

The availability of calcium in alfalfa preserved as
haylage or hay to growing dairy steers

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by

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SYMBOLS AND ABBREVIATIONS

Ca	Calcium
cc	Cubic centimeter(s)
DNA	Deoxyribonucleic acid
dig	Digestibility
DM	Dry matter
gal	Gallon(s)
GLM	Generalized Linear Models
g	Gram(s)
hr	Hour(s)
i.d.	Inner diameter
I	Intake
l	Liter(s)
Mg	Magnesium
ml	Milliliter(s)
min	Minute(s)
mo	Month(s)
OM	Organic matter
o.d.	Outer diameter
O ₂	Oxygen
%	Percent
P	Phosphorus
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
ret	Retention

I. INTRODUCTION

Calcium is a macromineral that is involved in numerous metabolic processes in the body. As factor XIV, calcium is an important component of the blood clotting mechanism. When bound to the protein calmodulin, calcium activates many enzymes requiring a protein kinase. At nerve cell synapses, calcium ions must enter the presynaptic neuron in order for release of transmitter vesicles to occur. In muscle, calcium binds to troponin during contraction. Calcium and phosphorus together form an essential part of the hydroxyapatite crystal of bone. Calcium also is an important component of the milk secreted by mammals to nourish their young. Because of the many vital processes in which calcium plays a major role, its concentration in plasma is highly regulated.

Forages are an important part of the diet of dairy animals. High quality legumes, such as alfalfa, contain liberal amounts of calcium, magnesium, and protein. Phosphorus, however, is present in amounts too low for adequate utilization of the calcium and therefore commonly is added to practical rations in the form of inorganic salts. The availability of calcium in feeds to dairy cattle is a significant factor in determining the dietary requirements.

This study was designed to measure the availability of calcium in alfalfa preserved as haylage or hay to dairy steers. Young animals were used because of their high calcium requirements for growth and for mineralization of bone. Digestibility coefficients for dry matter,

organic matter, and ash were used to evaluate the overall quality of forage utilization. Calcium availability in alfalfa was determined by conventional balance techniques. The availability of magnesium in alfalfa and phosphorus digestibility of the complete diet were studied in light of the interrelationships between these elements and calcium. Lastly, the glomerular filtration rates of steers were measured to test whether the more acidic haylage affects kidney function and, therefore, urinary excretion of calcium and calcium utilization.

II. LITERATURE REVIEW

A. History

1. Balance trials

Early studies of calcium absorption in cattle were balance trials in which the net retention of calcium (also known as calcium balance) was measured by subtracting calcium excretion in feces and urine from calcium intake. High producing dairy cows in peak lactation were used in these trials. Some experiments included three 20-day collection periods with dietary adjustment periods of 10 days, and required several men working around the clock in 12-hr shifts to ensure complete collection of all excretions from the animals (Forbes, 1915).

The results of extensive work by Forbes (1915, 1916) in which diets of timothy and alfalfa hay were fed alone or supplemented with calcium salts showed that the cows were in a critical state of negative calcium retention. Magnesium and phosphorus balances were almost always negative as well. Forbes (1919) concluded that the limiting factor was the process of assimilation of the mineral elements by the bones. Other negative calcium balances for lactating cows were reported by Hart et al. (1922a, 1922b) from studies of calcium and phosphorus in alfalfa. These findings were questioned by Meigs et al. (1919) who suggested that the collection of urine and feces created stressful conditions, and that mineral retentions in field situations were greater than in research trials.

It became clear from studies such as Huffman et al. (1930) that calcium retention in the dairy cow changes throughout lactation and the

dry period. Cows lost calcium during times of high milk production and accumulated calcium in later lactation and the dry period. Cows fed diets low in calcium utilized it more efficiently, and the total intake of calcium and phosphorus was more important than the calcium: phosphorus ratio in the utilization of these minerals. At the end of lactation, cows on a diet of alfalfa, corn silage, and grain were in positive calcium balance. Early work in calcium and phosphorus utilization and recommended supplementation levels were reviewed by Bohstedt (1932).

B. Isotope methods

Although many workers studied calcium utilization in the early years, they lacked the technology necessary to differentiate between calcium lost from body stores (endogenous calcium) and calcium from undigested plant materials (unabsorbed calcium). Beginning in the 1950s, radioisotopes have been used for this purpose. Advantages of this approach are the following: quantifying the daily endogenous mineral losses, estimating daily maintenance requirements, and determining true digestibilities that cannot be measured by conventional balance methods. Some disadvantages associated with the use of radioisotopes include handling of radioactive materials, loss of salvage value of animals, and disposal of wastes.

Hansard et al. (1952) describe a technique for studying calcium utilization using paired steers. One steer is given an oral dose of radioactive calcium (^{45}Ca) and the other an intravenous dose. The first

is for measuring the unabsorbed dietary calcium, and the second is for measuring the percentage of absorbed calcium that is reexcreted. After a 7-day collection period, the animals are slaughtered and the percentage of radioactivity appearing in the tissues and feces used to calculate a true digestibility value for calcium in the feedstuff.

A similar procedure for determining endogenous fecal calcium is presented by Visek et al. (1953). Daily intravenous injections of a constant amount of ^{45}Ca are given to cows, and the specific activity (s.a.) of calcium reaching the intestine is allowed to equilibrate with that of calcium circulating in the bloodstream. Endogenous calcium is calculated by the following formula:

$$\text{Endogenous calcium} = \text{daily fecal calcium} \times \frac{\text{s.a. fecal calcium (day n)}}{\text{s.a. blood calcium (day n-1)}}$$

Since the majority of the calcium administered intravenously is excreted in the feces within 24 hr after injection, blood radioactivity is measured on the day preceding feces collection.

Lofgreen and Kleiber (1953) used the technique of Visek et al. (1953) to investigate the availability of phosphorus in alfalfa hay. An apparent digestibility of 22% and a true digestibility of 91% were obtained with wether lambs. Lofgreen and Kleiber (1954) modified the procedure by substituting a single subcutaneous injection for the daily injections of ^{45}Ca . The true digestibility of phosphorus was 94%.

A review of work done on the biological availability of macro-minerals was compiled by Peeler (1972).

B. Dietary Factors

The absorption of calcium is influenced by many factors present in the feedstuff that were not examined closely by early workers, but studied in detail only recently. Characteristics of the diet that pertain to calcium absorption include carbohydrates, level of protein, acidity, and the presence of substances which combine with calcium and may decrease its digestibility.

1. Carbohydrates

The addition of grain to a forage-based ration generally improves the utilization of calcium. The practice of feeding rations consisting only of alfalfa hay to dairy cows existed in the western United States in the early twentieth century. Woll (1918) conducted an experiment that involved feeding alfalfa either alone or mixed with corn silage and various concentrates to study the effects of diet on body weight and dimensions, birth weight of calves, milk and butterfat production, and feed efficiency in terms of production. In all categories, the performance was better in animals fed mixed rations. The rations were isonitrogenous, but mixed rations were higher in energy than alfalfa alone. This might explain the differences obtained.

Turner et al. (1927) found positive calcium and phosphorus balances in one cow on a diet of good quality alfalfa hay and a grain mixture. Greater retentions of calcium and phosphorus were obtained with alfalfa than with clover, and with a calcium:phosphorus ratio of 1.25 than of 2.5.

Ellenberger and Newlander (1928) concluded that high-producing cows were not always in serious negative calcium and phosphorus balances, provided that a well-balanced supplementary grain mixture was fed in adequate amounts when a nonlegume forage was used.

Some positive calcium balances were reported by Haag et al. (1929) who fed dairy cows alfalfa hay with or without added starch, linseed oil meal, or disodium phosphate. Phosphorus supplementation improved the utilization of both calcium and phosphorus. Cows were always in negative phosphorus balances when diets restricted to alfalfa were fed. These workers suggested that the phosphorus content of alfalfa hay was inadequate for more than moderate milk production.

Hart et al. (1931) proposed that the feeding of fresh green grasses to dairy cows enhanced calcium absorption because of high levels of readily fermentable carbohydrates in the grasses. These carbohydrates would be metabolized to acid and increase the solubility of calcium in the intestinal tract. The results of feeding cerelese, a commercial preparation of glucose, to dairy cows did not support this hypothesis. They concluded that merely mixing sugar with the diet did not imitate the conditions existing in a green plant cell.

Hibbs and Conrad (1966) observed a marked improvement in calcium absorption and phosphorus balance when 3 to 5 pounds of grain per day were added to forage rations for dairy cattle. In a study of calcium utilization in dairy cows, Paquay et al. (1968) reported that alfalfa, clover, and pea straw resulted in poor utilization of calcium even though large amounts of feed were ingested. Supplementing the diets

with a ground mixture of cereals, especially barley, resulted in positive calcium digestibilities, while the feed intake was equal or less than with diets containing only forage. Varner and Woods (1972) found no significant changes in either calcium or phosphorus retention when calcium carbonate, starch, or both were added to a 30% roughage diet fed to steers.

Conrad and Hibbs (1973) conducted nine experiments using balance trials to study the absorption of calcium in dairy cows. Animals fed alfalfa-grass hay had lower calcium digestibilities than those receiving in addition a grain concentrate supplemented with bone meal and corn silage. The true digestibility for phosphorus was similar for both diets, but the apparent phosphorus digestibility was greater when bone-meal was fed. The additional calcium and phosphorus in bonemeal were important in increasing the absolute absorption of these minerals.

2. Protein

The level of protein in the diets of dairy animals is thought to influence the utilization of calcium. Monroe and Perkins (1925) found consistently greater calcium balances in high protein diets containing clover than in low protein diets containing timothy. The calcium content was also greater in the high protein diets, which may have confounded the results. Miller (1926) reported greater balances for calcium in lactating dairy cows with clover than with timothy in diets containing corn silage and a grain mixture. Although the protein content of the diets was equal, the authors believed that the quality of

the protein in clover was higher than the quality of the protein in timothy and the grain mixture. The calcium:phosphorus ratio was higher when clover was fed. The greater calcium balances in cows fed clover may be explained by the higher calcium content of this forage.

