.93	.18
Absorption, % of intake						
Stomach[a,b]	17.6	−24.6	−17.8	−55.5	7.2	6.8
Small int[a,b,d]	19.2	33.7	50.0	94.8	45.2	11.1
Large int[e]	−10.8	4.3	−12.7	−30.3	0	12.2
Total[b,d]	25.9	13.4	17.1	9.3	52.1	4.8

[a]Basal vs supplemented (P<.05)
[b]Dicalcium phosphate vs soybean meal (P<.05)
[c]Swine waste vs broiler litter (P<.05)
[d]Waste vs conventional supplements (P<.05)
[e]Dicalcium phosphate vs soybean meal (P<.1)

diet. A basically zero absorption was maintained in the large intestine by sheep fed the basal, broiler litter, soybean meal and swine waste diets. A high rate of absorption from the small intestine and low rates of loss from other intestinal segments resulted in higher total Ca absorption by sheep fed the soybean meal diet than by sheep fed the dicalcium phosphate diet (P< .05). Sheep fed the soybean meal diet absorbed more Ca than those fed any other diet.

Calcium absorption, calculated by difference was lower (P< .05) in sheep fed the waste diets than in sheep fed the conventionally supplemented diets (table 4). Absorption tended to be lowest from the swine waste diet, and serum Ca levels were lower (P< .05) in sheep fed the swine waste diet (table 5) than in sheep fed the broiler litter diet. However, serum Ca levels in all sheep were within normal range.

Magnesium Metabolism. Magnesium intakes were higher for sheep fed the supplemented diets, but were relatively equal across supplemented diets (table 8). Fecal Mg was higher in sheep fed supplemented diets (P< .05), primarily due to higher intakes. Sheep fed waste diets excreted more Mg via feces than those fed the conventional supplements (P<.01), and fecal Mg was lower in animals fed dicalcium phosphate compared to those fed soybean meal (P< .05).

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TABLE 8.   MAGNESIUM BALANCE IN WETHERS FED DIFFERENT SUPPLEMENTS

Item	None	Swine waste	Broiler litter	Dical phos	Soybean meal	SE
Intake, g/d[a]	1.00	1.56	1.42	1.21	1.48	.02
Excretion, g/d						
Fecal[a,b,c,d]	.50	.80	.72	.55	.72	.03
Urinary[c,e]	.34	.36	.37	.23	.35	.04
Total[a,b,c,d]	.84	1.16	1.05	.78	1.06	.04
Absorption						
Grams/d[a]	.49	.76	.71	.66	.77	.04
% of intake[e]	49.2	48.5	48.5	55.1	51.3	2.4
Retention						
Grams/d[a]	.15	.40	.37	.44	.42	.04
% of int[a,b,c]	15.1	25.4	25.1	36.3	28.1	2.9
% abs ret[a]	31.6	51.8	50.3	65.8	55.5	5.9

[a]Basal vs supplemented (P<.05)
[b]Waste vs conventional supplements (P<.01)
[c]Dicalcium phosphate vs soybean meal (P<.05)
[d]Swine waste vs soybean meal (P<.1)
[e]Waste vs conventional supplements (P<.1)

Expressed as g/d, Mg absorption was higher in sheep fed the supplemented diets (P< .05) than in sheep fed the basal diet. Expressed as percent of intake, sheep tended to absorb less Mg from the waste diets than from the conventionally supplemented diets (P< .1). Retention of Mg, using either system of measurement, was higher for animals fed the supplemented diets (P< .05).

Magnesium absorption, calculated by difference, tended to be lower by animals fed the waste diets than by animals fed the dicalcium phosphate and soybean meal diets (P< .1), but the rate of absorption was high on all diets. Serum Mg levels were similar across diets.

Expressed as g/d, absorption was higher (P< .05) from the stomach of sheep fed the supplemented diets and tended to be higher (P< .1) for sheep fed the waste diets, apparently due to a near zero value for the sheep fed the dicalcium phosphate diet (table 9). However, these differences were not seen when absorption was expressed as percent of intake. By either method of measurement, sheep fed the dicalcium phosphate diet absorbed less Mg from this region than sheep fed the soybean meal diet.

Sheep absorbed Mg from the small intestine on all diets. Expressed as g/d, absorption was higher by sheep fed supplemented diets (P< .05), but not when expressed as percent of

TABLE 9. MAGNESIUM INTAKE, FLOW, AND ABSORPTION IN WETHERS
FED DIFFERENT SUPPLEMENTS

Item	Supplement					SE
	None	Swine waste	Broiler litter	Dical phos	Soybean meal	
Intake, mg/d[a,b,c]	1.1	1.8	1.7	1.2	1.7	.02
Flow, g/d						
Stomach[a,b,c]	.76	1.28	1.14	1.16	.95	.06
Small int[b,d]	.57	.95	.79	.49	.62	.07
Large int[a,b,c]	.57	.91	.80	.61	.72	.04
Absorption, g/d						
Stomach[a,c,e]	.33	.47	.57	.07	.76	.05
Small int[a,c,e]	.19	.33	.35	.67	.33	.09
Large int	0	.04	-.02	-.12	-.10	.09
Total[a,c,e]	.51	.84	.91	.62	.99	.04
Absorption, % of intake						
Stomach[c]	30.2	26.9	32.8	5.5	44.3	3.6
Small int[b,c]	17.3	18.8	20.9	54.7	20.3	6.3
Large int	0	2.4	-1.3	-10.0	-6.6	7.1
Total[c]	47.5	48.1	52.6	50.2	57.9	2.6

[a]Basal vs supplemented (P<.05)
[b]Waste vs conventional supplements (P<.05)
[c]Dicalcium phosphate vs soybean meal (P<.05)
[d]Basal vs supplemented (P<.1)
[e]Waste vs conventional supplements (P<.1)

intake, indicating that absorption was in relation to in-take. Sheep fed the dicalcium phosphate diet absorbed more Mg from this area than sheep fed the soybean meal diet (P< .05).

Little Mg was transported in the large intestine, alt-hough sheep fed the dicalcium phosphate diet tended to ex-crete Mg into this area.

Copper Metabolism. Copper intakes were fairly high across diets, making detection of absorption differences difficult (table 10).
However, animals fed waste diets had higher apparent Cu ab-sorption than sheep fed the dicalcium phosphate and soybean meal diets (P< .01), primarily due to a very low rate of Cu absorption by animals fed the soybean meal diet. Less dif-ference was seen when absorption was expressed as percent of intake, indicating that some of the response was due to lev-el of intake. Similarly, Cu absorption was lower in g/d by sheep fed the swine waste diet than by sheep fed the broiler litter diet (P< .05), but not when expressed in percent of intake.

Copper was absorbed from the stomach of all sheep (table 11). Expressed as g/d, more Cu was absorbed from the sto-mach of sheep fed broiler litter than those fed swine waste (P< .05). However, expressed as percent of intake, less Cu

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TABLE 10. COPPER BALANCE IN WETHERS FED DIFFERENT SUPPLEMENTS

Item	None	Swine waste	Broiler litter	Dical phos	Soybean meal	SE
Intake, mg/d[a,b,c]	11.0	14.9	19.9	13.7	12.2	.8
Excretion, mg/d						
Fecal[a,d]	5.7	8.1	9.9	6.7	10.3	.8
Urinary	0	0	0	0	0	0
Total[a,d]	5.7	8.1	9.9	6.7	10.3	.8
Absorption						
Mg/d[b,c,d]	5.4	6.7	10.0	7.0	1.9	1.1
% of intake[d,e]	47.7	45.4	46.9	51.3	14.1	6.6
Retention						
Mg/d[b,c,d]	5.4	6.7	10.0	7.0	1.9	1.1
% of intake[d,e]	47.7	45.4	46.9	51.3	14.1	6.6
% abs ret	100	100	100	100	100	

[a]Basal vs supplemented (P<.05)
[b]Swine waste vs broiler litter (P<.05)
[c]Waste vs conventional supplements (P<.05)
[d]Dicalcium phosphate vs soybean meal (P<.05)
[e]Waste vs conventional supplements (P<.1)

