Effects of Phosphorus Supplementation on Grazing Beef Cattle

Scott Joseph Neil

Thesis submitted to the faculty of Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Masters of Science
In
Animal and Poultry Sciences

Mark A. McCann, Chair
Samer W. El-Kadi
Mark D. Hanigan
Rory O. Maguire

September 9, 2015
Blacksburg, VA

Key words: phosphorus, beef cattle, grazing

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ABSTRACT

Phosphorus (P) losses due to overfeeding of the mineral to livestock can contribute to surface water degradation. The objective of this study was to examine the impact to supplementing various levels of mineral P to grazing beef cattle. A producer survey and a research trial were conducted to examine the effects of supplementing mineral phosphorus (P) to grazing cattle. In the first study, mineral tags, producer surveys, and fecal, forage, and soil samples were collected from beef cattle operations in Virginia's Chesapeake Bay watershed. Samples (n=166) were collected from 120 producers in 11 counties. Soil test P results were based on Virginia Cooperative Extension soil test guidelines characterized as low (12 %), medium (37 %), high (35 %), and very high (16 %). Pasture grab samples contained 0.34 ± 0.12% P and forage P concentration increased ($P < 0.01$) across soil P categories going from low to very high. Fecal total phosphorus (TP) was lowly correlated ($R^2 = 0.18$, $P < 0.01$) to forage P concentration. Mineral supplements were categorized as nil (<1.0% P), low (1.0-<3.0% P), medium (3.0-<6.0% P), and high (>6.0% P). Fecal TP and inorganic phosphorus (Pi) concentration increased ($P < 0.01$) with mineral P levels. Fecal TP and Pi were lower ($P < 0.01$) when nil and low P mineral were supplemented as compared to medium and high P mineral. Soluble P (defined as fecal Pi/fecal TP*100) also increased ($P < 0.01$) with increasing mineral P content going from nil to high. All farms surveyed required little or no P supplementation in regard to beef cattle P requirements. The majority (82%) of producers were receptive to modifying mineral P supplementation practices based on

forage P levels. A 56-d study was also conducted with eight yearling Hereford steers (261±30 kg) grazing cool-season grass fall re-growth to determine the effects of varying levels of P supplementation on fecal P excretion. Treatments consisted of dicalcium phosphate supplemented at 0 (D1), 10.0 (D2), 20.0 (D3), or 30.0 (D4) g/d in a randomized 4x4 replicated Latin square design. These treatments provided an additional 0, 1.9, 3.7, and 5.6 g/d of P respectively. Two esophageally cannulated steers were used to collect forage samples for nutrient analysis. Forage P content was analyzed from hand collected samples. Forage P concentrations averaged 0.49% of dry matter (DM) across all periods. Chromic oxide (Cr_2O_3) was administered twice daily via gelatin capsule at 0630 and 1830 to serve as an external marker for determination of fecal dry matter excretion (DME). Indigestible NDF (iNDF) was used as an internal marker to determine dry matter intake (DMI). Due to the high forage P content, average P intake was in excess of National Research Council (NRC) requirements for all diets (D1 = 281%; D2 = 297%; D3 = 323%; D4 =348%). Orthogonal contrasts were performed to assess the relationship between treatment and P excretion. A linear response ($P < 0.01$) in daily inorganic P (Pi) excretion (0.054, 0.052, 0.062 and 0.063 g/kg of BW ± 0.003 for D1, D2, D3 and D4, respectively) was observed across treatments. Daily total P (TP) excretion increased linearly ($P < 0.01$) across treatments (0.080, 0.079, 0.092 and 0.093 g/kg of BW ± 0.003 for D1, D2, D3 and D4, respectively. When forage P content is sufficient to meet the requirement of grazing cattle, increasing P supplementation results in greater P excretion without additional benefits to growth or nutrient digestibility.