Braithwaite (1978) studied the effect of protein intake on calcium metabolism in the pregnant ewe. A decreased calcium retention was observed in protein-deficient animals as a result of decreased calcium absorption. This decrease in absorption was thought to be associated with a diminished concentration of intestinal calcium-binding protein. In a study with early-weaned lambs fed diets that differed in energy, protein, and calcium concentration, Davidson and McDonald (1981) found similar carcass mineral contents, although the growth rates were not the same.

3. Acidity

Forbes (1916) concluded that acid-base balance in normal cows does not affect retention of minerals but, to a limited degree, alters the routes of excretion. Urinary calcium was only slightly increased by more acid diets. This contrasts with the work of Hart et al. (1931) who observed increases in urinary calcium and a slight improvement in intestinal absorption of calcium when alfalfa hay was treated with hydrochloric acid. There was a greater net loss of calcium, however, than when untreated hay was fed.

Turner et al. (1932) postulated that the conjugate base of an acid that can maintain the solubility of calcium in the rumen might improve its uptake. Addition of sodium citrate to an alfalfa hay ration resulted

in a greater calcium balance, but this could be explained by increased intake of calcium and decreased milk yield.

More recently, Vagg and Payne (1970) observed increases in size of the exchangeable pool, urinary loss, and intestinal absorption of calcium in goats given ammonium chloride. Braithwaite (1972) reported similar findings with sheep. These observations suggest that increased pool size might result from an increased rate of pool turnover caused by an increase in absorption and excretion of calcium.

Verdaris and Evans (1976) examined the effects of acidity and calcium level on mineral metabolism in dry cows. More calcium was absorbed with higher dietary calcium and lower pH, and lower pH increased the percent absorption and retention. Dietary pH did not influence magnesium or phosphorus utilization, and treatments had no effect on blood minerals or urinary excretion. An excess of stable acid anions over alkaline cations in rations maintaining a positive balance for calcium in dairy cows increased the intestinal absorption of calcium (Lomba et al., 1978). This effect was not observed for rations causing a negative calcium balance. Phosphorus, along with chloride and sulfate, was the principal contributor to this effect. Kaentrakoon et al. (1980) studied renal excretion in ewes with ruminal infusions of potassium chloride, citric acid, or both. Little or no changes in urinary excretion of calcium and phosphorus were observed. Urinary magnesium increased temporarily when potassium chloride and citric acid were infused together.

In summary, the literature suggests that urinary excretion of calcium and intestinal absorption of calcium both increase in response to greater acidity of the diet, although the effect on calcium retention varies and is influenced by other factors as well.

C. Binding Substances

Many substances in the diet depress calcium absorption in some animals by forming insoluble compounds that are unavailable for absorption. These include corn oil, phytin, and oxalic acid.

1. Corn oil

The addition of corn oil to increase the caloric density of the ration often decreases the utilization of other nutrients, and this effect can be reversed by adding calcium. In studies with sheep, Grainger et al. (1957) found that the addition of corn oil to 5% of a corncob diet caused a significant decrease in the digestibilities of organic matter, protein, and cellulose that were improved by calcium supplementation. Tillman and Brethour (1958a) showed that 7.5% corn oil decreased the digestibility and increased the fecal excretion of calcium in sheep fed a ration containing alfalfa meal. Grainger et al. (1961) reported similar results with 7% corn oil in a corn cob diet. Also, they showed that 4.4 g of calcium or 6.2 g of iron corrected or partially corrected for the negative effect of corn oil on cellulose digestibility. They conjectured that the balance of free cations in the rumen determined the solubility of long chain fatty acids and affected microbial activity.

Fecal calcium was increased by the addition of calcium, but not by

the addition of corn oil or starch to a corn cob ration fed to sheep (Davison and Woods, 1961). Corn oil or calcium did not affect the digestibilities of cellulose, protein, or organic matter in an alfalfa hay diet. Davison and Woods (1963a) did not observe an increase in fecal calcium with corn oil addition to a corn cob ration fed to sheep. They concluded that calcium is important in the digestion of organic matter in the presence of dietary fat (Davison and Woods, 1963b).

Recently, Green and Body (1979) infused corn oil into the rumens of sheep fed white clover. Soaps of fatty acids were formed primarily in the large intestine and excreted in the feces. The acidity of rumen contents was not considered conducive to fatty acid soap formation. No effect on apparent absorption of calcium or magnesium was found, and plasma levels were normal. Most of the lipid was absorbed from the large intestine, and magnesium and calcium absorption were normal.

2. Phytin

Phosphorus, when present as phytic acid, is poorly utilized by non-ruminants such as swine and poultry, but has been shown by Reid et al. (1947) to be completely hydrolyzed in the alimentary tract of sheep under various conditions of calcium and phosphorus intake. The diets contained wheat chaff, oats, and bran as major sources of phytin phosphorus. When tested by an artificial rumen technique, calcium phytate was found to be as available to rumen microbes as inorganic phosphorus (Raun et al., 1956). Optimum pH for phytase enzyme activity was 5.5. In another study, Tillman and Brethour (1958b) fed sheep

monocalcium phosphate and calcium phytate to supply 70% of the phosphorus. The availabilities of both calcium and phosphorus were in the same order of magnitude in phytate as in monocalcium phosphate. Because ruminants are able to utilize the phosphorus and calcium in calcium phytate, it would seem that phytic acid has no effect on the absorption of calcium in these animals. However, Barth and Hansard (1961) reported decreased phytin phosphorus utilization in the presence of high calcium levels. Careful adjustment of the calcium:phosphorus levels was recommended for animals on high phytin diets.

3. Oxalic acid

The presence of oxalic acid salts in ruminant rations has been associated with decreased digestibility of calcium, and high levels of oxalate can cause death. Several conditions in the rumen may be responsible for the decreased calcium availability: alkalosis induced by the breakdown of soluble oxalates into carbonates and bicarbonates, interaction of soluble oxalates with dietary calcium forming insoluble calcium oxalate that is excreted in the feces, partial resistance of dietary calcium oxalate to hydrolysis, and binding of calcium with other organic molecules such as proteins and cell wall carbohydrates (Singh et al., 1978). Negi (1971) reported a complete hydrolysis of soluble oxalates in the rumen of bulls, and no oxalates were found in feces when 65 g of soluble oxalates were fed per day. However, when given the same quantity of oxalate as either calcium oxalate, or an equal amount of oxalate from calcium and potassium salts, only 62 and

48%, respectively, of the oxalate was hydrolyzed. The author concluded that alkalosis inhibited further hydrolysis of oxalate when soluble potassium oxalate was fed with calcium oxalate.

Campos et al. (1974) found that dietary calcium oxalate but not sodium oxalate, which is soluble, significantly decreased calcium absorption in goats. When infused into the duodenum, both decreased the absorption of calcium. The rumen microflora were thought to act less on the insoluble calcium salt. Calcium absorption from a perfused jejunal loop was reduced by both sodium oxalate and calcium oxalate. They concluded that although the duodenal pH in goats is low, the ability to absorb calcium from oxalate diets is attributed to the destructive ability of ruminal microbes because the goat intestine behaves similarly to that of a nonruminant. Sareen et al. (1978), in a study with buffaloes, observed a higher pH in rumen fluid and in urine, and greater bicarbonate levels in rumen fluid when 72 g of potassium oxalate were added to a basal ration that included wheat straw and corn. Both calcium and phosphorus balances were lower with oxalate.

Halogeton (Halogeton glomerulatus) is a western range plant that is high in oxalate. James and Butcher (1972) fed diets containing up to 36% halogeton (providing a dietary oxalate content of 6%) to sheep. No clinical abnormalities were reported in the sheep, although there was a slight hypocalcemia. As dietary oxalate increased, water intake and urine volume increased. They stated that the danger of high oxalate feeding seemed to involve acute rather than chronic poisoning.

Singh et al. (1978) reported greater urinary output of alkali,

bicarbonate, and oxalate with increasing oxalate intake in buffaloes. Urine volume and water intake were also greater, presumably a response to increased tonicity of urine. The percent true digestibility of calcium was decreased by oxalate. Greater than 50 g of oxalate per day was thought to decrease calcium digestibility by increasing the alkalinity of the digestive tract. Soluble oxalate was shown to be completely broken down in the rumen, and bicarbonate formed from hydrolysis of oxalate was absorbed into the blood and excreted in the urine. No interaction of calcium in feed with soluble oxalates was noted.

Ruminants can be adapted to high oxalate diets, as recent studies have shown. Allison et al. (1977) observed 10-fold and greater increases in the rate of in vitro oxalate metabolism by rumen microbes following a 3- to 4-day transition period from a diet of alfalfa to a diet of halogeton that contained 12% oxalic acid. These animals (two sheep and one cow) showed no signs of toxicity. Allison et al. (1981) isolated a bacterium that degrades oxalate to carbon dioxide and formate and depends on oxalate for growth from sheep fed increasing quantities of oxalate. Allison and Cook (1981) stated that bacteria using oxalate to meet growth requirements appear to be selected when dietary oxalate levels are increased, both in ruminants and nonruminants.

D. Physiological Factors

1. Age of animal

Age affects calcium absorption in both cattle and sheep. Hansard et al. (1954) studied calcium utilization in Hereford cattle ranging in

age from 10 days to over 15 years. Greater absorption of calcium occurred in younger animals, while practically none was absorbed in aged cattle. Little difference was found between retained calcium values for yearlings and mature animals. Endogenous calcium excretion did not vary when expressed as a fraction of body weight. In a later study, Hansard et al. (1957) reported that apparent and true digestibilities of calcium were more similar in young, growing calves than in mature animals. Age of animal was more important in determining calcium absorption than source of calcium when forages were compared with inorganic feedstuffs in availability of calcium to the animal.