TABLE 11. COPPER INTAKE, FLOW, AND ABSORPTION IN
WETHERS FED DIFERENT SUPPLEMENTS

Item	Supplements					SE
	None	Swine waste	Broiler litter	Dical phos	Soybean meal	
Intake, mg/d[a,b,c,d]	10.4	15.9	21.5	16.1	14.3	.03
Flow, mg/d						
Stomach[a,b,c,d]	6.3	11.2	14.7	12.0	9.4	.8
Small int[a,b,c,d]	1.7	1.9	6.7	1.1	3.5	.5
Large int[a]	6.1	8.7	11.1	7.1	10.4	1.2
Absorption, mg/d						
Stomach[e]	4.1	4.7	6.8	4.2	4.9	.8
Small int.[a,d]	4.6	9.3	8.1	10.9	5.9	.9
Large int	−4.4	−6.9	−4.4	−6.0	−6.9	1.3
Total[a,d,e,f]	4.3	7.2	10.4	9.1	3.9	1.2
Absorption, % of intake						
Stomach[g]	40.4	25.8	28.3	25.8	34.7	5.3
Small int[c,d]	44.4	61.9	38.4	67.4	40.3	6.8
Large int	−44.8	−44.3	−21.7	−37.6	−50.1	9.5
Total[d]	40.0	43.4	45.0	55.5	24.9	8.7

[a] Basal vs supplemented (P<.05)
[b] Waste vs conventional supplements (P<.05)
[c] Swine waste vs broiler litter (P<.05)
[d] Dicalcium phosphate vs soybean meal (P<.05)
[e] Swine waste vs broiler litter (P<.1)
[f] Waste vs conventional supplements (P<.1)
[g] Basal vs supplemented (P<.1)

was absorbed from this area in supplemented animals (P< .05).

Animals fed supplemented diets absorbed more Cu (g/d) from the small intestine than animals fed the basal diet (P< .05), but no difference was seen when absorption was expressed as percent of intake. Sheep absorbed a higher percentage of ingested Cu from the swine waste diet than the broiler litter diet (P< .05) from this area. Copper absorption in the small intestine was higher in animals fed the dicalcium phosphate diet than by those fed the soybean meal diet (P< .05).

Copper was secreted into the large intestine of all sheep. Total Cu absorption (g/d) was higher by sheep fed the supplemented diets (P< .05). Values tended to be higher for sheep fed the waste diets (P< .1) compared to those fed the conventional supplements. Absorption, expressed as percent of intake, tended to be higher by sheep fed the dicalcium phosphate diet than by sheep fed the soybean meal diet.

Iron Metabolism. Iron intakes were high across diets, making absorption differences difficult to detect (table 12). Sheep fed supplemented diets ingested more Fe than sheep fed the basal diet (P< .05). Fecal excretion did not differ across diets. Whether measured as mg/d or percent of intake, there were no differences in absorption across diets. Essentially all Fe absorbed was retained.

TABLE 12.   IRON BALANCE IN WETHERS FED DIFFERENT SUPPLEMENTS

| Item | Supplements | | | | | SE |
	None	Swine waste	Broiler litter	Dical phos	Soybean meal	
Intake, mg/d[a,b,c]	362.6	469.9	452.5	717.3	457.9	9.5
Excretion, mg/d						
Fecal	361.5	583.7	707.9	593.7	483.7	136.2
Urinary[a,b]	3.1	4.1	4.4	5.7	4.7	.5
Total[a,c,d]	364.6	587.8	712.3	599.4	488.3	25.3
Absorption						
Mg/d	1.1	-113.7	-255.4	123.7	-25.8	138.2
% of intake	-.3	-24.3	-59.6	-6.9	-6.3	32.4
Retention						
Mg/d[b,c]	-2.1	-117.9	-259.0	117.9	-30.5	130
% of intake	-1.2	-25.2	-60.3	-7.8	-7.3	32.4
% abs ret	121.9	104.1	103.5	100.1	98.4	12.7

[a]Basal vs supplemented (P<.05)
[b]Waste vs conventional supplements (P<.05)
[c]Dicalcium phosphate vs soybean meal (P<.05)
[d]Swine waste vs broiler litter (P<.1)

Less Fe was absorbed from the stomach (P< .05) and small intestine (P< .1) of animals fed the waste diets than animals fed the conventional supplements (table 13). However, considerable secretion into the large intestine occurred across diets. Total Fe absorption tended to be higher in animals fed swine waste than those fed broiler litter (P< .1). A net loss was recorded for animals fed the broiler litter diet.

Zinc Metabolism. More Zn was ingested by animals fed supplemented diets (P< .05), and animals ingesting the swine waste diet tended to have the highest intake of this element (table 14). Excretion values were similar across treatments. Expressed as percent of intake, sheep ingesting the waste diets tended to be in less negative Zn absorption and retention status than sheep fed the dicalcium phosphate and soybean meal diets (P< .1). Essentially all Zn absorbed was retained across diets. Animals on all diets were in negative Zn balance.

Expressed as g/d or percent of intake, less Zn was secreted into the stomach of sheep fed supplemented diets than from sheep fed the basal diet (table 15) (P< .1). All supplemented sheep absorbed Zn from the small intestine. Animals fed supplemented diets secreted more Zn into the large intestine than those fed the basal diet (P< .05). Expressed

TABLE 13. IRON INTAKE, FLOW, AND ABSORPTION IN WETHERS FED
DIFFERENT SUPPLEMENTS

| Item | Supplements | | | | | |
	None	Swine waste	Broiler litter	Dical phos	Soybean meal	SE
Intake, mg/d[a,b,c,d]	451.9	655.8	466.5	590.2	434.9	4.6
Flow, mg/d						
Stomach[a,b,c,d]	361.5	565.1	495.5	546.2	318.7	21.7
Small int[b]	353.4	557.9	520.5	352.6	269.8	60.9
Large int[e]	385.5	625.6	750.4	628.4	464.2	111.5
Absorption, mg/d						
Stomach[b,c,d]	90.4	90.7	-29.0	44.0	116.2	20.8
Small int[f]	8.1	7.3	-25.0	193.6	48.9	63.3
Large int	-32.0	-67.7	-229.9	-275.8	-194.4	98.9
Total[g]	66.5	30.2	-283.9	-38.2	-29.2	111.5
Absorption, % of intake						
Stomach[a,b,c,d]	19.9	12.2	-6.0	7.2	24.7	3.7
Small int[f]	1.7	-1.7	-5.2	33.1	7.2	12.0
Large int	-6.6	-8.2	-48.9	-46.9	-39.6	20.0
Total[g]	15.0	2.4	-60.1	-6.5	-7.7	23.4

[a]Basal vs supplemented (P<.05)
[b]Waste vs conventional supplements (P<.05)
[c]Swine waste vs broiler litter (P<.05)
[d]Dicalcium phosphate vs soybean meal (P<.05)
[e]Basal vs supplemented (P<.1)
[f]Waste vs conventional supplements (P<.1)
[g]Swine waste vs broiler litter (P<.1)

TABLE 14. ZINC BALANCE IN WETHERS FED DIFFERENT SUPPLEMENTS

Item	Supplements					SE
	None	Swine waste	Broiler litter	Dical phos	Soybean meal	
Intake, mg/d[a,b,c]	33.6	58.1	46.3	40.5	35.2	2.9
Excretion, mg/d						
Fecal	164.9	125.6	174.9	176.3	174.4	33.6
Urinary	.76	.55	.69	1.17	.55	.27
Total	165.7	126.2	176.5	177.5	175.0	30.6
Absorption						
Mg/d	-131.3	-67.5	-128.7	-135.8	-139.3	33.2
% of intake[d]	424.6	-125.2	-277.4	-328.8	-385.4	82.5
Retention						
Mg/d[b,d]	-132.1	-68.1	-140.2	-137.0	-201.2	30.4
% of intake[a]	-427.4	-126.2	-303.1	-331.5	-537.5	77.1
% abs ret	101.1	95.7	100.5	100.8	100.9	2.8

[a]Waste vs conventional supplements (P<.05)
[b]Swine waste vs broiler litter (P<.1)
[c]Basal vs supplemented (P<.05)
[d]Waste vs conventional supplements (P<.1)