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CHAPTER 1: INTRODUCTION

Phosphorus (P) is an essential element for both plants and animals (Haan et al., 2007). In agriculture, P deficiency will lead to reduced crop yields and animal performance. However, overfeeding of the mineral adds an unnecessary expense and also poses a threat to watershed ecosystems (Knowlton et al., 2004). Excess P runoff into the watershed, where it is a limiting nutrient for growth, can cause uncontrolled algae blooms in a process known as eutrophication (Sharpley et al., 1994). This process is characterized by increased aquatic plant growth, water oxygen depletion, pH variability, and a breakdown in the hierarchical aquatic food system. As the numbers of algae and aquatic plants increase, the increase in decaying organic matter depletes water oxygen levels, especially in deeper water, leading to the reduction in numbers of desirable species. Historically, most of the attention and research efforts have focused on reducing P losses in confined animal operations and dairies. While manure output in cow/calf operations is not as concentrated, over-supplementation of P still has implications for the producer and the environment.

Inorganic phosphorus (Pi), also known as phosphate, is the most potentially harmful form of P because it is highly water soluble (Sharpley and Moyer, 2000). In cattle, a portion of Pi is normally lost through endogenous processes, however, manure Pi fractions increase as P intake increases past maintenance requirements (Dou et al., 2002). This effect is magnified when overfeeding of P is accomplished by supplementing it in an inorganic mineral form. Two studies were conducted to examine the impact of supplementing P to grazing beef cattle in a mineral form. The first study surveyed producers and collected samples from beef cattle operations in Virginia's Chesapeake

Bay watershed to document current mineral feeding practices and gather information on the phosphorus status of grazing beef cattle. A second study was conducted in a controlled setting to quantify the effects of supplementing grazing steers with mineral P.

CHAPTER 2: LITERATURE REVIEW

Phosphorus Requirements of Beef Cattle

The National Research Council (NRC) for nutrition has established minimum P intake levels for beef cattle. Maintenance recommendations are 16 mg P/kg live weight (LW) with an additional 3.9 g P per 100 g protein gain. Lactating cattle require 0.95 g P/kg of milk produced in addition to maintenance requirements (NRC, 1996). These recommendations are commonly used by producers in the U.S. as targets to ensure adequate P nutrition of their cattle. However, several studies have questioned whether or not the listed NRC requirements are too high and provided lower values (Call et al., 1978; Coates et al., 1992; Ternouth et al., 1996). Part of the reason for the disagreement stems from the differing absorption coefficients (AC) of various P sources. The NRC used an (AC) of 0.68 in its requirement calculations; however Challa et al. (1989) reported an AC of 0.50-0.60 for low quality roughages, 0.75-0.80 for endogenous P, and 0.80-0.90 for mineral P. Therefore it seems that a strategic practice would be to estimate dietary P requirements using ACs for specific feed sources rather than use a static value.

Ternouth et al. (1996) examined cattle consuming either barley straw-based diets or tropical pasture forage, and observed an AC of 0.75-0.85 across diets. The authors came to the conclusion that that 0.75 was a "suitably conservative estimate." Coates and Ternouth (1992) investigated a wide range of P feeding levels in heifers grazing tropical pastures and reported P absorption coefficients between 0.64 and 0.92. The highest AC values were associated with heifers grazing unfertilized pastures and receiving no supplementation while the lowest values were observed in heifers grazing fertilized pastures and receiving inorganic P supplementation. While the Droughtmaster breed used in this study is generally better adapted to low P environments, a logical conclusion is

that cattle are able to more efficiently utilize low P diets by increasing mineral absorption in order to meet requirements.

In a two year study, Call et al. (1978) reported that two groups of 48 Hereford heifers fed above (174%) and below (66%) NRC requirements did not exhibit any differences in growth or health. A second study by Call et al. (1986) in which Hereford heifers were fed either a low P (6 to 12.1 g/day based on BW) or a high P diet (20.6 to 38.1 g/day based on BW) demonstrated that growth and reproductive measures were similar between the two groups. However, when supplementation in the low P group was reduced even further, clinical signs of deficiency became apparent within 6 months. The study concluded that 12 g P/day throughout the year was adequate for 450 kg Hereford cows and is lower than the 16.1 recommended throughout the year by the NRC for 450 kg cows producing 20 lb milk/day (NRC 1996).