In Jersey steers (Garces and Evans, 1971), younger animals absorbed calcium at a maximum rate sooner than older animals when switched from a low to a high calcium diet. Amount absorbed over a 12-week period also was greater in the younger steers. The results of Braithwaite and Riazuddin (1971) demonstrated that calcium absorption as a percentage of intake was greater in young, growing sheep than in mature wethers. The amount of calcium absorbed depended on the amount ingested, but the percentage absorbed did not change significantly in young animals. In contrast, the amount of calcium absorbed in mature animals was not related to intake, and the percentage absorbed was lower at a greater level of intake. Rate of absorption in mature animals decreased slightly with age. Fecal endogenous losses were related to the amount absorbed and decreased with age, unlike in cattle, where it did not change when corrected for body weight (Hansard et al., 1954). The greater absorption of calcium in younger sheep was related to a lower rate of bone

resorption, and total absorption was greater at a higher level of calcium intake. Retention of calcium depended on the amount absorbed.

2. Sites of absorption of calcium

Since the early 1960s, many workers have investigated the sites of calcium absorption in the gastrointestinal tract. While the primary site of calcium absorption in the rat and other nonruminant species is the proximal duodenum (Harrison and Harrison, 1969), other sites have been shown to be important in ruminants. Chandler and Cragle (1962) studied the movement of calcium in calves using radioactive cerium, ^{144}Ce , as an indigestible marker. No absorption of calcium was found in the reticulo-rumen, endogenous secretion occurred in the omasum, and much was absorbed in the abomasum. Calcium absorption occurred at a lower rate in the duodenum, was greater in the jejunum, and was constant throughout the remainder of the tract. In sheep, Phillipson and Storry (1965) likewise found the rumen epithelium impermeable to calcium, even with levels infused at 6 times the normal concentration. No net absorption occurred in the duodenum, while the proximal jejunum and middle ileum showed positive calcium absorption directly related to its concentration in solution. The low pH of the abomasum maintains much of the calcium in an ultrafilterable form (Storry, 1961) and increased pH results in binding of calcium to the digesta. The pH of the digesta increases as it moves along the small intestine, the solubility of calcium decreases, and the total calcium concentration of digesta thus increases (Ben-Ghedalia et al., 1975). This results in a net absorption of calcium from

the proximal part of the small intestine, and a net secretion of calcium into the distal portion of the small intestine (Yano et al., 1979).

Some absorption of calcium from the large intestine in ruminants has been reported (Grace et al., 1974), and it also may occur in the rat cecum (Nellans and Goldsmith, 1981).

3. Excretion of calcium

Plasma calcium is excreted primarily in the feces. After an intravenous dose of ^{45}Ca was given to cattle, 15% of the activity was recovered in feces and only .3% in urine (Hansard et al., 1952).

Endogenous fecal calcium excretion increases with age (Hansard et al., 1954) and decreases with lower dietary calcium (Braithwaite, 1974).

Urinary calcium in sheep does not change with age (Braithwaite and Riazuddin, 1971). When calcium-depleted sheep were offered calcium at high and low intakes, excretion did not change with intake but was less than that of sheep from a previous study replete with calcium (Braithwaite, 1975).

Magnesium intake can affect fecal calcium excretion in rats (Clark, 1968; Levine et al., 1981), but the results with ruminants have generally been nonsignificant (House and Mayland, 1976; Reid et al., 1979). Magnesium has a variable effect on urinary calcium excretion. In some studies, magnesium increased urinary calcium (House and Mayland, 1976; Clark, 1968; Levine et al., 1981), while in other studies (O'Kelley and Fontenot, 1973; Reid et al., 1979), it had no effect. Chicco et al. (1973) reported that dietary magnesium increased urinary calcium in sheep.

Calcium:phosphorus ratio had no effect on metabolic fecal calcium in sheep (Lueker and Lofgreen, 1961) when phosphorus intake was adequate, but a diet low in phosphorus gave a small increase in metabolic fecal calcium, possibly because of increased bone resorption to compensate for the phosphorus deficiency (Young et al., 1966a). Chicco et al. (1973) showed that sheep on low calcium diets had greater fecal calcium excretion when phosphorus was high than when phosphorus was low. On high calcium diets, supplemental phosphorus resulted in a decrease in fecal calcium. In all cases, plasma calcium was decreased by adding phosphorus to the diet. Low dietary phosphorus increased urinary calcium in sheep (Young et al., 1966a) and rats (Clark, 1968).

Vagg and Payne (1970) found no change in fecal endogenous loss of calcium when ammonium chloride was used to induce a mild acidosis in goats and cattle. Verdaris and Evans (1976) studied the influence of dietary calcium and pH on mineral balance in Holstein cows and showed no change in urinary excretion of calcium with either treatment. In other studies using ammonium chloride to reduce dietary pH, increased urinary calcium resulted from treatment (Vagg and Payne, 1970; Horst and Jorgenson, 1974; Braithwaite, 1972).

Visek et al. (1953) found a trend toward increased urinary excretion of calcium with a high protein diet in cattle. This calciuric effect is well-known in rats and humans, and is thought to be the result of renal acid excretion (Lutz and Linkswiler, 1981; Schuette et al., 1980; Whiting and Draper, 1980) and increased glomerular filtration rate

(Schuette et al., 1980; Kim and Linkswiler, 1979). Fractional renal reabsorption was decreased when increased protein was fed (Allen et al., 1979) despite no change in the GFR. Metabolism of sulfur-containing amino acids produces two equivalents of hydrogen ions per mole of sulfate oxidized, but the levels found in practical diets of humans are not enough to affect renal calcium clearance (Block et al., 1980).

E. Vitamin D

1. Dietary

Vitamin D has long been recognized as being essential for the proper absorption and retention of calcium and phosphorus from the diet as well as for mineralization of bone. Forbes (1915) suggested that, to a large extent, a factor different from calcium controlled the retention of calcium in lactating dairy cattle fed rations containing timothy or clover hay. Meigs (1922) stated that a vitamin present in alfalfa hay but absent in timothy hay and corn silage was important for maintaining milk production in cows. Supplementing the timothy hay ration with calcium carbonate simply delayed a decline in milk production not observed in animals on the alfalfa hay diet. Hart et al. (1922a) reported positive calcium and phosphorus balances in milking cows fed dry alfalfa hay, corn silage, and a grain mixture. The hay used had been cured without prolonged exposure to sunlight. Better retention of calcium occurred, however, in animals fed fresh green alfalfa. In contrast, Hart et al. (1922b), in a later investigation with alfalfa hay that had been dried in windrows with four days exposure to the sun, found

small negative calcium and phosphorus balances in cows. These workers concluded that a vitamin present in the hay was destroyed in the curing process. Timothy hay diets did not give positive calcium or phosphorus balances, even with the addition of bone meal to make up for some deficiencies of these minerals.

Later, Rupel et al. (1931) reported that the dairy calf is susceptible to rickets when adequate levels of vitamin D are not present in the diet, and that codliver oil and sunlight exposure are effective sources of the vitamin. In a review of calcium and phosphorus requirements of farm animals, Bohstedt (1932) stated that, when animals are fed rations containing large amounts of sun-cured roughages, high levels of vitamin D are ingested, and the animals can utilize the limited amounts of calcium and phosphorus present more efficiently. Also, greater variation in the calcium:phosphorus ratio is tolerated. Bethke (1932) stressed that, even with adequate levels of vitamin D, calcium and phosphorus content and their relative proportions in the diet are important for achieving maximum production, especially in growing animals. Similar information was provided in a review of early literature by Huffman (1934). Using balance studies, Wallis et al. (1935) found that calcium and phosphorus in cattle were absorbed in a ratio of 2:1 irrespective of mineral content of the ration. (This same ratio exists in normal bone.) They proposed that a young, growing calf may store excess dietary vitamin D that is later metabolized when deficiencies in the diet exist. Exactly how this might occur was not explained by the authors.

2. Metabolism

Vitamin D can be acquired by absorption from the diet or synthesized in the skin from 7-dehydrocholesterol upon exposure to ultraviolet radiation. The major form of vitamin D in plants is D_2 , while D_3 predominates in animal tissue. Vitamin D entering the body through the intestine is transported via the lymphatics to the bloodstream (Dobson and Ward, 1974), while vitamin D produced photochemically in the skin enters the extracellular fluid and becomes available for further metabolism (Horst and Reinhardt, 1983). Vitamin D is associated with the chylomicron fraction in the lymphatics and with a plasma binding globulin (Holick and Clark, 1978) in the blood. Plasma vitamin D accumulates in the liver, where it is hydroxylated at the 25-position by a mixed function monooxygenase, which is under negative feedback (allosteric) control by high levels of 25-hydroxy D. Circulating levels of 25-hydroxy D are the best indication of the vitamin D status of an animal. This metabolite is further hydroxylated in the kidney mitochondria, this time at the 1-position to form 1,25-dihydroxy D ($1,25-(OH)_2 D$), by an enzyme system requiring NADPH, O_2 , and magnesium (DeLuca, 1980). Conditions of hypophosphatemia, hypocalcemia, and hyperparathyroidism stimulate the activity of the enzyme system. The $1,25-(OH)_2 D$ is the most biologically active metabolite of the vitamin and is considered to be the hormonal form of vitamin D.

3. Actions

The $1,25-(OH)_2 D$ metabolite acts to increase calcium absorption from the small intestine and also the colon (DeLuca, 1980). It acts in

conjunction with parathyroid hormone to cause resorption of calcium from bone and mineralization of new bone. In the kidney, $1,25-(\text{OH})_2 \text{D}$ may be involved in calcium reabsorption from the distal convoluted tubules, but no agreement on this has been reached. Vitamin D metabolites have been found in greater levels in the small intestine at sites where active absorption of calcium was occurring (Walling et al., 1974), providing additional evidence for their major role in calcium absorption.

4. Mode of action

The mode of action of $1,25-(\text{OH})_2 \text{D}$ is typical of a steroid hormone. Upon entering the cell, it binds to a cytosolic receptor and passes into the nucleus where it stimulates RNA polymerase. This in turn causes transcription of DNA and produces a generalized trophic effect on the enterocyte (Bikle et al., 1979). The hormone increases the permeability of the cell membrane to calcium, perhaps by changing the composition of membrane phospholipids or by insertion of calcium carrier proteins. Active vitamin D exerts a post-transcriptional effect on synthesis of alkaline phosphatase and calcium-activated ATPase, enzymes involved in the active transport of calcium at the brush-border and basolateral membranes, respectively. Adenylate cyclase may serve as a second messenger of vitamin D.