TABLE 15. ZINC FLOW AND ABSORPTION IN WETHERS FED
DIFFERENT SUPPLEMENTS

| Item | Supplements | | | | | SE |
	None	Swine waste	Broiler litter	Dical phos	Soybean meal	
Intake, mg/d[a,b,c,d]	35.8	49.8	61.1	40.9	35.3	.9
Flow, mg/d						
Stomach[e]	193.2	123.9	138.2	125.7	134.8	30.5
Small int[a]	197.2	111.6	111.2	81.0	81.0	30.2
Large int	177.8	132.0	201.1	191.4	179.9	39.4
Absorption, mg/d						
Stomach[e]	−157.5	−74.1	−77.1	−84.8	−99.5	30.2
Small int	−4.0	12.3	27.0	44.7	53.8	27.9
Large int[a]	19.4	−20.4	−89.9	−110.4	−98.9	30.7
Total	−142.0	−82.2	−140.0	−150.5	−144.6	38.9
Absorption, % of intake						
Stomach[a]	−441.0	−148.9	−130.3	−203.8	−266.9	75.9
Small int	−10.1	24.5	47.6	121.6	140.1	72.8
Large int[a,b]	51.2	−40.7	−147.2	−278.6	−263.2	74.8
Total[f]	−399.9	−165.1	−229.9	−360.9	−389.9	99.7

[a] Basal vs supplemented (P<.05)
[b] Waste vs conventional supplements (P<.05)
[c] Swine waste vs broiler litter (P<.05)
[d] Dicalcium phosphate vs soybean meal (P<.05)
[e] Basal vs supplemented (P<.1)
[f] Waste vs conventional supplements (P<.1)

as percent of intake, animals fed the waste diets secreted less Zn into the large intestine than sheep fed the dicalcium phosphate and soybean meal diets (P< .05). However, no differences were found in total Zn absorption across diets.

Discussion

Phosphorus. Balance data indicates a trend for higher P absorption from swine waste and broiler litter than from dicalcium P and soybean meal, but differences were small and not significant. At this level of P intake, sheep were apparently able to regulate their P absorption to meet their requirements. Positive absorption values for mature wethers indicate that animals were replacing body stores lost during the depletion period.

Sheep fed supplemented diets showed higher apparent absorption in g/d (P< .01) than sheep fed the basal diet but less difference was shown when absorption was expressed as percent of intake (P< .1). True absorption was not measured, so endogeneous secretion could have masked true absorption in sheep fed the basal diet. Field et al. (1977) found that for lambs fed poultry litter apparent P absorption ranged from 23 to 74%, but true absorption, from 49 to 90%.

The trend for higher P excretion in the urine of waste-fed wethers may reflect efficiency of P absorption or amount absorbed in excess of requirements. Urinary P excretion tended to be higher for animals fed supplemented diets, and highest for animals fed the swine waste diet. Field et al. (1983) found that animals excreting appreciable amounts of P in the urine were the most efficient in absorbing P. They suggested that P not required by the body is excreted first via saliva, then as the secretory capacity of the salivary glands is approached, serum inorganic P levels rise above the renal threshold for P excretion (about 6.57 mg/dl) and P is lost via urine.

The amount of P secreted via saliva will be affected by salivary P concentration and the total amount of saliva produced (Tomas, 1974), which will depend on type of feed and rumination time. Our sheep were fed a finely ground silage, which may have resulted in low rumination time and more P lost via the urine. Secretion of P anterior to the site of P absorption conserves P and helps to insure a constant supply of P for rumen microorganisms, important to animals which evolved on forages which tend to be high in Ca but limiting in P.

Animals may have adapted to the low P basal diet, maximizing absorption and conservation mechanisms during the de-

pletion periods. Field et al. (1983) reported endogeneous excretion values of 17.3 mg/kg body weight and 24.0 mg/kg body weight for sheep fed a low P and an adequate P diet, respectively. If these values are used in calculating P absorption by sheep fed different diets, calculated true absorption for the basal, swine waste, broiler litter, dicalcium phosphate and soybean meal diets are 84.7, 85.7, 81.0, 66.7, and 75.0 percent, respectively. The high rate of absorption shown by animals fed the basal diet would indicate that animals have increased the efficiency of their absorptive mechanisms in a homeostatic response to reduced P supply. Young et al. (1966) reported that sheep depleted of P increased their rate of P absorption from the small intestine for at least 11.5 d after being returned to a replete diet.

Poppi and Termouth (1979) found lower serum P values, but no difference in liveweight or bone P between wethers fed a low P basal diet and those fed supplemented diets. Sheep ingested 1.56 g P/d from the basal diet, similar to the amounts supplied by our basal diet, and they were of comparable weights. They suggested that 1.56 g/d might be an adequate intake for their animals. Field et al. (1975), feeding young lambs .13% P and varying levels of Ca, found that lambs reduced growth rate according to nutrient supply,

but were never in negative P balance. Our animals were mature, and had no requirement for net bone deposition, milk production, or fetal growth. During the depletion period they may have maximized absorption and conservation mechanisms, thus succeededing in adapting to the amount of P contained in the basal diet.

Similar levels of preintestinal P secretion have been reported by other researchers (Pfeffer et al., 1970; Ben-Ghedalia et al., 1975; Dillon and Scott, 1979; Yano et al., 1979; Ben-Ghedalia et al., 1982). The secretion has been shown to be due to P secretion from serum via saliva (Tomas and Somers, 1974; Scott et al., 1984). Sheep fed the supplemented diets secreted more P in g/d than the sheep fed the basal diet (P< .1), and sheep fed the swine waste diet secreted more than sheep fed the broiler litter diet (P< .05). This may have been due to the relationship between serum inorganic P and secretion of P via the salivary glands. Parotid salivary P secretion has been found to be linear with serum P (Manas-Almendros et al., 1982) and is affected by the rate of removal of P by salivary secretion (Tomas, 1974) and the P gradient from the saliva to the serum.

Expressed as percent of intake, sheep fed the low P basal diet had the highest rate of recycling and of absorption

from the small intestine. Phosphorus homeostasis has been postulated to be controlled by a combination of recycling via saliva, and adjustment of the absorptive efficiency of the small intestine for P (Scott et al., 1984). The absorptive efficiency of the small intestine is increased in sheep in response to reduced P intake (Young et al., 1966; Braithwaite, 1979; Scott et al., 1984). A carrier mediated transport system for P has been postulated (Care et al., 1980), although a combination of diffusion plus active transport has also been suggested (Braithwaite, 1979).

The trend for sheep fed the dicalcium P diet to show the lowest apparent P absorption could mean either that the P from this supplemental source was utilized more efficiently, thus requiring less, or that it was less well retained. Phosphorus from this diet was absorbed from the small intestine quite efficiently (table 3), then was secreted in saliva, urine, and via the large intestine, resulting in lower apparent absorption and lower retention. Sheep fed this diet also tended to have lower serum inorganic P values than sheep on the other supplemented diets, indicating a rapid rate of loss and poor retention.

Appreciable amounts of P are absorbed or secreted in the large intestine. Sheep fed the swine waste diet, which had the most positive P balance, absorbed 12.8% of their P in-

take from the large intestine. In contrast, animals fed the dicalcium phosphate diet secreted 37.2% of P intake into this area. The net effect was to equalize apparent P absorption across the supplemented diets. The large intestine may play an important role in P balance in ruminants.

Overall, absorption and retention of P was as good from waste diets as from conventional sources. Partitioning of absorption was somewhat different, but net absorption tended to equalize across supplemented diets.

Calcium. The extremely high rate of Ca absorption, expressed either as g/d or percent of intake, found in sheep fed the soybean meal diet may have been due to the high protein level of that diet. Some nonruminant data suggest that Ca absorption increases in response to a high protein diet (McCance et al., 1942; Hall and Lehmann, 1944; Desikachar and Subrahmanyan, 1949; Chu et al., 1975) although the effect is not always found and has been suggested to occur only in mature animals (Howe and Beecher, 1981). However, unless the P intake is raised (Linkswiler et al., 1981) nonruminants develope hypercalciuria which often results in negative net Ca absorption, an effect which was not seen with these animals.

The high rate of Ca absorption found in sheep fed the basal diet may have been a compensation for lower intake.