Ternouth et al. (1996) calculated a maintenance requirement of between 8.6-16.6 mg/P kg BW for intakes between 1.0% and 2.5% of BW. This is lower than or equal to NRC estimates. In contrast, Challa et al. (1989) reported maintenance requirement of 19 mg P/day per kg LW. This is a 19% increase over NRC recommendations and would result in an extra requirement of 1.7 g P/day for a 550 kg cow. The higher observed value may have been due to the use of metabolism crates in the Challa study. Behavioral and environmental differences associated with the use of metabolism crates may lead to results that are different than values observed in a more natural setting. In conclusion, it is likely that the P requirements established by the NRC using a fixed absorption coefficient are too high in many cases. A better practice would be to estimate

requirements based off the P availability of the feed source and the animal's genetic ability to recover P from the diet.

There are several dietary factors which can affect P requirements. In cattle consuming a high nitrogen (N) diet, a P deficiency can become even more pronounced as the higher N content increases growth while P intake remains low. This subsequently increases P demand in order to keep up with increased tissue retention, stretching a limited supply even further (Bortolussi et al., 1996). Trace minerals such as Fe, Al, and Mg can bind to P to form insoluble phosphates and prevent its absorption in the intestine (McDowell, 2003). These factors should be taken into account when N or trace mineral intake of animals is above requirements.

Functions of Phosphorus in the Body

Phosphorus is the second most abundant mineral found in the body of cattle and plays a major role in normal function and support. Approximately 2 kg of P are present in cattle weighing 300 kg with the majority (80-85%) found in bone in the form of hydroxyapatite (McDowell, 2003). Skeletal P occurs in a roughly 1:1.7 ratio to calcium and not only is important in structurally supporting the animal body, but also serves a major mineral reserve for times of increased requirement or dietary deficiency (Ternouth, 1990).

Outside of the skeletal system, P is also found in the phospholipid component of cell membranes where it serves to maintain the integrity of the cells. The element is required by carbohydrate catabolism to form phosphate bonds in the ATP molecule as a form of energy storage. Phosphorus aids in nerve signal transmission by serving as a component of the myelin sheath. Coenzymes of several B vitamins require

phosphorylation for proper function. In the cell nuclei, P is a component of DNA where it is involved in the maintenance of genetic information. Consequently, it also plays a role in gene expression as a component of RNA (Ammerman et al., 1983; McDowell, 2003). Without P, the body could not structurally support itself, store energy, or transfer genetic information and therefore would cease to function.

Intake/Digestion

Animals ingest P in one of three forms. Inorganic P naturally occurs in many feeds and forages and is the form found in traditional cattle mineral supplements. The other two forms are both classified as organic phosphorus (Po). The first is phytate phosphorus which is the most common form stored in plants, being especially high in seeds. The other form consists of P that is bound to phospholipids, phosphoproteins, and nucleic acids within the plants (Bravo et al 2003a).

In ruminants, P entering the stomach is composed of a dietary source and an endogenous fraction. The endogenous fraction originates almost entirely from P recycled in the saliva (Coates and Ternouth, 1992). Endogenous P is nearly all Pi which is highly absorbable (Care, 1994) while dietary P from forage and grains is mostly in the organic form and is not immediately available for uptake (Bravo et al., 2003a). In non-ruminants, absorption of phytate P is very low. However, microbes in the gut of ruminants produce phytase, the enzyme that converts phytate into its inorganic form. Phytase is also found in some feedstuffs including rye and wheat (Eeckhout and De Paepe, 1993; McDowell, 2003). The action of this enzyme increases P available for absorption and allows ruminants to utilize low quality roughages.

Phosphate that passes out of the rumen enters into the abmasum where pH is greatly reduced. At this low pH (3-4), 98% of P takes on a monovalent form ($H_2PO_4^-$) which is available for uptake by the small intestine (Shirazi-Beechey et al., 1996). As digesta moves through the small intestine, the pH rises and less P is in a form available for absorbtion.

Phosphorus kinetic studies are inherently difficult to perform due to the relative unpredictability of endogenous P flow. The complex factors that influence this flow of P are discussed in the following section. The amount of dietary P available for uptake in the intestine, not only depends of the composition of the diet, but also the level of phytase activity in the rumen. In the intestine, luminal pH and flow rate also affect digestibility. Due to the many factors which play a role in P digestion, accurately predicting P retention based on intake can be difficult. However, recent studies have made progress in mapping the flow of P throughout the bovine. Feng et al. (2015) found that almost 95% of phytate P was broken down into its inorganic form in dairy cattle and noted that total tract P digestibility was near 41%.