The concentration of calcium inside the enterocyte must be kept low or toxicity will occur. Mitochondria are stimulated at high calcium levels to take up calcium and phosphorus and may migrate to the basolateral membrane and release calcium to an enzyme pump that exchanges it

for sodium. Bikle et al. (1979) postulate that calcium binding protein (CaBP) participates in release of calcium from the mitochondria to the calcium pump. Spencer et al. (1978) and Harmeyer and DeLuca (1969) have shown that calcium transport begins several hours before detectable levels of CaBP are present. Actinomycin D and cycloheximide, two inhibitors of protein synthesis, were unable to block calcium uptake despite inhibition of CaBP synthesis (Bikle et al., 1978).

Calcium also may be transported through the cell in vesicles that coalesce with lysosomes to form secondary lysosomes in which any binding proteins associated with calcium are digested. Calcium is released from the cell by exocytosis. Another means for calcium to pass through the intestinal epithelium is through channels between cells which also can form within a cell by confluence of the vesicles described earlier (Wasserman, 1981). This is known as the paracellular route of calcium transport and may be independent of vitamin D (Levine et al., 1981).

5. Regulation of calcium absorption

A picture of the overall regulation of calcium absorption and retention now can be presented. In a young, growing animal, accretion of calcium into bone occurs at a rapid rate and exceeds calcium resorption. Thus, the animal maintains a high level of absorption to meet growth requirements, limited only by the availability of dietary calcium (Braithwaite, 1976). An older animal does not increase its absorption of calcium, even with increased intake, until the calcium from exchangeable pools in the bone have been depleted to the extent that the animal cannot

maintain plasma calcium. Increased absorption of calcium might not occur in parturient animals in time to prevent severe hypocalcemia (Braithwaite, 1976). Decreased plasma levels of calcium and phosphorus promote conversion of vitamin D to $1,25-(OH)_2 D$, and decreased plasma calcium also stimulates parathyroid hormone release. Calcium absorption from the intestine then proceeds at a high rate until all the losses have been met. That calcium absorbed in excess of requirements is excreted in the feces and to a lesser extent in the urine.

F. Interrelationships of Calcium, Phosphorus, Magnesium, and Vitamin D

There is much information in the literature on the interactions of calcium, phosphorus, magnesium, and vitamin D in calcium absorption in ruminants. The findings are by no means always consistent regarding these interactions.

1. Calcium level of the diet

Animals fed a low calcium diet will adapt by reducing fecal excretion of calcium and increasing calcium absorption (Jacobson et al., 1972). It is generally believed that increasing the dietary level of calcium will increase the retention while decreasing the percentage absorption in animals (Reid et al., 1979; Hines et al., 1982; Verdaris and Evans, 1976; Visek et al., 1953; Braithwaite and Riazuddin, 1971) depending on age and calcium status. Jacobson et al. (1972) stated that, in older animals, the ability to mobilize minerals from storage pools in the bone in response to decreased dietary intake is less than

in younger animals. A substantial increase in apparent absorption of calcium in steers occurred when the animals were taken off a low calcium diet and given a diet high in calcium (Garces and Evans, 1971). The amount of absorbed calcium declined after 6 weeks on the high calcium diet.

2. Calcium:phosphorus ratio

In nonruminants, the ratio of calcium:phosphorus of the diet is important because these animals do not grow well when rations containing high ratios of calcium:phosphorus are fed. Ruminants, however, are able to tolerate ratios as high as 7:1 with no adverse effects (Wise et al., 1963), but ratios less than 1:1 give decreased nutrient conversion and performance. The explanation offered by these workers is that ruminants have eaten forages containing high calcium:phosphorus ratios and their digestive processes have evolved to the extent that the animal can utilize high levels of calcium. Young et al. (1966b) suggest that the lower pH of the proximal small intestine in ruminants prevents precipitation of tricalcium phosphate, and thus enables the animal to absorb calcium and phosphorus more efficiently when high ratios of calcium:phosphorus are ingested in the feed. Colovos et al. (1958) found reduced digestibility of carbohydrate and protein when high levels of calcium and low levels of phosphorus resulted from limestone supplementation of cattle. Retention of calcium is dependent upon the amount of dietary phosphorus, in excess of that needed for soft tissue formation, available for synthesis of new bone (Henry et al., 1960). Ricketts et al. (1970) fed Holstein cows ratios containing 1:1, 4:1, or 8:1

calcium:phosphorus and found highest absorption and utilization efficiencies in cows on 1:1 diets. Greatest apparent retention of calcium occurred in cows on 4:1 diets. The calcium:phosphorus ratio of the diet did not affect amounts absorbed by sheep (Lueker and Lofgreen, 1961) but absorption was directly related to intake of calcium or phosphorus. Diets used contained calcium:phosphorus ratios of 0.8:1, 2.8:1, or 6.0:1. Manston (1968) measured absorption of calcium in dry, nonpregnant cows using balance and isotope techniques. Absorption of calcium increased with a greater calcium:phosphorus ratio, but only for a few days. Heifers on diets of 2:1 calcium:phosphorus showed better absorption than those on 1:1 diets from weaning through first pregnancy.

3. Calcium, phosphorus, and vitamin D

Low levels of dietary phosphorus have improved the absorption of calcium and increased the levels of CaBP (Thomasset et al., 1977) and $1,25-(\text{OH})_2 \text{D}$ (Tanaka and DeLuca, 1973) in rats. Low phosphorus diets maintained serum calcium in rats given low calcium diets with or without supplemental vitamin D or $1,25-(\text{OH})_2 \text{D}$ (Thomasset et al., 1977). However, Hibbs and Conrad (1966) reported that adequate phosphorus was necessary for vitamin D to increase calcium absorption in cattle. This was attributed to the unavailability of calcium and phosphorus in the diets fed. Calcium absorption was closely related to phosphorus balance when adequate levels of phosphorus were provided in the ration. The results of Kichura et al. (1982) with dairy cows suggest that low levels of phosphorus activate the gut in the presence of high dietary levels

of calcium.

4. Calcium and magnesium

The interactions of calcium and magnesium are complex, and contradictory findings between ruminants and nonruminants have been reported. A more positive calcium balance in rats given supplemental magnesium was reported by Clark (1968), but level of magnesium had no effect on calcium balance in wethers (Hjerpe, 1968). There was no effect on apparent absorption or retention of calcium when magnesium-fertilized alfalfa was given to lambs (Reid et al., 1979). In contrast, Chicco et al. (1973) noted decreased calcium utilization in cattle when magnesium levels of the diet were increased. In the rat, Levine et al. (1981) found that magnesium caused decreased calcium absorption in vitamin D deficient animals, while $1,25-(OH)_2 D$ prevented the fall in absorption. Vitamin D was less effective. It was proposed that calcium and magnesium share certain cellular transport sites that are activated by vitamin D or $1,25-(OH)_2 D$. Care and Van't Klooster (1965) suggested that calcium and magnesium may share the same carrier in the small intestine. However, magnesium in sheep is absorbed more distally in an area where calcium is secreted (Yano et al., 1979) in the small intestine.

V. MATERIALS AND METHODS

A. Digestibility Trial

Alfalfa in the early bloom stage from the Iowa State University farms was harvested in mid-August, 1981, and preserved as either haylage or hay. Alfalfa for haylage was chopped (10-20 cm) after wilting to 50% dry matter in the field. It was ensiled in 220 l (55 gal) barrels double-lined with plastic garbage sacks. After packing with alfalfa, the sacks were secured with two knots each after as much air as was possible was excluded by hand. At least 3 mo were allowed for fermentation of the haylage before feeding to the steers. Typical pH of haylage as fed was 4.8. Hay was baled in rectangular bales after 2 days in windrow, barn-dried for 3 mo, and then chopped (10-20 cm) at 90% dry matter.

Eight young, growing Holstein steers, weighing an average of 135 kg, were assigned randomly to hay or haylage diets in a crossover design (Cochran and Cox, 1957) with two treatments (diets) and eight replications (steers). The dry matter composition of the diets is given in Table 1, and the chemical analysis in Table 2. Diets were formulated to meet requirements of growing dairy bulls for energy, calcium, and phosphorus (N.R.C., 1978). Cracked corn, monosodium phosphate, and trace mineralized salt were combined as a premix. The alfalfa and premix were each weighed in a plastic sack and mixed by shaking prior to feeding the steers. Totals for each diet were not equal because analysis of haylage after ensiling showed it contained more moisture

Table 1. Composition of experimental diets

Ingredient ^a	Diet	
	Hay	Haylage
	g	
Alfalfa hay	1510.3	0.0
Alfalfa haylage	0.0	1305.7
Cracked corn	496.1	496.1
Monosodium phosphate	23.0	23.0
Trace mineralized salt ^b	5.5	5.5
Total	2034.9	1830.3

^aExpressed on a dry matter basis.

^bListed to contain .250% Mn, .200% Fe, .100% S., .033% Cu, .0025% Co, .007% I, .005% Zn, and 96.0% to 99.0% NaCl.

Table 2. Chemical analysis of experimental diets

Component ^a	Diet	
	Hay	Haylage
	%	
Organic matter	92.4	92.3
Acid detergent fiber	26.9	25.1
Crude protein	16.6	16.6
Ether extract	11.9	11.1
Ash	7.57	7.70
Calcium	.85	1.01
Magnesium	.28	.24
Phosphorus	.48	.53

^aExpressed on a dry matter basis.

than before ensiling.

Steers were fed daily at 8 AM and 5 PM, and water was provided to them at all times. Each steer was allowed to adapt to each diet for 10 days in a pen prior to a 7-day digestibility trial; total collection of urine and feces was performed. For the digestibility trial, each animal was transferred from its pen to a stanchion-type metabolism stall designed for the clean separation of urine and feces. Steers were handled in pairs because only two metabolism stalls were available for use. Both metabolism stalls were provided with a water bucket and a feeder. Because of slight differences in the metabolism stalls, one of the feeders was a rubber bowl and the other a wooden manger built by the author. Each animal stood on a metal grate that was kept as free of feces as possible during the trial. Enough room was provided in the metabolism stalls for the animals to stand up or to lie down. Urine drained into a plastic jug placed underneath each metabolism stall.