Sheep can increase the rate of Ca absorption in response to reduced Ca supply (Braithwaite, 1974; Abdel-Hafeez. et al., 1982), and between Ca intakes of 40 to 100 mg/d/kg body weight, sheep have been shown to be able to adjust Ca absorption to their requirement (Braithwaite, 1979). Our intakes fell within this range.

Phosphorus deficiency has been shown to reduce Ca absorption (Young et al., 1966; Abdel-Hafeez et al., 1982), which did not occur in our study. However, Young et al. (1966) used a much more severe P deficiency in their sheep than we used in ours. Abdel-Hafeez et al. (1982) did find a small drop in absorptive efficiency of Ca by sheep fed a mildly deficient diet similar to our basal diet. However, our animals were fed the basal diet for a much longer period than those in the Abdel-Hafeez study, and it is possible that the sheep had adapted to the low P basal diet.

Both secretion (Ben-Ghedalia et al., 1975; Greene, 1981; Ben-Ghedalia et al., 1982) and absorption (Grace et al., 1974; Dillon and Scott, 1979) of Ca in the preintestinal area of sheep have been reported by other researchers. The concentration of soluble Ca in the rumen may be an important factor; this would be affected by the amount and type of Ca ingested. Assuming a rumen capacity of 13 to 23 liters and a rumen turnover time of 12 h, (Breazile, 1971), then a ru-

men Ca concentration higher than standard serum Ca values could be calculated to exist in sheep in Grace et al., (1974) and one lower than serum Ca in the sheep of Greene (1981) and Ben_Ghedalia et al. (1975). A low rate of Ca absorption from the stomach of the sheep fed waste was reported by Ben-Ghedalia et al. (1982). Calcium may be complexed with organic matter in the rumen of sheep fed waste, and thus not be available for absorption. Less organic matter was digested in the stomach of sheep fed waste, which may have resulted in less Ca being released into solution in this region. The net Ca secretion by sheep fed the dicalcium phosphate diet may have been due to a low solubility of dicalcium phosphate in neutral rumen conditions (Durand and Kawasaki, 1980). Feeding the same absolute amount of Ca in several different diets, Pfeffer et al. (1970) found a small net secretion with an organic Ca source, but a small net absorption when part of the Ca was supplied by calcium carbonate. If absorption is diffusion dependent, it could occur by paracellular routes, or by facilitated transport across cell membranes. Diffusion of ionized Ca unassisted by carrier molecules across cell membranes is unlikely.

Dillon and Scott (1979) showed that developement of forestomach Ca absorption paralleled developement of the rumen and of forestomach Mg absorption in the weanling lamb. It

is possible that Ca competes with Mg for the Mg transport system postulated by Martens (1978). The low rate of preintestinal Mg absorption by sheep fed the dicalcium phosphate diet may be due to transport carriers being occupied in effecting the high rate of Ca secretion in this area. Conversely, sheep fed the soybean meal diet showed virtually no transfer of Ca across the stomach, but had the highest rate of Mg transfer. Moderate levels of absorption or excretion of Ca by sheep on the basal, swine waste, and broiler litter diets were associated with moderate rates of preintestinal Ca transfer. Both Ca and Mg are members of the s, 2A subgroup of the Periodic Table, and both are active primarily as ions within the body. Although they are certainly not used interchangeably in metabolic reactions, they share enough characteristics to be competative for a transport mechanism.

All animals absorbed substantial amounts of Ca from the small intestine. Both absorption (Ben-Ghedalia et al., 1975; Dillon and Scott, 1979; Yano et al., 1979; Ben-Ghedalia et al., 1982) and secretion (Pfeffer et al., 1970; Grace et al., 1974) of Ca in the small intestine of sheep have been reported. Calcium and Mg are absorbed in the upper small intestine (Ben-Ghedalia et al., 1975; Yano et al., 1979) but secreted in the lower small intestine (Ben-Gheda-

lia et al., 1975). If this secretion is part of the homeostatic control of body stores, then a high intake might cause increased secretion, resulting in net secretion across the small intestine for both minerals. A relatively limited supply of Ca in our diets may have increased absorption in the small intestine.

Secretion and absorption in the large intestine seemed to be affected by the amount of Ca which had been absorbed in previous intestinal sections. Animals appear to be capable of absorbing Ca from the large intestine if they have not been able to meet their requirement by absorption from earlier segments, but the large intestine seems to primarily serve as a route of Ca excretion. Sheep appear capable of absorbing Ca in excess of requirements, and secreting the excess.

The fact that all animals were in positive Ca balance suggests that Ca stores were lost, possibly through bone resorption during the P depletion, and were being replaced. Retention of Ca between animals fed the basal, swine waste, and broiler litter diets were similar, despite different intakes, suggesting control of absorption and retention.

Although sheep fed waste diets have lower net Ca absorptions, retention expressed as g/d indicates that waste can supply the dietary requirement. Calcium from waste may be

better retained than Ca from an inorganic source such as dicalcium phosphate.

Magnesium. Magnesium absorption, expressed as percent of intake, was similar to that found by Ben-Ghedalia et al. (1982). They measured Mg absorption of 51% from a soybean meal supplemented diet, and 41.6% from a poultry litter supplemented diet. Although results are variable, addition of starch to a diet has been shown to increase the Mg absorption (Giduck and Fontenot, 1984) The substantial levels of starch used both in our diets and in Ben-Ghedalia et al. (1982) may have increased the rate of Mg absorption from these diets.

Essentially no Mg was absorbed preintestinally by sheep fed the dicalcium phosphate diet, which could be due to interference by the high rate of Ca transfer. The substantial preintestinal absorption of Mg evidenced by sheep fed all diets except the dicalcium phosphate diet agrees with reports by other researchers (Pfeffer et al., 1970; Grace and Macrae, 1972; Axford et al., 1975; Martens, 1978; Dillon and Scott, 1979). Magnesium absorption from this region did not differ significantly between diets. Formation of insoluble magnesium phosphate may have limited preintestinal Mg absorption, although similar levels of preintestinal P secretion did not correspond with similar decreases in Mg absorp-

tion in sheep fed the swine waste and dicalcium phosphate The low values for preintestinal absorption of Mg by sheep fed the dicalcium phosphate diet are not likely to be purely due to indicator error since there was not a proportional reduction in values for all other minerals determined by the same indicator. diets.

Variable absorption (Grace et al., 1974; Ben-Ghedalia et al., 1982) and secretion (Grace et al., 1974; Greene , 1981) of Mg in the small intestine has been reported. Magnesium status and intake may determine net Mg absorption or secretion. Our sheep were fed approximately at maintenance, which may have minimized secretion and maximized net absorption in the small intestine. There was no indication that Mg and Ca absorption from this area might be competitive at the levels which we fed. Sheep fed the dicalcium phosphate diet had the highest level of Ca absorption in the small intestine, and absorbed the largest amount of Mg from this area as well.

With the exception of sheep fed the dicalcium phosphate diet, sheep absorbed little or no Mg from the large intestine. Both zero (Ben-Ghedalia et al., 1982) and positive absorption (Grace et al., 1974; Greene, 1981; Ben-Ghedalia et al., 1982) have been found by other researchers.

Partitioning of absorption was different with animals fed the different diets, but the net effect was to equalize percent apparent Mg absorption across the diets.

It seems clear that absorption of minerals can vary along the intestinal tract but still give similar net results. The methods of coordination of uptake or secretion in different parts of the tract are presently unknown. Most studies have examined the role of the small intestine in mineral absorption, and, particularly in nonruminants, the small intestine tends to be the area of most active absorption and most important control mechanisms. However, the full role of the large intestine in mineral homeostasis should prove an important field of study in the next few years.

Copper. The high rates of Cu absorption and retention by sheep from all diets agree with findings by other researchers (Fontenot et al., 1972; Fontenot and Webb, 1975; Van Rysden, 1981; Suttle et al., 1978; Olson et al., 1984). Preintestinal absorption expressed as percent did not differ among animals, suggesting that absorption was in relation to intake. Although Cu is considered to be antagonistic to Ca absorption (Georgievskii, 1984), a fairly high rate of Cu absorption from the small intestine of animals fed the dicalcium phosphate diet corresponded with a high rate of Ca and Mg absorption from this region. Mineral antagonism may be acting at another site than than the small intestine.