Salivary Phosphorus

To completely understand P homeostasis in ruminants, it is important to recognize the role that saliva plays. In non-ruminants, the kidneys work to maintain P homeostasis by excreting excess P in the urine. In ruminants, the P absorption threshold of the renal tubules is much higher, and the parotid glands become the major site for removal of P from the blood (Care, 1994; McDowell, 2003). This mechanism is in place as it offers a solution to several unique challenges inherent to the ability of ruminants to maintain healthy microbial populations within the gut.

The first role of salivary P is to supply rumen microbes with an adequate available P supply regardless of dietary P status in order to maintain proper rumen function (Challa et al., 1989). Studies on several strains of rumen microbes revealed that microbial growth is stopped in the absence of phosphate (Bryant et al., 1959). Witt and Owens (1983) reported on a very low P diet (0.07%), cattle rumen P concentrations remained at 208 mg/L, significantly higher than the minimum 75-100 mg/L needed for proper rumen microbial function (Komisarczuk et al., 1987). Also, no reduction of in vitro dry matter disappearance was observed on the low P diet although there was a reduction in total P retention, indicating that bone reserves were mobilized to maintain salivary P concentrations.

A long term study by Williams et al. (1991) demonstrated that rumen P concentrations were not different (273 mg/L) between cows on a low P diet (0.12%) and a high P diet (0.20%) even after 564 days. However, bone P and body weight gains were reduced on the low P diet (Williams et al., 1989) indicating that cattle were mobilizing reserves and shunting P away from growth to maintain rumen P flow. Work by Challa et al. (1989) showed that ruminants consuming P deficient diets continue to secrete salivary P levels at levels adequate to meet baseline microbial demands (8.6 mg P/day per kg LW).

A secondary function of salivary P is to serve as a buffer against a pH drop caused by the production of volatile fatty acids (VFA) in the rumen (Care, 1994). While P is not the only buffering agent in the saliva, sodium bicarbonate being found in higher quantities, its action is still required by the ruminant. Without this buffering effect, rumen

pH would eventually drop below the level at which most microbes could effectively function, limiting diet digestibility (McDougall, 1948).

Saliva flow is highest when animals are eating and ruminating but remains at a fairly constant rate when animals are at rest (Wheeler, 1980). It has been shown that in animals consuming roughage diets, chewing and ruminating time are greatly increased over those consuming concentrate diets (Care, 1994). This ultimately results in lower flows of P into the rumen of the latter group, partly explaining the drop in pH as VFA production outpaces buffer entry into the rumen (Briggs et al., 1956).

In cattle, daily saliva flow is between 33-190 L per day, depending on animal size and diet (Wheeler, 1980), with total daily salivary P flow around 30-60 g (Breves and Schroder, 1991). The total salivary P flow is a function of salivary concentration and salivary volume. Flow rate is influenced mainly by dietary factors. As stated earlier, ruminants consuming diets high in roughages have greater salivary flow than those consuming concentrates. Multiple sources have shown that cattle experiencing a P deficiency reduce DMI (Karn, 2000; McDowell, 2003). This reduction in DMI is usually attributed to a diminished rumen microbial function. However, this is possibly a mechanism to reduce endogenous P losses from saliva although it often accelerates deficiency symptoms by limiting P intake even further.

Control of salivary P concentration is more complex. Bravo et al. (2003a) found that P concentration was inversely related to salivary volume. A direct relationship between total P intake and salivary P concentration has been established (Scott et al., 1985), most likely a consequence of the direct relationship between P intake and blood plasma P. As blood plasma P rises, more P is removed by the saliva producing glands and

salivary P concentration increases (Coates and Ternouth, 1992). Ruminants are able to concentrate P from the blood in saliva producing glands. Breves and Schroder (1991) indicated a salivary P to plasma P ratio between 12:1 and 16:1, while a review by Bravo et al. (2003b) noted a ratio between 6:1 and 13:1.

The flow of salivary P into the rumen is higher than dietary P except in cases where very high P diets are fed (Care, 1994). Bravo et al. (2003a) reported that dietary P only exceeds salivary P when the ration contains greater than 0.66% P. Diets this high in the element are uncommon in grazing cattle. On low P diets, salivary flow can make up as much as 80% of daily P intake (Care, 1994).