Body weights of steers were recorded at the beginning of the first and at the end of the second collection periods. Urine and feces were sampled daily, frozen, and later composited for analysis. Thorough mixing of feces before sampling was done by hand with a small shovel. About 1% of the total feces was used to obtain a representative sample. Prior to analysis, thawed feces were blended in a vertical pedestal type mixing machine.¹ Urine was collected in 50 ml plastic bottles. A running total of feces and urine was kept during the collection period.

¹Triumph Manufacturing Co., Cincinnati, Ohio.

Refusals were collected at the end of each collection period, and a subsample was frozen for later analysis. Refusals and feces were dried to a constant weight in a forced-air oven at 50 C and ground in a Wiley mill through a 20-mesh screen.

All feed and feces samples were analyzed for dry matter by drying as described above. Ash was determined by incinerating all feeds and feces for 12 hr at 600 C. Organic matter in feeds and feces was calculated as dry matter minus ash. Crude protein in feeds was determined by the Kjeldahl method (AOAC, 1975). Ether extract for feeds was measured by standard procedures (AOAC, 1975). Acid detergent fiber in feeds was determined according to the technique of Goering and Van Soest (1970). Oxalic acid analysis in hay, haylage, and corn was by gas chromatography (Salanitro and Muirhead, 1975). Calcium (Willis, 1960a) and magnesium (Willis, 1960b) in feeds, feces, and urine were determined by atomic absorption spectrophotometry. Phosphorus was analyzed using a colorimetric procedure (Fiske and Subbarow, 1925).

B. Measurement of Glomerular Filtration Rates

After each 7-day collection of urine and feces in four of the steers, inulin clearance tests were conducted to measure glomerular filtration rates (GFR). Each steer was weighed and placed in a metabolism stall (identical to those used for the digestibility trial, except that a rubber mat was provided on which the animal could stand). For infusion, an inulin solution was prepared by adding 40 g of inulin to 1 l of sterile .9% physiological saline solution. The mixture was heated without boiling until all the inulin was dissolved. The left

and right jugular veins of each animal were catheterized with Angiocaths² (1.7 mm diameter x 13.3 cm long). No anesthetic was required. Silastic³ extension catheters (15.9 mm i.d., 3.2 mm o.d.) filled with 23 units/ml of heparinized saline were connected to each jugular catheter and capped immediately to prevent aspiration of air into the veins. Subsequently, the cap on the extension catheter on the right side was replaced with a three-way valve to allow for sampling of blood and flushing of the catheter.

Immediately prior to each infusion, a zero-time blood sample was obtained from the right jugular vein. After first removing and discarding the heparinized saline in the extension catheter and a small amount of blood, a clean syringe was used to withdraw 10 ml of blood. The catheter then was flushed with 5 ml of heparinized saline. The inulin solution was infused into the left jugular vein through a length of flexible Tygon⁴ plastic tubing (6.4 mm i.d., 17.2 mm o.d.) using a finger pump⁵ capable of providing a flow rate of 1 ml/min.

No priming dose of inulin was given. Blood samples were taken at 30 min intervals for 6 hr. Blood was transferred to test tubes containing 2 drops of a 50,000 units/ml heparin solution to prevent clotting. All blood samples were centrifuged, and the plasma removed and frozen in vials for later analysis of inulin. A 1 ml aliquot of the infusion

²The Deseret Co., Sandy, Utah 84070.

³Dow Corning Corp., Midland, Michigan 48640.

⁴Norton Plastics and Synthetics Division, Akron, Ohio 44309.

⁵Sigmamotor, Middleport, New York.

solution was diluted 1:10 with water and frozen for later analysis.

Urine was not collected. Inulin was assayed according to the colorimetric method of Hultman (1974).

GFR was calculated using the following formula:

$$\text{GFR} = \frac{\text{infusion rate} \times [\text{inulin}] \text{ infusion solution}}{\text{average } [\text{inulin}] \text{ plasma} - [\text{inulin}] \text{ plasma at time 0}}$$

The rationale behind this formula is that, at equilibrium, plasma concentration of inulin reaches a constant value, and the rate of inulin entering plasma is equal to the plasma clearance of inulin. Since inulin is neither absorbed nor secreted by the kidney tubules, its rate of clearance is equal to the rate of formation of glomerular filtrate. The infusion rate was determined by dividing the difference in weight of the container of inulin solution at the time of sampling of blood by the time between samples in minutes. One gram was considered to represent a volume of 1 ml of infusion solution. Average values for plasma inulin and the infusion rate were obtained from readings over the last 2 hr of the infusion period, because preliminary work with the other four steers (results not shown) indicated this was the most reliable.

C. Statistical Analysis

All data were analyzed on a computer by Procedure GLM of the Statistical Analysis System (Barr et al., 1979). A simplified analysis of variance is given in Table 3. The period effect measures variation attributed to differences between periods of the digestibility trial,

Table 3. Analysis of variance

Source	Degrees of freedom
Animal	7
Period	1
Sequence	1
Period*Sequence	1
Error	5
Total	15

while the sequence effect measures any residual effect due to feeding one diet after the other. The period by sequence interaction is the treatment effect.

The following parameters were studied combining data for both diets and all animals to determine any linear relationships:

Dry matter excretion in feces and dry matter intake

Dry matter retention and dry matter intake

Dry matter digestibility and dry matter intake

Organic matter excretion in feces and organic matter intake

Organic matter retention and organic matter intake

Organic matter digestibility and organic matter intake

Ash excretion in feces and ash intake

Ash retention and ash intake

Ash digestibility and ash intake

Calcium excretion in feces and calcium intake

Calcium excretion in urine and calcium intake

Calcium retention and calcium intake

Calcium digestibility and calcium intake

Calcium digestibility and calcium excretion in urine

Calcium retention and calcium excretion in urine

Magnesium excretion in feces and magnesium intake

Magnesium excretion in urine and magnesium intake

Magnesium retention and magnesium intake

Magnesium digestibility and magnesium intake

Magnesium digestibility and magnesium excretion in urine

Magnesium retention and magnesium excretion in urine

Phosphorus excretion in feces and phosphorus intake

Phosphorus excretion in urine and phosphorus intake

Phosphorus retention and phosphorus intake

Phosphorus digestibility and phosphorus intake

Phosphorus digestibility and phosphorus excretion in urine

Phosphorus retention and phosphorus excretion in urine

VI. RESULTS AND DISCUSSION

A. General

The alfalfa haylage used in this experiment was of high quality, as determined from its texture and aroma. About 90% of the haylage recovered from the silos was not spoiled. That portion which was spoiled with mold was discarded and not fed. The steers consumed the haylage more readily than the hay, and more hay than haylage was spilled from the feeders and wasted as a result. The 220 l (55 gal) barrels lined with plastic garbage sacks made simple, useful, and convenient silos for this experiment. The metabolism stalls proved adequate for the degree of precision required. However, the drainage hole for urine occasionally needed to be cleared of fecal debris and hair.

In Table 4 are the number of feedings each steer received during each collection period. Several steers were given the wrong diets

Table 4. Diets given to steers for each collection period

Period	Diet ^a	Steer							
		8549	8626	8629	8636	8558	8559	8570	8635
1	Hay	14	15	15	12	0	0	0	1
	Haylage	0	0	0	1	14	14	14	13
	Total	14	15	15	13	14	14	14	14
2	Hay	1	0	0	0	13	14	14	10
	Haylage	13	10	10	10	1	0	0	0
	Total	14	10	10	10	14	14	14	10

^aBoth diets also contained cracked corn and a mineral mixture.

inadvertently by the workers, but in no instance was any steer given more than one incorrect diet throughout the length of the experiment. The total numbers of feedings were not always equal for several reasons. One feeding was missed in the first collection period for steer 8636. Steers 8626 and 8629 were fed in the morning of the last day before they were removed from their stalls, resulting in an extra feeding. The second collection period for steers 8626, 8629, 8635, and 8636 lasted only 5 days due to a shortage of diets. These steers were fed twice daily, as were all steers in this experiment. The second collection period for steers 8635 and 8636 followed an adaptation period of only 6 days, also because of a diet shortage. All errors in feeding were accounted for in the calculation of intakes for each steer.

During its first collection period, steer 8549 developed lacerations on its right hindleg below the hock joint, and was treated for 4 days with Combiotic¹ (an antibiotic combination of penicillin and dihydrostreptomycin in an aqueous suspension) at the rate of 10 cc per day. Average gain for all animals was 22.6 kg, or about 750 g per day.

B. Digestibility Trial

1. Dry matter

Dry matter data are summarized in Table 5. Both intake and fecal excretion were greater in steers on the hay diet, and these were linearly related. The regression equation was:

¹Pfizer Corp., New York, New York 10017.

$$\text{DM in feces} = .503 (\text{DM intake}) - .679$$

$$r = .633$$

$$P < .009$$

Table 5. Effect of diet on dry matter utilization

Diet	Dry matter (kg/day)			Digestibility (%)
	Intake	Feces	Retention	
Hay	3.86	1.29	2.69	66.6
Haylage	3.63*	1.12**	2.51	69.2
Standard error	.07	.03	.14	.9

*P<.05.

**P<.02.

Roffler et al. (1967) used lactating dairy cows to compare alfalfa-brome forage stored as wilted silage, low-moisture silage, and hay. In two of three trials, the dry matter intake of low-moisture silage was greater than that of hay. In addition, low-moisture silage fed cows had the highest production of 4% fat-corrected milk and cows fed hay had the lowest. Epley (1968) concluded from a study of the literature that sheep and cattle fed ensiled forage consumed less dry matter and gained at slower rates than animals fed hay. In his experiment with sheep fed alfalfa, dry matter intake of hay and haylage was greater than that of silage. Sutton and Vetter (1971) reported that daily dry matter intake of alfalfa in lambs, when expressed on a body weight basis, did not differ between hay, low-moisture, or high-moisture silages. In the present experiment, the reduced dry matter intake of haylage compared with that of hay is most probably a result of the

lower amount of haylage dry matter offered to the steers, rather than a property of the feed itself.