An inverse relationship between Cu and Ca net transfer was seen in the large intestine. A very high rate of excretion from the large intestine resulted in a much lower net Cu absorption by animals fed the soybean meal diet than by animals fed any other diet. Sheep fed the soybean meal diet had shown a very low rate of P and Ca transfer in the large intestine. Since net absorption of Cu and Ca are known to be antagonistic (Underwood, 1977; Georgievskii, 1984), the low Ca secretion may have allowed greater Cu excretion into this region. Differences in net absorption tended to be heavily influenced by excretion into the large intestine (table 12). A low rate of excretion into this area by sheep fed the broiler litter diet resulted in a net Cu absorption approximately equal to that by animals fed the swine waste diet, although animals fed the swine waste diet absorbed more Cu in the stomach and small intestine.

Expressed as percent of intake, little difference was seen in Cu absorption in sheep fed different diets, but more difference was noted when Cu absorption was expressed as g/d, which might indicate that sheep absorb Cu in response to intake and lack control mechanisms. However, partitioning of absorption was very different by sheep fed different diets. Evidence for redistribution of body stores has been found (Olson et al., 1984), and it appears that instead of

excretion via the bile, Cu is primarily excreted into the large intestine of sheep. This would seem to be a less efficient means of eliminating excess Cu, which may be the reason sheep are quite sensitive to high Cu levels.

Iron. Considering standard errors, both absorption and retention were basically zero for all sheep except the sheep fed the broiler litter diet. Adult animals are known to have very low Fe requirements since Fe released during hemoglobin catabolism is reused (Georgievskii, 1984). Therefore, a zero balance in mature replete animals would be expected. Since both Fe and Zn are antagonistic to Cu absorption (Underwood, 1977; Miller, 1979), Fe absorption by sheep fed the broiler litter diet may have been depressed by Cu antagonism. It is also posible that valence states or donor ligands are limiting Cu absorption from the broiler litter diet, since both are known to be important (Miller, 1979), but the Cu level in the diet appears to have a marked effect.

Lower secretion into the large intestine of sheep fed the swine waste diet may have been due to the release of Fe into the intestinal lumen as a result of organic matter digestion in this area. Alternatively, it could be an adaptive response to conserve Fe since lowered secretion was found in sheep fed the basal diet as well.

Zinc. Zinc is secreted into the preintestinal area of animals fed all diets. This element is secreted via saliva and has been suggested to move across the rumen wall (Georgievskii, 1984). On both mass and percent basis, more Zn was recycled by animals fed the basal diet than by those fed supplemented diets, suggesting a homeostatic response to Zn supply (P< .01) similar to that found with P. Sheep may need to control secretion into the rumen to maintain Zn levels for resident microflora.

The large variation in Zn absorption from the small intestine precluded detection of differences in absorption between diets. The high Cu and Fe levels in all diets may have reduced absorption of Zn from the small intestine, and would also have reduced reabsorption of Zn secreted into the preintestinal area.

Although large standard errors make interpretation difficult, the large intestine seemed to be the main area of absorption by sheep fed the basal diet. If secretion and absorption across the first two intestinal segments are averaged, they are -98.6% for sheep fed the waste diets, and -95.2 % for those fed the positive control diets. However, sheep fed waste diets secreted less Zn into the large intestine, which resulted in a less negative net Zn absorption by these sheep. The low rate of Zn secretion into the

large intestine found in sheep fed the waste and the basal diets may have been related to a high rate of organic matter digestion increasing Zn availability. Zinc absorption is known to be reduced by high Ca intakes (Miller, 1979). Therefore, the high rate of Ca absorption from the large intestine of animals fed the swine waste diet may have reduced Zn transport in the large intestine. It is apparently possible to absorb Zn from the large intestine, but this seems to be more frequently an area for secretion. The control mechanisms for this secretion are not presently understood.

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CHAPTER III

JOURNAL ARTICLE 2

THE EFFECT OF LEVEL AND SOURCE OF PHOSPHORUS ON

CELLULYTIC BACTERIA NUMBERS IN THE RUMEN OF SHEEP

Summary

A trial was conducted with 15 wethers surgically equipped
with duodenal cannulas to study the relationship between
cellulytic bacterial levels in the rumen of sheep fed diffe-
rent levels and sources of P and the degree of cellulose
digestion in these sheep. Animals were fed a low P basal
diet until serum inorganic P averaged 5.5 mg/dl, which was
taken as evidence of a mild depletion. Then they were ran-
domly assigned to one of the following diets: low-P basal,
basal + swine waste, basal + broiler litter, basal + dical-
cium phosphate, basal + soybean meal. The trial consisted
of a 7-d preliminary period, a 7-d collection of urine and
feces, and a 6-d sampling of duodenal and ileal digesta and
feces. Rumen fluid was sampled for analyses of cellulolytic
bacterial populations at the end of the depletion period and
after sheep had been fed experimental diets for at least

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21-d. Diets, duodenal samples and feces were analyzed for cellulose content, ADF and lignin. More cellulolytic bacteria were found in sheep fed diets supplemented with P ($P < .1$), indicating that P was limiting to growth of cellulolytic bacteria in animals fed the basal diet. The highest levels of bacteria were isolated from sheep which demonstrated the greatest amount of P recycling. However, no difference was seen in digestion of cellulose either in the stomach or across the intestinal tract. The ADF fraction of the diets tended to be degraded less in the rumen than in the lower intestinal tract. Increased cellulolytic bacterial populations may have been utilizing alternate carbon sources in the diets. Phosphorus recycling does appear to have an important influence on the resident cellulolytic bacteria populations.

Key words: Phosphorus, Sheep, Bacteria, Cellulose, Mineral

Introduction

Rumen bacteria require P in order to grow (Durand and Kawashima, 1980) and in vitro experiments have shown increased digestion of cellulose with increased P concentration in medium (Hall et al., 1961). Cellulytic bacteria form the basis of the rumen ecosystem of animals fed a forage diet.

Degradation products of cellulose digestion supply energy requirements of associated rumen bacteria. Therefore, anything which limits the rate of cellulose digestion will affect the population dynamics of noncellulolytic bacteria as well. Experimental evidence concerning whether the host animal or resident microbial population are first affected by a P deficiency has been ambiguous. A complicating factor is that the phosphorus requirement is determined not only by the P but also by the Ca and Mg concentrations in the media, (Durand and Kawashima, 1980).

Dry matter digestibilities were lower for lambs fed a normal Ca:low P diet than for lambs fed a normal Ca:normal P diet (Field et al., 1975), suggesting reduced cellulolytic activity. Addition of Ca has been shown to lower P solubility in the rumen (Nel and Moir, 1974).

Phosphorus is recycled extensively into the rumen via saliva (Tomas, 1974; Scott et al, 1984), reducing the likelihood of a P deficiency for the rumen bacteria. However, P secretion depends on both amount of salivary secretion and serum P levels (Tomas, 1974). Recycling of P is particularly important in meeting the P requirements of cellulolytic bacteria. Degredation products of cellulose help supply the energy requirements of associated rumen bacteria. Stimulation of cellulose degradation by addition of P has been

shown in vitro (Hall et al., 1961). Wier et al. (1958) reported that the P content of hay limited its digestibility. This suggested that P might be a limiting nutritent for cellulolytic bacteria during low P intakes by the host animal.

The effect of level and source of P on the cellulolytic bacterial populations in the rumen and on digestibility of organic components was studied in wethers fed different amounts and sources of P.

Materials and Methods

A detailed description of the trial procedure is given in Chapter II. All data concerning bacterial populations are from samples taken in the second trial. Briefly, animals were placed on a low P basal diet supplying approximately one-half their P requirement until serum inorganic P averaged 5.5 mg/dl, which was taken as evidence of a mild deficiency. At this time, rumen fluid was sampled via stomach tube just before the evening feeding, a time when cellulolytic bacterial populations should have peaked (Leedle et al., 1982). Rumen fluid was quickly strained through 2 layers of cheesecloth, then pipetted into sterile 1 ml freezer tubes containing 1 to 2 drops of DMSO as a cryoprotectant. Tubes were immediately frozen in liquid nitrogen, and stored in a -80 C degree freezer until innoculation.