In ruminants, salivary P excretion is the foremost method by which the animal regulates P homeostasis. The kidneys serve as a secondary regulator and only play a role once plasma P exceeds the renal threshold value for reabsorption into the blood (McDowell, 2003). Challa et al. (1989) stated this threshold was reached at 2.3 mmol/L. Care (1994) noted an similar value of 2.7 mmol/L. This threshold provides an alternate route for P excretion once the mechanism controlling salivary P excretion has been saturated. Reabsorption of salivary P in the intestine is in the range of 70-80% (Challa et al., 1989; Bravo et al., 2003a). Therefore, as long as there is salivary secretion, obligatory endogenous P losses will occur. Cattle go into negative P balance once these obligatory endogenous losses exceed dietary P absorption. However, by recycling P in the saliva, ruminants are able recover and preserve a majority of the mineral while still maintaining homeostasis.

Phosphorus Absorption

Phosphorus is absorbed throughout the digestive tract, however; the majority of uptake occurs in the duodenum and jejunum (McDowell, 2003). Under normal conditions, 70-80% of dietary P will be absorbed and absorbtion is driven by both active and passive transporters (McDowell, 2003). Several studies by Ternouth and Coates suggested that when P intake was near requirement, the AC would be between 0.75 and 0.85 for cattle consuming forage diets, regardless of stage of growth or lactation (Ternouth et al., 1996; Ternouth and Coates, 1997). Ternouth described the normal range of daily intake as between 10-60 mg/kg live weight, stating that 0.75 is a suitably conservative absorbtion coefficient and is higher than studies done previously. Bravo et al. (2003a) provided an estimate of absorbability equal to 0.72 for dietary P and 0.77 for endogenous sources.

Others have noted that the AC for P is higher in low P diets and and decreases as more P is added to the diet. (Coates and Ternouth, 1992) When P was supplemented in a mineral form, the increase in AC was even greater. This is most likely due to the fact that mineral P is the inorganic form which is more easily absorbed as it does not require the action of phytase prior to uptake. Studies by Challa et al. (1989) argued that P absorbtion increased as more P was added to a low P diet, however these studies used mineral P to supplement low quality roughage diets and absorbtion most likely increased due to the higher availability of mineral P. Maximum P absorbtion efficiency was 0.85 at 50-60 mg/day per kg BW which is in agreement with the work done by Ternouth. Comparitively, the AC of the unsupplemented roughage diet used in these trials was between 0.50 and 0.60.

Stomach

Under normal conditons, absorption of P in the stomach of ruminants is negligible. However, it appears that the rumen epithelium do have the ability to passively absorb P under certain conditions. Scarisbrick and Ewer (1951) observed the transport of radioactively labeled P from the rumen into the blood when the compartment was emptied of all contents but concluded that no net transport out of the rumen occurred. Parthasarathy et al. (1952) agreed that there was no net transport of P across the rumen epithelium, observing movement of P into and out of the rumen.

Later work by Breves et al. (1988) in sheep showed significant movement of P across the rumen epithelium that was directly related to ruminal P concentrations and the author identified an electrical gradient as the driving force. This study indicated that up to 29% of daily P intake could be absorbed in the reticulo-rumen. In a similar study, Beardsworth et al. (1989) showed that rumen P uptake increased as P concentrations in the rumen increased. Concentrations observed ranged from 2 to 38 mmol/L and absorption rates ranged from -2.9 to 57.7 μmol/L per min across treatments. However, workers in the latter two experiments removed all contents and washed the rumen prior to infusing the P solution. This does not accurately represent the normal state of the rumen and as demonstrated by Scarisbrick and Ewer, absorption under these conditions is different than when the rumen is full.

Little work has been done examining P absorption from the omasum and abomasum. Engelhardt and Hauffe (1975) demonstrated the omasum to be a non-factor in P absorption in sheep. While it is generally assumed that the physiological mechanisms of P absorption are similar between cattle and sheep, an experiment by Edrise and Smith (1986) using cattle showed significant removal (10-40%) of P by the omasum. It should

be noted that the omasum is much larger in proportion to the rest of the stomach in cattle than it is in sheep, possibly accounting for this difference (Scott et al., 1985). Absorption from the abomasum is generally considered to be negligible.