No differences between diets were observed for retention or digestibility of dry matter. Retention of dry matter increased with intake according to the regression equation:

$$\text{DM retention} = .921 (\text{DM intake}) - .846$$

$$r = .575 \qquad P < .02$$

Digestibility of dry matter was greater in period 1 than in period 2 ($P < .03$) and fecal excretion was greater in period 2 than in period 1 ($P < .02$). These results suggest that dry matter utilization decreased with age, but there were only 17 days between the start of period 1 and the start of period 2. Therefore, the significance of this finding is questionable. No sequence or animal effects on dry matter utilization were statistically significant.

Dry matter digestibility for sheep and cattle fed alfalfa ensiled at 34% or 43% dry matter (McGuffey and Owens, 1979) averaged 58.5% in covered silos and 56.1% in uncovered silos, both values lower than the 69.2% found in this experiment. Digestibility of dry matter was greater in lambs fed hay than in lambs fed low-moisture and high-moisture silages made from alfalfa (Sutton and Vetter, 1971). In dairy cattle, dry matter digestibility of alfalfa hay was greater than that of direct-cut silage, which was greater than that of haylage (Gordon et al., 1961). There was no difference in dry matter digestibility between the hay and haylage diets fed to steers in my experiment. The dry matter

digestibility of 66.6% for the hay diet was greater than the 61.0% obtained by Reid et al. (1979), but lower than the 69.5% reported by Leibholz and Hartmann (1972). Both studies were with sheep fed alfalfa hay.

2. Organic matter

Organic matter results are shown in Table 6. As with dry matter, intake and fecal output of organic matter were greater when steers were

Table 6. Effect of diet on organic matter utilization

Diet	Organic matter (kg/day)			Digesti- bility (%)
	Intake	Feces	Retention	
Hay	3.57	1.15	2.42	67.8
Haylage	3.35*	1.00**	2.35	70.2
Standard error	.06	.03	.06	.9

*P<.05.

**P<.02.

given the hay ration. Likewise, digestibility was greater in period 1 (P<.03) and fecal output greater in period 2 (P<.02). Fecal output of organic matter was significantly correlated with intake:

$$\text{OM in feces} = .506 (\text{OM intake}) - .675$$

$$r = .639$$

$$P < .008$$

No differences between diets were observed for retention or digestibility of organic matter.

Retention of organic matter increased with intake. The regression

equation was:

$$\text{OM retention} = .496 (\text{OM intake}) + .667$$

$$r = .631 \quad P < .009$$

No animal or sequence effects on organic matter utilization were statistically significant.

Merchen and Satter (1983) found better organic matter utilization in lactating cows fed 40% dry matter haylage than in those fed hay. Digestibility coefficients were 73.6% for haylage and 68.7% for hay. Leibholz and Hartmann (1972) reported an organic matter digestibility of 75% for alfalfa hay fed to sheep. In a study with sheep and cattle (McGuffey and Owens, 1979), alfalfa silage from covered bunkers had a mean organic matter digestibility of 60.2% compared with 57.8% for silage from uncovered bunkers.

3. Ash

Results for ash utilization are listed in Table 7. Fecal excretion of ash was greater when steers were on the hay diet and digestibility

Table 7. Effect of diet on ash utilization

Diet	Ash (g/day)			Digesti- bility (%)
	Intake	Feces	Retention	
Hay	292	139	153	52.1
Haylage	279	120**	159	57.0*
Standard error	5	3	6	1.3

*P<.04.

**P<.01.

was greater when steers were fed haylage. Variation between animals was significant ($P < .03$) for fecal excretion of ash. Ash retention was not highly correlated with intake, as illustrated by the regression equation:

$$\text{Ash retention} = .537 (\text{ash intake}) + 2.52$$

$$r = .446$$

$$P < .09$$

No effects of period or sequence on ash metabolism were statistically significant. Gordon et al. (1961) reported ash digestibilities of 43.4% for alfalfa hay, 40.6% for haylage, and 45.9% for direct-cut silage fed to dairy cows.

4. Calcium

The results for calcium are shown in Table 8. Steers on the haylage diet consumed more calcium because the haylage contained more

Table 8. Effect of diet on calcium utilization

Diet	Calcium (g/day)				Apparent availability (%)
	Intake	Feces	Urine	Retention	
Hay	32.7	19.0	.05	13.7	41.9
Haylage	36.5**	21.9	.03*	14.6	40.0
Standard error	.6	1.3	.01	1.3	3.7

* $P < .05$.

** $P < .01$.

calcium than did the hay. Less urinary excretion of calcium occurred in steers given haylage, but there was no significant linear correlation between urinary calcium output and intake, in agreement with the results of Grace et al. (1974) from a study with sheep. Fecal calcium increased with intake according to the following regression equation:

$$\text{Ca in feces} = .843 (\text{Ca intake}) - 8.69$$

$$r = .540 \qquad P < .03$$

A direct relationship between fecal output of calcium and calcium intake has been reported for sheep (Powell et al., 1978; Leibholz, 1974; Braithwaite and Riazuddin, 1971) supporting the concept that ruminants adapt to increased dietary calcium by decreasing net calcium absorption from the intestine (Miller, 1975).

No differences between diets were found for the apparent availability of calcium, which averaged 40.9%. This value is similar to the 45% used by the National Research Council for the true availability of calcium in typical diets fed to dairy cattle (NRC, 1978) and indicates that calcium in the alfalfa was readily available to the steers, and not affected by the method of preservation used. No animal, period, or sequence effects on calcium utilization were statistically significant.

Hansard et al. (1954) reported a daily endogenous fecal calcium excretion of approximately 16 mg/kg body weight for cattle. If this value is used to calculate total endogenous calcium excretion/day, estimated true availabilities for calcium in my experiment are 48% in

hay and 46% in haylage.

Hansard et al. (1957) found apparent and true availabilities for calcium in alfalfa of 18% and 41%, respectively, when fed to Hereford calves from 5 to 7 mo of age and weighing an average of 154 kg.

Leibholz (1974) observed 39% retention of dietary calcium in crossbred sheep fed alfalfa. Reid et al. (1979) fed recut alfalfa to sheep at ad libitum or restricted intake levels. The apparent availability of calcium, hereafter referred to simply as availability, was greater (22.6% versus 13.6%) when sheep consumed alfalfa at the restricted level.

Other forages have been studied by various procedures to measure the availability of calcium to ruminants. In India, cowpeas are fed in the summer and berseem in the winter as legume forages for cattle. Agrawal and Talapatra (1970), using growing male Harijana calves, found an availability of 45% for calcium in cowpeas and 50% for calcium in berseem. Joyce and Rattray (1970) reported an availability of 26% for calcium in lambs fed either clover or ryegrass. With the comparative slaughter method for estimating retention, the estimated true availabilities ranged from 35-50%. Grace et al. (1974) found similar results with 1- to 2-year-old wether sheep. The availability values averaged 10% at a low organic matter intake and 26% at a higher organic matter intake. According to Grace and Wilson (1972), plasma levels of calcium in sheep and cattle indicated no difference in the availability of calcium from ryegrass or clover. Stevenson and Unsworth (1978), using re-entrant cannulated sheep, reported a 31% availability

for calcium from direct-cut grass silages, while Powell et al. (1978) found a 29% availability for calcium in lambs fed various grasses.

Reid et al. (1978) observed a higher availability for calcium in legumes than in grasses with 5- to 10-mo-old wether lambs. The higher calcium content of legumes and the high availability of calcium in alfalfa show it to be a good dietary source of calcium for cattle.

Oxalate analysis of the feeds, given in Table 9, indicates that as much as 23.5% of the calcium in haylage and 32.7% of the calcium in

Table 9. Oxalate analysis of feeds

Item	Corn	Hay	Haylage
Oxalate (%)	.171	.807	.718
Moles oxalate/kg	19.0	89.7	79.8
Calcium (%)	.122	1.10	1.36
Moles calcium/kg	3.05	275	340
Fraction of calcium combined with oxalate	100.0	32.7	23.5

hay can be bound to oxalate anions ($C_2O_4^{-2}$). Ensiling of the alfalfa may have decreased the quantity of oxalate present by acid hydrolysis or microbial breakdown to carbonates and bicarbonates. Ward et al. (1979) stated that up to 33% of the calcium in alfalfa may be combined with oxalate. These workers suggested that calcium in alfalfa is less available to cattle than inorganic sources of calcium because of the presence of insoluble calcium oxalate in alfalfa.

5. Magnesium

The results for magnesium are given in Table 10. Both intake and retention of magnesium were greater when steers were fed the hay diet.

Table 10. Effect of diet on magnesium utilization

Diet	Magnesium (g/day)				Apparent avail-ability (%)
	Intake	Feces	Urine	Reten-tion	
Hay	11.0	6.3	.007	4.6	43
Haylage	8.5**	5.4	.005	2.8*	36
Standard error	.2	.4	.001	.4	3

*P<.02.

**P<.01.

A higher magnesium intake level for steers fed hay was expected because the hay contained more magnesium than did the haylage, and the dry matter intake of hay was greater also. Joyce and Rattray (1970), in studies with sheep fed perennial ryegrass, found that magnesium retention was related to magnesium intake. Although retention of magnesium was greater in steers on the hay diet, no difference between diets for magnesium availability was noted. This was probably due to a high degree of variation between animals on a given diet.

There was a significant linear relationship between intake and urinary excretion of magnesium. The regression equation was:

$$\text{Mg in urine} = .001 (\text{Mg intake}) - .004$$

$$r = .528$$

$$P < .04$$

In a study with cattle (Kemp et al., 1961), urinary magnesium excretion was significantly related to magnesium absorbed in excess of that secreted into milk.

The relationship between fecal excretion of magnesium and intake was less apparent:

$$\text{Mg in feces} = .489 (\text{Mg intake}) + 1.07$$

$$r = .449 \qquad P < .09$$

L'Estrange et al. (1967) found significant linear correlations of urinary and fecal magnesium with magnesium intake. Animal, period, and sequence effects on magnesium utilization were not statistically significant in my experiment.