Animals were transferred to the experimental ensiled diets for the duration of the trial. Diets included a low P basal, and basal supplemented with equal amounts of P from each of the following: swine waste, broiler litter, dicalcium phosphate or soybean meal. Phosphorus concentrations of the diets ranged from .15 to .31 %. After at least 21 d on the experimental diets, rumen fluid was taken again following the same protocol.

Bacterial populations were estimated by means of MPN tubes. The medium described in Leedle and Hespell (1980) for estimation of cellulolytic bacteria was adapted as following: no agar was used, no cellobiose was added in order to make the test more stringent, 5 % of the cellulose slurry was used, and cysteine was added before autoclaving (table 16). Endigeneous carbohydrate in the rumen fluid was removed by preincubation for three d. The cellulose slurry consisted of Whatman filter paper which had been wet ball milled for 3 d, and was a gift from Dr. Robert Hespell. The medium was tubed under oxygen free carbon dioxide, and all innoculations were performed under carbon dioxide. Medium pH was adjusted to 6.8 after autoclaving by means of a sterile 8 % sodium carbonate solution, and tubes were incubated for at least 5 d before use to insure sterilty.

TABLE 16.   MEDIUM FOR MPN TUBES

Item	Percent of Medium
Trypticase	.2
Mineral One[a]	10
Mineral Two[b]	10
Hemin (0.01%)	1
VFA[c]	1
Resazurin (0.1%)	.1
Rumen Fluid Soln[d]	30
Distilled H_2O	35
Na_2CO_3 (8%)[e]	5
Na_2S/cysteine (2.5%/2.5%)	1
Cellulose slurry	5

[a] .6% K_2HPO_4 in distilled H_2O
[b] .6% KH_2PO_4, .6% $(NH_4)_2SO_4$, 1.2% NaCL,
   .255% $MgSO_4$, .7 H_2O and .17% $CaCL2H_2O$
[c] 17 ml acetic, 6 ml propionic, 4 ml butyric,
   1 ml isobutyrate, 1 ml n-valeric, 1 ml isovaleric,
   1 ml DL-α-methyl-butyric, adjusted pH to 7.5 with
   NaOH and dilute to 100 ml
[d] 35 ml each of Mineral One and Two, 300 ml of
   distilled H_2O, 2 ml of 2.5% (wt/vol) L-cysteine
   hydrochloride and 300 ml rumen fluid incubated for
   5 days at 37°C.
[e] Prepared as a sterile (121°C, 15 min; equilibrated
   with CO_2, 30 min) solution and added after autoclaving

Samples were thawed at room temperature and diluted for innoculation in dilution blanks prepared according to the Anaerobe Laboratory Manual (1977) in a range from 10-3 to 10-8. Dilutions were shaken on a vortex mixer for 10 sec before further use to insure thorough mixing. Tubes were inspected by phase microscopy after 4 wk of incubation, but due to low bacterial numbers, were allowed to incubate for 2 more wk. After 6 wk, bacterial numbers from samples taken from sheep fed the supplemented diets equaled those reported for cellulolytic bacteria isolated from a steer (Leedle et al., 1982). Visual inspection suggested considerable degredation of substrate, so all tubes were examined both by wet mount under phase microscopy and by gram stained preparations under light microscopy. Populations of bacteria in end point tubes were rated from +1 to +4 by visual estimation. A subsample of tubes was examined 2 wk later, but no evidence of a further increase in bacterial numbers was seen, so all data in this report area drawn from the 6 wk incubation.

Data were analyzed using the general linear models procedure of Statistical Analysis System (SAS, 1979). The statistical model included effects of treatment, trial, block, and treatment*trial interactions. Block was deleted from the final model because of lack of significance. Contrasts

tested were: basal vs supplemented diets, waste supplemented diets vs dicalcium phosphate and soybean meal diets, dicalcium phosphate vs soybean meal diets and broiler litter vs swine waste diets.

Results

The tubes contained a heterogeneous bacterial population, with proportionately more single and short chain gram positive cocci visible under phase microscopy, and more rods apparent in gram stained preparations. The rods tended to occur in large clumps, and were often associated with media particles which were assumed to be cellulose particles. Although biochemical identification was not performed, when examined under light microscopy by gram stain, gram positive cocci occured in both single cell and short chains typical of Ruminococcus albus and in the long chains suggestive of Ruminococcus flavefaciens. There were a large number of gram negative rods morphologically similar to Bacteroides succinogenes, and some gram negative curved rods suggestive of Bacteroides fibrisolvens. A small proportion of bacteria such as Trepenemia were present; these may have been using the end products of the cellulose degraders as energy sources. Nutritional interdependence is known to occur between rumen bacteria (Miura et al., 1980; Miura et a., 1983).

For sheep fed all diets, numbers of bacteria tended to increase from the end of the depletion period to the end of the experimental period for all diets (table 17). Numbers of bacteria in animals fed different diets were not significantly different as measured by phase microscopy or by gram-stained preparations due to large variations. However, the increase in numbers during the trial calculated by averaging the two methods showed that bacteria increased to a greater degree in the rumen of sheep fed the supplemented diets (P< .1), with slightly more found in the sheep fed the swine waste, as compared to sheep fed the broiler litter diet (P< .1) There was a trend for animals fed the swine waste and the dicalcium phosphate supplemented diets to contain higher levels of cellulolytic bacteria and to show a greater increase in bacterial numbers during the trial. Levels of bacteria in animals fed the broiler litter, soybean meal and the basal diet were markedly lower at the end of the trial.

Logrhythmic transformation of the raw data indicated similar levels of bacteria in the rumen of all sheep at the end of the depletion period (table 18). However, as measured by phase microscopy, more bacteria were isolated from sheep fed the supplemented diets than from those fed the basal diet by the end of the trial (P< .05). When measured by

TABLE 17. CELLULOLYTIC BACTERIAL NUMBERS IN WETHERS

FED DIFFERENT SUPPLEMENTS

Method	Time	Supplements					SE
		None	Swine waste	Broiler litter	Dical phos	Soybean meal	
		----------------10 x 6-------------------					
Phase:	initial[a]	1.5	42.4	9.0	4.0	4.3	18.6
	final[b]	10.8	95.0	30.0	127.0	53.0	45.7
	diff	9.3	52.6	21.0	123.0	48.7	46.8
Gram stain:	initial	9.6	7.2	4.2	1.1	5.8	3.4
	final	37.0	78.3	53.2	105.8	1.1	38.4
	diff.	27.4	71.1	49.0	104.7	-4.7	40.1
Averaged:	initial	5.5	24.8	6.6	2.5	5.0	9.8
	final	23.9	86.7	41.6	116.3	27.2	27.6
	diff[c,d]	18.4	61.9	35.0	113.7	22.2	34.7

[a]Basal vs supplemented (P<.1)
[b]Swine waste vs broiler litter (P<.1)
[c]Prior to feeding experimental silage
[d]At end of trial

TABLE 18. LOGARITHMIC TRANSFORMATION OF CELLULOLYTIC
BACTERIA NUMBERS IN WETHERS FED DIFFERENT SUPPLEMENTS

		Supplements					
Method	Time	None	Swine waste	Broiler litter	Dical phos	Soybean meal	SE
		----------------10×6----------------					
Phase:	before[a]	14.2	13.9	15.7	14.6	14.8	.65
.	after[b]	16.2	18.1	17.2	18.4	17.3	.56
Gram stain:	before	15.6	15.2	14.7	13.9	14.8	.73
	after[c]	16.2	18.0	17.6	18.1	13.9	.81
Averaged:	before	14.9	14.5	15.2	14.2	14.8	.50
	after[b]	16.2	18.0	17.4	18.2	15.6	.43

[a]Swine waste vs broiler litter (P<.1)
[b]Basal vs supplemented (P<.05)
[c]Waste vs conventional supplements (P<.1)

gram stained preparations, more bacteria were found in sheep fed the waste supplemented diets than in those fed the dicalcium phosphate and soybean meal supplemented diets (P< .1), primarily due to lower cellulolytic bacteria levels found in the sheep fed the soybean meal diet.

If values for the two methods are averaged, by the end of the experimental period more bacteria were found in sheep fed the supplemented diets than those fed the basal diet (P< .05). However, the increase in numbers during the trial was not in itself significant between diets.