Most of these experiments observed diffusion of P from the blood into the rumen during the infusion of no or low P solutions. This may be another mechanism by which the ruminant copes with providing P to microbes during times of dietary deficiency, comparable to the role of saliva. While it has been shown that the reticulo-rumen can passively absorb P, in most situations it should be assumed that secretion of P into the rumen from the blood results in little to no net uptake from this organ.

Breves et al. (1988) demonstrated that P absorption across the rumen wall was greater in P depleted sheep due to the higher potential difference between the blood and rumen contents. In lactating cattle, plasma P is significantly lower than in non-lactating animals due to the high demand of P associated with milk production. Under these conditions, the ratio of plasma P to dietary P is lowered (Ternouth and Coates, 1997) and therefore the potential difference would be greater. While no paper has been found to support this hypothesis, it is possible that under these conditions, the increase in the gradient between rumen P and blood P would allow for greater absorption across the rumen wall. However, it should be concluded that more work needs to be done examining P absorption from the reticulo-rumen in animals with full stomachs.

Small Intestine

The majority of P absorption takes place in the small intestine. The duodenum and proximal jejunum, where pH is the lowest, are the most active sites for uptake (Pfeffer et al., 1970; Grace et al., 1974; Cohen, 1980; Schroder et al., 1995). Absorption throughout

the small intestine is controlled by both active and passive transporters (Cohen, 1980; Care 1994; Bravo et al., 2003a). Passive absorption rate is reliant on luminal P concentration, therefore, passive absorption only exceeds active absorption on very high P diets due to active transporters becoming saturated. During P deficiency, the energy cost of P transport across the intestinal epithelium is higher due to decreased absorption by passive action (Cohen, 1980). However, low P diets are often also energy deficient, possibly accelerating deficiency symptoms.

Although there is disagreement about whether P shortage leads to an increase in whole-tract P absorption efficiency, multiple authors have demonstrated marked increases in P absorption across the small intestine epithelium by active transporters during times of prolonged defiency (Scott et al., 1985; Shirazi-Beechley et al., 1991; Shirazi-Beechley et al., 1996). It is important to note that all of these studies were done using sheep, however, the physiological mechanisms should translate to cattle. Shirazi-Beechey et al. (1991) observed a 4-fold increase in P uptake by the brush border membrane of sheep fed a prolonged low P diet compared to those fed adequate P. While P transport across the reticulo-rumen epithelium was thought to be be due to an electrical gradient, this was not the case in the small intestine.

Phosphorus transport across the intestinal epithelium is pH dependent with higher absorption at more acidic levels (Shirazi-Beechey et al., 1991; Care, 1994). In the duodenum and proximal jejenum, normal pH is in the range of 3-4. At this pH, 98% of orthophosphate is in the monovalent form ($H_2PO_4^-$) suggesting that this is the preferred form for uptake (Shirazi-Beechey et al., 1996). It has also been reported that Na^+ plays a major role in P uptake. Similar to studies by Shirazi-Beechey et al., Schroder et al. (1995)

used isolated ovine small intestinal epithelium segments from both P depleted and P repleted sheep and found that in the absence of Na^+, active P transport across the brush border membrane was reduced to 34% of its normal rate. The author concluded that Na^+ was most likely not directly involved as a P co-transporter, but instead served as an H^+ antiporter to maintain the low pH in the intestinal lumen and therefore improve absorption efficiency.

Circulating vitamin D seems to play some role in regulating P uptake. Reducing Ca in the diet leads to an increase in circulating 1,25 dihydroxy vitamin D and in turn results in an increase in both Ca and P absorption efficiency (Care, 1994). However, P deficiency by itself does not increase circulating levels of 1,25 dihydroxy vitamin D in ruminants, indicating the role of vitamin D levels on enhancing P uptake during P deficiency is regulated by some other mechanism (Shirazi-Beechley et al., 1996). Schroder et al. (1990) used goat small intestine epithelium to demonstrate that during P depletion, receptor affinity for 1,25 dihydroxy vitamin D is enhanced. While this proves that vitamin D does play a role in upregulating P absorption from the intestine, the exact mechanisms by which it acts are still unclear. One probable means is an increase in expression of a specific binding protein due to activity by the vitamin (Care, 1994).