The availability of magnesium in hay (43%) and haylage (36%) as determined for dairy steers is higher than most values reported in the literature. Reid et al. (1979), using growing wether lambs, found availabilities for magnesium in regrowth alfalfa of 32.9% when fed at a restricted intake level and 27.9% when fed ad libitum. Availabilities for magnesium in 18-mo-old sheep consuming grass silages averaged 42% (Stevenson and Unsworth, 1978). The average magnesium availability was 36.6% for grasses fed as cut herbage to growing lambs (Powell et al., 1978). Rook and Campling (1962) used urinary magnesium values to determine magnesium availabilities for a variety of legume and grass forages fed to lactating dairy cattle. Availabilities ranged from 5 to 30% and were not affected by levels of calcium or phosphorus in the diet.

Several studies have compared the magnesium availability of

legumes to that of grasses. Joyce and Rattray (1970) found no differences in magnesium availability between ryegrass and clover fed to sheep. Grace and Wilson (1972) showed white clover contained less available magnesium for cattle than did ryegrass. They suggested that dietary factors in the clover reduced the availability of magnesium for cattle. Sheep used in this study received more magnesium when clover was fed, and the availability ranged from 25 to 38%. Plasma magnesium levels were also higher in sheep on white clover than in sheep on ryegrass. Similarly, Reid et al. (1978), using lambs fed at a standardized level, found higher availabilities for magnesium from legumes than from grasses. The values averaged 32% and 27%, respectively. Grace et al. (1974) reported lower magnesium availabilities in wether sheep fed ryegrasses or clover. These availabilities did not differ between forages, and averaged 18% on a low and 29% on a high organic matter intake level.

Many studies have shown that magnesium availability increases with maturity of forage (Powell et al., 1978; Kemp et al., 1961; Rook and Campling, 1962; L'Estrange et al., 1967) and decreases with intake of nitrogen (Rook and Campling, 1962; Stillings et al., 1964; Kemp et al., 1961). The present experiment used alfalfa from the same cutting and the nitrogen intake of steers was equalized. Therefore, the effects of maturity of forage and nitrogen intake on magnesium availability were not tested.

6. Phosphorus

Phosphorus utilization results are presented in Table 11. No significant correlation between fecal excretion of phosphorus and phosphorus

Table 11. Effect of diet on phosphorus utilization

Diet	Phosphorus (g/day)			Retention	Digestibility (%)
	Intake	Feces	Urine		
Hay	18.8	7.8	6.9	4.2	58
Haylage	19.2	8.1	5.4	5.7	58
Standard error	.3	.5	.6	.5	3

intake was found. This differs from the work of Leibholz (1974) and Powell et al. (1978). Urinary phosphorus increased with the digestibility of phosphorus. The regression equation was:

$$\% \text{ P digestibility} = 2.93 (\text{P in urine}) + 40.0$$

$$r = .697$$

$$P < .004$$

This linear relationship shows that urinary excretion of phosphorus can be used to compare phosphorus digestibilities for alfalfa haylage and hay diets in steers, and is in agreement with Braithwaite (1975) who showed that urinary phosphorus excretion increased with absorbed phosphorus in sheep.

There was no difference in phosphorus digestibility between the two diets as expected because the corn mix provided nearly all of the phosphorus. There was a wide variation in phosphorus digestibility and

fecal phosphorus between animals, and this probably contributed to the similarity in phosphorus digestibility values between diets. Supplemental phosphorus was provided as sodium monophosphate because the alfalfa contained low levels of this mineral. Chicco et al. (1965), using data from in vitro studies with cellulolytic rumen microorganisms, reported that sodium orthophosphate was equal to calcium orthophosphate and sodium metaphosphate as a source of phosphorus.

Retention of phosphorus was negatively correlated with urinary excretion of phosphorus, according to the regression equation:

$$P \text{ retention} = -.42 (P \text{ in urine}) + 7.51$$

$$r = .571 \qquad P < .02$$

Approximately 56% of the excreted phosphorus was in feces and 44% in urine. Retention of phosphorus was not related to intake ($P > .8$) in contrast to the results of Joyce and Rattray (1970) from a study with sheep. Urinary phosphorus excretion was greater ($P < .04$) in steers fed haylage in period 1 and hay in period 2. No animal or period effects on phosphorus utilization were statistically significant.

C. Glomerular Filtration Rates

Glomerular filtration rates (GFR) are shown in Table 12. When expressed on a body weight basis, there was no significant variation between animals. Values based on metabolic body weights ($BW^{.75}$) were also similar. No differences due to the effects of diet were noted.

Table 12. Results of inulin clearance studies in steers

Item	Steers								Mean	S.E.
	8626		8629		8636		8635			
	Hay	Hay-lage	Hay	Hay-lage	Hay	Hay-lage	Hay	Hay-lage		
GFR (ml/min)	480	500	500	540	540	570	500	500	516	3
GFR/ kg BW	3.5	3.5	3.8	3.8	4.1	4.0	3.4	3.5	3.7	.1
GFR/ kg BW ^{.75}	11.9	12.2	13.2	12.8	13.9	13.9	12.0	12.2	12.8	.1

Using the double-isotope, single injection method for estimating renal function, Mercer et al. (1978) found a mean GFR for adult cattle of 2.8 ml/(kg x min). Dalton (1968) used inulin and sodium thiosulfate clearances to calculate an average GFR for neonatal calves of 2.3 ml/(kg x min). The mean GFR value of 3.7 ml/(kg x min) in my study seems high in comparison to the results of these studies. However, Campbell and White (1967) concluded that the clearance of inulin in cattle is a characteristic of the animal and is not related to its body surface area or weight. It was difficult to maintain a constant flow of inulin into the animals in my experiment, and variation in the infusion rate could have resulted in misleading values for the GFR. The low plasma inulin levels resulting from low infusion rates made analysis for inulin difficult at best. Campbell and White stated that inulin levels below 5 mg/100 ml were difficult to measure, and that clearances obtained were lower. If the inulin levels in plasma had been underestimated, the

clearances obtained in my investigation would be too high. Therefore, an infusion rate of about 3 ml/min instead of the 1 ml/min which was used probably would have resulted in higher plasma inulin levels and allowed for a more accurate assay of inulin. No priming dose of inulin was administered to the steers, prolonging the period necessary to achieve a plateau in plasma inulin concentration. Other workers (Campbell and White, 1967; Grignolo et al., 1982; Bailey, 1978) used priming doses of inulin in their studies. Difficulties with the animals and with the infusion equipment were encountered in measuring GFR. If the steers had been anesthetized with xylazine, it would have been possible to collect urine by catheterization of the bladder. This might have been more accurate, because the equation for calculating the GFR would have included the rate of filtration and not the rate of infusion. Nevertheless, any effects of the anesthetic on glomerular filtration would constitute a source of error.

VII. SUMMARY AND CONCLUSIONS

Eight Holstein steers were used in a crossover experiment to measure the apparent availability of calcium in alfalfa preserved as haylage or hay. Diets consisted of cracked corn, a mineral mixture to provide trace minerals and phosphorus, and either hay or haylage made from the same cutting of alfalfa. Apparent availability of magnesium in alfalfa, and digestibilities of phosphorus, dry matter, organic matter, and ash in the complete diet were also studied. Glomerular filtration rates (GFR) in four of the steers were determined using inulin clearance procedures. The following conclusions were made:

- (1) Retention and fecal excretion of both dry matter and organic matter were related to intake. Retention decreased and fecal excretion increased from period 1 to period 2.
- (2) Ash digestibility was higher in steers fed haylage.
- (3) Although the intake of calcium was greater in steers fed haylage, urinary excretion of calcium was lower. Urinary excretion of calcium was not related to calcium intake.
- (4) Fecal excretion of calcium was significantly related to intake.
- (5) Apparent availabilities for calcium from hay and haylage were similar and averaged 41%.
- (6) As much as 33% of the calcium in hay, but no more than 24% of the calcium in haylage, may have been present as calcium oxalate.
- (7) Urinary excretion of magnesium increased with magnesium intake.
- (8) The apparent availability of magnesium was not affected by

treatment and averaged 40%.

- (9) Digestibility of phosphorus increased and retention of phosphorus decreased with urinary excretion.
- (10) Urinary phosphorus excretion was greater in steers fed haylage in period 1 and hay in period 2.
- (11) GFR averaged 3.7 ml/(kg x min) and did not differ between diets or among animals.

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X. APPENDIX

Table 13. Chemical analysis of feeds

Component ^a	Source		
	Corn	Hay	Haylage
Organic matter	93.93	91.94	91.67
Acid detergent fiber	--	36.3	35.2
Crude protein	9.96	19.0	19.1
Ether extract	10.28	12.43	11.36
Ash	6.07	8.06	8.33
Calcium	.12	1.10	1.36
Magnesium	.14	.33	.27
Phosphorus	1.26	.21	.24

^aExpressed as a percent of dry matter.