These values, particularly the combined values, correspond to the amount of P recycled into the rumen by sheep fed the different diets. Data in Chapter II show that the largest quantity of P was secreted into the stomach of sheep fed the swine waste and the dicalcium phosphate diets, with less P secreted into the stomach of sheep fed the basal, broiler litter and soybean meal diets.

Expressed as percent of intake or g/d, there was no difference in the amount of cellulose digested in the stomach of sheep fed basal or supplemented diets (table 19). Sheep fed the dicalcium phosphate diet tended to digest less cellulose than sheep fed the soybean meal diet (P< .1).

Sheep fed the waste diet digested less of the ADF fraction in the stomach than sheep fed the conventionally sup-

TABLE 19. DIGESTIBILITY OF DIETARY COMPONENTS AT THE
DUODENUM OF WETHERS FED DIFFERENT SUPPLEMENTS

| Item | Supplements | | | | | SE |
	None	Swine waste	Broiler litter	Dical phos	Soybean meal	
Cellulose						
G/d	99.0	107.2	88.6	85.9	116.6	14.3
% of intake[a]	48.5	48.1	42.4	40.7	59.5	6.7
ADF						
G/d[b,c]	94.7	83.8	11.4	81.2	126.4	20.6
% of intake[a,b,c]	37.1	32.0	6.0	31.0	52.9	8.1
Lignin						
G/d[d]	2.1	−1.4	−4.2	.4	11.0	5.0
% of intake[d]	4.6	−2.9	−8.5	1.1	26.6	10.5

[a]Dicalcium phosphate vs soybean meal (P<.1)
[b]Waste vs conventional supplements (P<.05)
[c]Swine waste vs broiler litter (P<.05)
[d]Waste vs conventional supplements (P<.1)

plemented diets (P< .05). Animals fed the swine waste diet digested more of this dietary fraction than animals fed the broiler litter diet (P< .05). When expressed as percent of intake, more ADF tended to be digested in the stomach of sheep fed the soybean meal diet than sheep fed the dicalcium phosphate diet (P< .1), but not when expressed as g/d of fiber degraded.

Essentially no lignin was digested in the stomach of these wethers. More lignin was digested in the large intestine of sheep on all diets, although there was little difference between sheep fed different diets.

Digestion of dry matter and of all dietary components, when measured across the intestinal tract, was higher for sheep fed the conventional supplements than for sheep fed the waste diets (P< .05) due to a higher digestibility by sheep fed the soybean meal diet (table 20). Sheep fed the basal, swine waste, broiler litter and dicalcium phosphate diets had very similar measures for all digestibility values calculated. Sheep fed supplemented diets did digest a higher proportion of the NDF in the diet than sheep fed the basal diet (P< .05).

Animals fed the soybean meal diet tended to digest a higher percentage of dietary dry matter in the stomach (table 21) than animals fed other diets. Sheep fed the waste

TABLE 20.   APPARENT DIGESTIBILITY OF DIETS OF WETHERS
FED DIFFERENT SUPPLEMENTS

| Components | Supplements | | | | | SE |
	None	Swine waste	Broiler litter	Dical phos	Soybean meal	
	------------------%------------------					
Dry matter[a]	71.3	71.4	69.4	68.9	81.7	1.2
ADF[a]	56.8	51.2	55.5	58.0	63.8	2.0
NDF[a,b,c]	55.5	60.5	59.2	56.0	75.3	2.1
Cellulose[a,d]	65.4	62.3	62.6	64.3	74.1	2.5
Lignin[a,c]	43.1	33.8	40.6	44.8	50.7	2.4

[a]Waste vs conventional supplements (P<.05)
[b]Basal vs supplemented (P<.05)
[c]Swine waste vs broiler litter (P<.1)
[d]Dicalcium phosphate vs soybean meal (P<.05)

TABLE 21.   DRY MATTER DIGESTIBILITIES IN DIFFERENT
INTESTINAL SEGMENTS

| Item | Supplements | | | | | SE |
	None	Swine waste	Broiler litter	Dical phos	Soybean meal	
	----------------------%--------------------					
Stomach[a]	39.2	32.1	36.1	22.9	50.7	4.6
Small int[b]	11.6	12.1	20.1	41.8	23.6	8.0
Large int[b,c]	20.6	27.2	13.2	4.2	7.4	5.5
Total[a,b]	71.3	71.4	69.4	68.9	81.7	1.2

[a]Dicalcium phosphate vs soybean meal (P<.05)
[b]Waste vs conventional supplements (P<.05)
[c]Swine waste vs broiler litter (P<.1)

diets digested less dry matter in the small intestine (P<
.05) but more in the large intestine (P< .05) than animals
fed the conventionally supplemented diets. Dietary dry mat-
ter digestion was high across diets, but tended to be high-
est by sheep fed the soybean meal diet.

Discussion

Since the two methods of enumeration tended to measure
different populations within the cellulolytic species, these
results could indicate a shift of populations in sheep fed
the soybean meal diet, with reduced numbers of rods (Bacter-
iodes and Butyrivibrio) and higher numbers of cocci (Rumino-
coccus). High rumen ammonia concentrations have been shown
to increase proteolytic bacterial numbers (Wallace, 1979); a
similar alteration in species proportions could be occuring
in these animals.

The apparent increase in bacterial numbers with increas-
ing P supply to the rumen indicates that P is a limiting
nutrient for cellulolytic populations. Despite larger num-
bers of cellulolytic bacteria found in the rumen of sheep
fed the supplemented diets, there was no difference in the
degree of cellulose digestion measured either in the stomach
or across the intestinal tract. Approximately one-half of
dietary cellulose was digested in the stomach of sheep fed

all diets, with another 15 percent digested in the large in-
testine. It is possible that rumen bacteria are capable of
digesting only this proportion of the amount of cellulose
presented to them under the given conditions of passage rate
and alternate substrate source. However, it seems likely
that the additional cellulolytic bacteria present in supple-
mented sheep are utilizing alternate carbon sources. The
addition of glucose or cellobiose (Hiltner and DeHority,
1983) stimulates the rate of cellulose digestion. Addition
of soluble carbohydrate to cellulose broth shortens the lag
phase of cellulose digestion, with both soluble carbohyd-
rates and cellulose being degraded simultaneously (Hiltner
and DeHority, 1983), indicating that catabolite regulatory
mechanisms would not be operating with these substrates
(Russell and Baldwin, 1978). Continued cellulose digestion
in the presence of easily degraded substrate may be due to
an apparently constitutive production of cellulase by the
major cellulolytic species, B. succinogenes, R. albus and R.
flavefaciens (Hiltner and DeHority, 1983). Cellulytic
bacteria are capable of using more than one carbon and ener-
gy source, and the higher bacterial levels in the swine
waste and dicalcium phosphate fed sheep may have been using
some of the more easily degraded dietary components. Sub-
stantial levels of starch are present in all the experimen-

tal diets. Cellulytic bacteria have been shown to be capable of utilizing starch as a substrate (el-Shazly et al., 1961).

The smaller populations of cellulolytic bacteria in sheep fed the basal diet may have been using available P more efficiently to maintain a high rate of cellulose digestion than those in sheep fed the supplemented diets. Some species of intestinal bacteria have been shown to be capable of increasing the production of alkaline phosphatase when grown in media containing limiting concentrations of P (McComb et al., 1979). Cellulytic bacteria in sheep fed the basal diet may have increased their efficiency of P utilization by a mechanism such as this. However, the P supplied was aparently not enough to support larger microbial populations.

Waste supplementation tended to shift the site of organic matter digestion from the stomach and small intestine to the large intestine. Sheep fed the basal diet also tended to digest more of their dietary dry matter in the large intestine. More digestion in this area tended to be associated with a higher rate of calcium and phosphorus absorption (Chapter II). This could have been simply due to microbial degredation releasing the minerals from the organic matrix and increasing their luminal concentration. The minerals would then be available for absorption by diffusion or by carrier mechanisms.

A substantial portion of total digestion of lignin and ADF occurs in the large intestine, emphasizing the importance of bacteria in this area. These dietary components may be rendered more subject to microbial attack by exposure to acid and enzymes found in the stomach and small intestine.