Although it has been clearly shown that ruminants can alter facilitated uptake of P from the intestine during deficiency, the exact mechanisms still remain unclear. It is most likely that vitamin D activity plays some role. Increases in circulating levels of 1,25 dihydroxy vitamin D, either due to Ca shortage or external injection, increase P absorption although it has been shown that circulating levels do not increase in response to a P shortage. During P deficiency, the affinity of vitamin D receptors in the intestine is

increased leading to enhanced absorption of P. Based on this knowledge, vitamin D supplementation could be used to enhance P extraction from diets low in the mineral or to limit losses from high P diets. It has also been shown that dietary P level affects expression of carrier proteins in the intestine. Possible carrier proteins that have been suggested to play a role in P uptake include Na^+/H^+ antiporters, H^+/P symporters, or Na+/P symporters. Based on this information it might be expected that animals consuming Na^+ deficient diets would have lower P absorption efficiency, however, more work needs to be done examining the exact mechanisms involved in P uptake from the small intestine.

Colon/Cecum

It appears that the large intestine of ruminants does absorb P to some degree research in cattle has been limited. Work by Pfeffer (1970) using reentrant cannulas placed at the terminal ileum in sheep showed that P absorption from the large intestine was between <1% and 16.6% of the total P leaving the stomach. However, there was large variation between animals and diets making it unclear as to what was responsible for these differences. A study by Grace et al. (1974), also using sheep, had similar results and indicated that absorption from the organ was between 0% and 18% of P leaving the stomach. There was much less variation in this study and the author attributed rises in absorption to increases in dietary P and the subsequent increase in P entering the colon. The mechanisms by which P absorption occurs in the large intestine are unclear.

Previously cited studies suggest that absorption in this area is passive due to the increases observed as P concentration rises. However, Breves and Schroder (1991) cited work which postulated that at low concentrations, P absorption in the colon was due to

active transport. All of the cited studies were done in sheep and although it is usually assumed that the mechanisms observed in this animal is similar to those in cattle, this may not always be the case.

Recent work in cattle found that dairy cattle consuming diets containing 0.43% , 0.48%, and 0.54% P respectively absorbed 8.4%, 12.3%, and 9.6% of Pi entering the colon. (Ray et al., 2013). A model developed by Feng et al. (2015) determined that for a 600 kg dairy cow consuming 23 kg of DM/d containing 0.33% P, 11.7% of TP entering the large intestine would be absorbed. It is evident that P uptake can occur in the colon of cattle but it should be noted that lactating dairy cows consume diets with significantly higher P concentrations than beef cattle which could lead to an increase in P leaving the small intestine and entering the large intestine. More work needs to be done to better understand the role of the large intestine in P absorption in beef cattle.

Phosphorus Excretion

In contrast to humans and carnivores, there is very little P excretion in the urine in ruminants (McDowell, 2003). However, urinary P can become significant in cattle consuming high P diets where plasma P levels exceed the capability of renal tubules to reabsorb the mineral (Challa et al., 1989). The authors of this study found that this resorption capacity was saturated when blood serum P levels exceeded 2.3 mmol/L. This concentration threshold is in the range of 16-30 mmol/dL as reported in a review by Bravo et al. (2003b). Cattle consuming high concentrate diets tend to excrete more urinary P than those grazing or consuming roughage diets. This is due to the high levels of P typically found in concentrate diets combined with the lower salivary secretion required for consumption compared to forage diets (Ternouth, 1990). When salivary

removal of P from the blood is reduced, blood P levels subsequently increase to the point where renal reabsorption capacity is overwhelmed.

The main route of P excretion in the ruminant is through the feces (McDowell, 2003). Fecal P is made up of both a dietary fraction, and an endogenous fraction (Bravo et al., 2003b). The contribution of dietary P fraction to fecal P is determined by the amount of P consumed, and the P absorbtion coefficient of the diet. As stated earlier, dietary P absorption is affected both by the form of P in the diet and the P concentration of the diet. The other fraction of fecal P, endogenous P, primarily originates from the saliva (Challa et al., 1989; Bravo et al., 2003b). Under normal conditions, the AC of salivary P remains fairly constant, indicating that salivary flow is the main determinant of endogenous P losses (Challa et al., 1989). The factors affecting salivary P flow, including roughage intake and blood plasma P, were previously discussed in the "Salivary Phosphorus" section.