Table 14. Body weights of steers

Body weights (kg)	Steer							
	8549	8558	8559	8570	8626	8629	8635	8636
Initial	138	122	134	115	128	125	128	125
Final	152	143	163	158	143	144	147	147
Mean	145	132	148	137	137	135	138	136
Gain	14.1	20.9	28.1	42.7	15.4	19.1	18.2	22.2

Table 15. Average daily values for digestibility trial

Item	Intake	Feces	Urine	Retention	Digestibility (%)
	g				
Dry matter	3740	1210	--	2600	67.9
Organic matter	3460	1080	--	2390	69.0
Ash	286	130	--	156	54.5
Calcium	34.6	20.5	.04	14.1	40.9
Magnesium	9.7	5.8	.006	3.7	39.7
Phosphorus	19.0	7.9	6.2	4.9	58.1

Table 16. Average daily values/kg body weight for digestibility trial

Item	Intake	Feces	Urine	Retention
	mg/kg			
Dry matter	27000	8740	--	18800
Organic matter	25000	7800	--	17300
Ash	2060	939	--	1130
Calcium	250	148	.3	102
Magnesium	70	42	.04	27
Phosphorus	37	57	45	35

Table 17. Totals of feed, feces, and urine from digestibility trial

Diet	Steer	Feed offered	Feed refused	Feed intake	Feces	Urine
		kg				—l—
Hay	8549	28.49	2.56	25.93	8.12	59.2
	8558	28.28	.13	28.15	9.25	53.6
	8559	22.49	1.13	27.36	9.66	36.6
	8570	28.49	2.66	25.83	9.29	41.9
	8626	30.52	3.30	27.22	7.79	37.4
	8629 ^a	30.52	.95	29.57	9.49	34.5
	8635 ^a	20.35	.30	20.05	7.83	30.9
	8636	26.25	2.17	24.08	7.74	36.8
Haylage	8549	25.83	.06	25.77	7.58	54.2
	8558	25.62	1.17	24.45	7.57	38.4
	8559	25.62	.17	25.45	7.13	44.7
	8570	25.62	.17	25.45	6.24	37.2
	8626 ^a	18.30	.12	18.18	6.08	33.9
	8629 ^a	18.30	.08	18.22	5.71	25.6 ^b
	8635	25.83	.18	25.65	8.81	69.1
	8636 ^a	18.30	.13	18.17	6.32	36.5

^aTrial lasted 5 days.^bUrine production for 4 days.

Table 18. Mineral composition of feed refusals and feces from digestibility trial

Diet	Steer	Feed refusals				Feces			
		Ash%	Ca%	Mg%	P%	Ash%	Ca%	Mg%	P%
Hay	8549	8.21	1.0	.21	.381	13.48	1.5	.38	.957
	8558	6.54	.79	.20	.351	9.54	1.6	.51	.257
	8559	7.04	1.0	.24	.406	10.38	1.0	.38	.493
	8570	6.55	.77	.21	.200	9.89	1.5	.43	.412
	8626	8.63	.85	.24	.427	10.65	1.5	.46	.616
	8629	9.95	1.1	.29	.362	10.77	1.6	.60	.699
	8635	7.53	.95	.28	.394	10.02	1.7	.63	.671
	8636	7.52	.91	.22	.279	12.33	1.4	.45	.755
Haylage	8549	8.30	1.1	.21	.270	2.01	1.6	.37	.676
	8558	7.94	.81	.26	.710	9.87	2.3	.47	.659
	8559	6.69	.95	.16	.284	11.29	2.3	.50	.791
	8570	8.05	.88	.20	.581	10.68	2.0	.50	.678
	8626	6.75	.76	.19	.347	10.03	2.0	.52	.555
	8629	8.70	1.0	.25	.442	11.48	1.8	.51	.746
	8635	8.81	1.2	.24	.325	10.39	1.9	.47	.849
	8636	7.01	.83	.21	.372	10.40	1.9	.52	.825

Table 19. Dry matter utilization data from digestibility trial

Diet	Steer	Dry matter (kg/day)			
		Intake	Feces	Retention	Digestibility (%)
Hay	8549	3.70	1.16	2.54	68.7
	8558	3.49	1.08	2.41	69.0
	8559	3.64	1.02	2.62	72.0
	8570	3.64	.89	2.75	75.5
	8626	3.89	1.11	2.78	71.4
	8629	4.22	1.36	2.86	67.9
	8635	3.66	1.26	2.40	65.7
	8636	3.44	1.11	2.33	67.9
Haylage	8549	3.68	1.08	2.60	70.6
	8558	4.02	1.32	3.70	67.1
	8559	3.91	1.38	2.53	64.7
	8570	3.69	1.33	2.36	64.0
	8626	3.64	1.22	2.42	66.6
	8629	3.64	1.14	2.50	68.7
	8635	4.01	1.57	2.44	61.0
	8636	3.63	1.26	2.37	65.2

Table 20. Organic matter utilization data from digestibility trial

Diet	Steer	Organic matter (kg/day)			
		Intake	Feces	Retention	Digestibility (%)
Hay	8549	3.41	1.00	2.41	70.7
	8558	3.23	.97	2.26	69.8
	8559	3.36	.90	2.46	73.1
	8570	3.36	.80	2.56	76.3
	8626	3.60	.99	2.61	72.4
	8629	3.91	1.21	2.70	69.0
	8635	3.38	1.13	2.26	66.7
	8636	3.18	.97	2.21	69.5
Haylage	8549	3.40	.95	2.45	72.0
	8558	3.72	1.20	2.52	67.8
	8559	3.61	1.24	2.37	65.7
	8570	3.41	1.20	2.21	64.9
	8626	3.36	1.09	2.26	67.4
	8629	3.37	1.01	2.36	70.0
	8635	3.71	1.41	2.30	62.0
	8636	3.36	1.13	2.22	66.3

Table 21. Ash utilization data from digestibility trial

Diet	Steer	Ash (g/day)			
		Intake	Feces	Retention	Digestibility (%)
Hay	8549	278	156	122	43.8
	8558	269	107	162	60.3
	8559	280	115	165	59.0
	8570	280	95	185	66.0
	8626	289	119	171	59.0
	8629	317	146	171	53.9
	8635	282	131	151	53.6
	8636	261	136	125	47.7
Haylage	8549	283	130	153	54.1
	8558	305	126	179	58.7
	8559	297	143	154	51.7
	8570	283	131	152	53.6
	8626	280	122	158	56.5
	8629	280	131	149	53.2
	8635	304	157	147	48.3
	8636	280	131	149	53.1

Table 22. Calcium utilization data from digestibility trial

Diet	Steer	Calcium (g/day)				Apparent availability (%)
		Intake	Feces	Urine	Retention	
Hay	8549	30.8	16.9	.07	13.8	45.1
	8558	34.6	21.1	.04	13.4	38.8
	8559	32.9	13.8	.06	19.0	58.0
	8570	31.6	19.9	.05	11.6	36.9
	8626	32.9	16.7	.04	16.2	49.3
	8629	35.4	21.7	.10	13.7	38.8
	8635	33.9	26.7	.02	7.3	21.2
	8636	29.4	15.5	.02	13.8	47.4
Haylage	8549	36.6	17.3	.03	19.2	52.6
	8558	35.6	24.3	.02	11.3	31.7
	8559	36.7	23.4	.05	13.2	36.2
	8570	36.8	17.8	.03	18.9	51.5
	8626	36.8	24.3	.03	12.5	33.9
	8629	36.8	20.6	.02	16.2	44.1
	8635	36.3	24.2	.02	12.2	33.5
	8636	36.7	23.5	.02	13.3	36.1

Table 23. Magnesium utilization data from digestibility trial

Diet	Steer	Magnesium (g/day)				Apparent availa- bility (%)
		Intake	Feces	Urine	Reten- tion	
Hay	8549	10.7	4.4	.006	6.3	58.9
	8558	8.1	5.1	.004	3.0	37.1
	8559	8.5	5.1	.006	3.4	39.8
	8570	8.5	4.5	.003	4.0	47.4
	8626	11.1	5.1	.007	6.0	54.0
	8629	11.9	8.1	.014	3.7	31.4
	8635	8.7	5.9	.005	2.7	31.5
	8636	9.7	5.0	.005	4.8	48.9
Haylage	8549	8.7	4.0	.005	4.7	54.1
	8558	11.2	6.7	.008	4.4	39.7
	8559	11.1	5.2	.005	5.8	52.6
	8570	10.6	5.7	.004	4.9	46.4
	8626	8.5	6.3	.005	1.5	25.5
	8629	8.5	5.8	.004	1.9	31.4
	8635	11.3	9.8	.003	1.0	12.8
	8636	8.5	6.5	.005	1.4	22.9

Table 24. Phosphorus utilization data from digestibility trial

Diet	Steer	Phosphorus (g/day)				Apparent digestibility (%)
		Intake	Feces	Urine	Retention	
Hay	8549	18.0	11.1	3.9	3.5	38.3
	8558	18.2	7.1	7.9	3.2	60.9
	8559	19.3	8.1	6.5	4.8	58.3
	8570	19.3	6.0	7.8	5.4	68.6
	8626	18.8	6.9	4.3	7.6	63.5
	8629	20.3	9.5	9.6	1.2	53.3
	8635	19.3	10.7	3.1	5.6	44.7
	8636	17.1	8.6	4.3	4.2	50.0
Haylage	8549	19.4	7.3	4.1	8.0	62.3
	8558	19.3	3.4	12.5	3.5	82.4
	8559	18.7	6.8	8.3	3.6	63.7
	8570	18.6	5.5	8.3	4.9	70.7
	8626	19.3	6.7	4.3	8.3	65.1
	8629	19.3	8.5	4.2	6.7	55.9
	8635	19.2	10.5	3.8	4.9	45.2
	8636	19.3	10.4	5.5	3.4	46.1

Table 25. List of significant regressions

Item	Regression equation	Coefficient	P>F
Dry matter	DM feces = .503(DMI) - .679	.633	.009
	DM ret = .921(DMI) - .846	.575	.02
Organic matter	OM feces = .506(OMI) - .675	.639	.008
	OM ret = .496(OMI) + .667	.631	.009
Ash	Ash ret = .537(ASHI) + 2.52	.446	.09
Calcium	Ca feces = .843(CaI) - 8.69	.540	.04
Magnesium	Mg urine = .001(MgI) - .004	.528	.04
	Mg feces = .489(MgI) + 1.07	.449	.09
Phosphorus	P dig = 2.93(P urine) + 40.0	.679	.004
	P ret = -.42(P urine) + 7.51	.571	.03

Table 26. Data from inulin clearance studies

Diet	Steer	Infusion rate ^a	Infusion [inulin] ^b	Plasma [inulin] ^b	GFR ^c
Hay	8626	.87	3860	8.3	3.4
	8629	.74	3270	9.8	3.7
	8635	.96	3390	7.8	3.4
	8636	1.17	3550	10.0	4.1
Haylage	8626	1.11	3550	9.1	3.5
	8629	1.17	4000	14.4	3.8
	8635	1.15	3900	9.4	3.5
	8636	.96	3350	7.3	4.0

^aml/min.^bmg/100 ml.^cmg/(kg x min).