Cellulytic bacterial levels apparently responded to both P intake and to the degree of P recycling into the rumen; factors affecting recycling of P via saliva must also be considered when determining microbial requirements. Although increased cellulolytic populations did not result in increased cellulose digestion by sheep fed our diets, larger bacterial numbers might improve the rate or degree of cellulose digestion in animals fed a more normal high fiber diet such as hay.

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CHAPTER IV

GENERAL DISCUSSION

In our study, the small intestine was the major site for absorption of P, a result which agrees with previous researchers (Pfeffer et al., 1970; Ben-Ghedalia et al., 1975; Dillon and Scott, 1979; Ben-Ghedalia et al., 1982). The trends for rate of absorption from the small intestine and secretion into the rumen were similar, which supports the suggestion by Field et al. (1983) that sheep efficient in absorbing P will excrete it primarily into the rumen via saliva, then into urine.

Phosphorus absorption from the small intestine measured in our sheep tends to be higher than values reported by other researchers (Pfeffer et al., 1970; Grace et al., 1974; Poppi and Termouht, 1979; Greene, 1981). However, Pfeffer et al. (1970), Grace et al. (1974) and Poppi and Termouth (1979) were using mature, replete animals. Poppi and Termouth fed one group of sheep a low P control diet, and these sheep had P absorption values from the small intestine similar to values reported here. Greene (1981) used older lambs fed a replete diet. In these cases, absorption would

be at a lower level than was found in our sheep due to lack
of stimulation of production of
1,25-dihydroxycholecalciferol. In contrast, Ben-Ghedalia et
al. (1982), using younger lambs, reported P absorption
values very similar to those recorded here. Production of
1,25-dihydroxycholecalciferol would be enhanced by growth
hormone in younger animals.

The low rate of apparent absorption and retention of P
from the dicalcium phosphate diet was not expected, since
this compound has been traditionally considered a highly
available Ca and P source. Solubility of this material in
different parts of the gastrointestinal tract may be
important. Dicalcium phosphate has been suggested to be
relatively insoluble in neutral rumen conditions (Durand and
Kawashima, 1981). Although our results indicate that it is
highly available to cellulytic bacteria, if it is not in
solution it would not have access to transport mechanisms in
the rumen.

Passage through the acidic stomach region would tend to
solubilize this compound. The small intestine would be
presented with a large amount of highly available Ca,
resulting in a high rate of Ca absorption from the small
intestine which was, in fact, observed in sheep fed the
dicalcium phosphate diet. However, serum ionized Ca levels

are subject to strict homeostatic controls. Any rise in serum Ca resulting from this high rate of absorption would cause increased excretion in order to maintain proper serum Ca levels, resulting in the increased fecal and urinary Ca and the low rate of absorbed Ca which was retained by animals fed this diet.

Braithwaite (1979) suggested that P retention is related to Ca retention in sheep. This is reasonable since bone is the primary deposition site for both minerals in mammals. The low rate of Ca retention may have limited the rate of P retention in sheep fed the dicalcium phosphate diet. A tendency for lower P retention is supported by a tendency for lower serum inorganic P values for sheep fed this diet.

Both Ca and Mg were absorbed from the small intestine of these sheep. As discussed in Chapter II, ruminants absorb both Ca and Mg from the upper small intestine but secrete it in the lower small intestine (Ben-Ghedalia et al., 1975). Whether net absorption or secretion occurs may depend upon the mineral status of the animal and the experimental protocol used.

Many previous researchers supplied Ca to experimental animals at a rate which would have depressed the parathyroid-vitamin D mechanism. Pfeffer et al. (1970) fed adult wethers very high levels of Ca and P, and relatively

high levels of Mg. They found no net Ca absorption from the small intestine, and a tendency for Mg secretion into this area. At high levels of Ca ingestion in adult animals, the parathyroid and 1,25- dihydroxycholecalciferol mechanisms would not have been active, so Ca and Mg excretion would have exceeded absorption. Grace et al. (1974) used a similar feeding regimen with adult sheep, and also found Ca and Mg secretion in the small intestine. Greene (1981) fed more moderate Ca levels to older lambs, and found net Ca absorption, but net Mg secretion, in this intestinal section.

Dillon and Scott (1979) fed younger lambs, which would have a more active absorptive mechanism, moderate levels of Ca and Mg. They reported that Ca was absorbed primarily in the small intestine, but that Mg was absorbed in both the stomach and the small intestine, with a slight secretion in the large intestine. Ben-Ghedalia et al. (1982) also reported Ca and Mg absorption from the stomach, small intestine and the large intestine. They had supplied fairly high amounts of Ca and P in their diets, but if these lambs were actively growing they would have required the minerals for bone deposition and continued active absorption despite intakes.

The degree of absorption from the small intestine could be affected by the mineral status and growth stage of the animal. If either factor stimulats the production of 1,25-dihydroxycholecalciferol, absorption of Ca from the small intestine will be increased and Mg may be similarly affected. The ratio between 1,25-dihydroxycholecalciferol and 24-25-dihydroxycholecalciferol in the serum might might be a useful indicator of the degree of repletion in the animals under study.

Our study used a P depletion to equalize the P status of the sheep. This mild induced P deficiency would probably have stimulated bone resorption by these animals in an attempt to maintain P homeostasis. Such bone loss would have resulted in Ca and Mg loss as well. Considering the marginal amounts of these elements in the basal diet, the animals may not have been able to replace the losses. Therefore, when sheep were placed on the experimental diets, the wethers may have been in mild deficeincy for all three elements. This possibility is supported by the rather high rates of absorption (for adult sheep) which were measured. Mild deficiencies are known to increase the rate of absorption of Ca and P from the small intestine of ruminants (Young et al., 1966; Braithwaite, 1976; Braithwaite, 1979; Abdel-Hafeez et al., 1981), presumably by an increase in

1,25-dihydroxycholecalciferol production. This vitamin D metabolite has been found to increase Mg absorption from the small intestine of rats (Georgievskii, 1983). The mild dietary P deficiency, combined with a possible Ca deficiency, may have maximized absorption mechanisms and reduced Ca and possibly Mg secretion in this intestinal segment, resulting in a greater rate of absorption than of secretion.

More information is needed on the interaction in ruminants between the different minerals discussed in the present paper. The relationship between absorption in different segments of the gastrointestinal tract appears to be complex and deserves investigation. The large intestine appears to important in absorption in some cases, particularly when absorption from earlier intestinal segments has been inadequate. The effect of mineral status and stage of growth appear to be quite important.

It appears that ruminants are capable of making effective use of the P and Mg from ensiled poultry litter and swine waste. They are also capable of absorbing enoungh Ca from these wastes to meet their requirement. Animal wastes, given the mild treatment of ensiling, offer considerable promise as mineral sources for ruminants.

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UTILIZATION OF PHOSPHORUS AND OTHER MINERALS FROM ENSILED BROILER LITTER AND SWINE WASTE

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Animal Science

(ABSTRACT)

Two metabolism trials were conducted with 15 wethers surgically equipped with duodenal and ileal cannulas to study the absorption of P and certain other minerals from swine waste and broiler litter. The effect of source and level of P on rumen cellulytic bacterial populations was also determined. Animals were fed a low P basal diet until serum inorganic P averaged 5.5 mg/dl, then randomly assigned to the following diets: low P basal alone, or supplemented with swine waste, broiler litter, dicalcium phosphate, or soybean meal. Trials consisted of a 7-d preliminary period, a 7-d collection of urine and feces and a 6-d sampling of duodenal and ileal digesta, and feces. Animals fed the waste diets tended to absorb more P than those fed the

conventional supplements. Calculated by difference, sheep absorbed more P from swine waste and broiler litter than from dicalcium phosphate and soybean meal (P< .1). Less Ca was absorbed from the waste diets than from the conventionally supplemented diets (P< .05). Expressed as g/d, there was no difference in Mg absorption between waste and conventional diets. Sheep fed waste tended to digest more dry matter and a higher percentage of ADF in the large intestine. Lignin was primarily digested in the large intestine of all sheep. More cellulytic bacteria were isolated from the rumen of sheep fed the supplemented diets (P < .05). Both P intake and P recycling appear to be important influences on cellulytic bacteria in the rumen. Both broiler litter and swine waste appear to be good sources of P and Mg for ruminants.

Key words: Swine Waste, Broiler Litter, Sheep, Ensiled, Cellulose, Bacteria, Waste.