In a study using penned cattle, Bortolussi et al. (1996) found that animals consuming high roughage, low P diets (5.8-6.5 mg/kg LW), endogenous fecal losses (9.8-11.5 mg/kg LW) ranged from 155-177% of P intake. Animals in the same trial consuming high P diets (41 mg/kg BW) had total endogenous P losses twice as high as the low P group, but these losses only equaled 50% of intake. Work by Coates and Ternouth (1992) in grazing cattle also found that endogenous fecal losses (9.7-27.5 mg/kg LW) increased with total P intake. It should be noted that in both penned and grazing cattle, the lower value for endogenous losses was ~10 mg/kg. This value is representative of "obligatory losses" which occur even in cattle consuming diets containing zero P (Coates and Ternouth, 1992; Ternouth et al., 1996).

It can be concluded that changes in fecal P excretion are a result of those factors which contribute to maintaining P homeostasis, salivary P secretion and P absorption efficiency. In low P diets, endogenous fecal P losses can exceed P intake causing the animal to be in negative P balance. Fecal P excretion is expected to increase as dietary P levels rise although compostion of the diet can affect the magnitude of this increase.

Forage Phosphorus

There are multiple factors which contribute to forage P levels including, soil P content, soil pH, plant species, plant maturity, and grazing pressure (Haan et al., 2007). A study done in Iowa by Haan et al. (2007) found that forage P concentration in smooth bromegrass pastures was adequate throughout the year to meet the NRC requirements of spring calving cows producing moderate levels of milk. Average forage P concentrations of grazed paddocks were highest in April (0.22% of DM) and lowest in November (0.13% of DM). The observed decrease in P content through the grazing season was in agreement with work by Rauzi et al. (1969). The authors did find that P content temporarily increased immediately after harvesting a mature paddock for hay as the less mature re-growth contained greater concentrations of P.

Morris et al. (1982) examined differences between growth and P uptake of warm and cool season grasses in high P and low P soils. While dry matter yields were nearly double for warm season grasses in low P soils, P concentrations were significantly reduced compared to cool season grasses (0.07 to 0.11% vs. 0.14 to 0.22%). During the first year of establishment, yields were lower for warm season grasses and P concentrations ranged from 0.19% to 0.23%. The next year warm season grass yields were significantly higher, however, P concentrations were lower and in the range of

0.11% to 0.19%. Across both years, the P concentation of cool season grasses growing in high P soils was similar and in the range of 0.22% to 0.29%.

The highest P requirements throughout the year for young growing cattle, pregnant replacements, and mature beef cows receiving a diet containing 60% TDN (roughly equivalent to high quality pasture) are 0.18%, 0.27%, and 0.20% of DM respectively (NRC, 1996). Phosphorus research utilizing grazing animals is generally limited due to the difficulties in quantifying P intake in a pasture setting.

One such study, conducted by Brokman et al. (2008) examined P intake and P excretion patterns in young, growing Holstein steers. Pastures used in the trial consisted of fertilized, cool season grasses and legumes which contained between 0.30% and 0.36% P throughout the grazing periods. Forage P content was estimated via hand-grab samples and grazing steers receiving no supplemental P had intakes of 126% and 133% of NRC recommendations across years, assuming a fairly conservative P absorption coeffiecient of 64% (see 'Phosphorus Requirements' section). Growth was no different between this group and those receiving mineral supplementation of P however, P excretion was 30-58% higher in those receiving mineral P.

A study by Coates and Ternouth (1992) examined P balances in heifers grazing low P pastures (LP), low P pastures and supplemented with mineral P (LPS), or high P fertilized pastures (HPF). While P intakes were lower than NRC requirments on all diets, with the LP treatment being reduced compared to LPS and HPF, heifers were able to adjust the P absorption efficiency to allow for greater utilization of the deficient diets. Although no differences were observed in bone density between treatments, growth was highest in the LPS treatment. The two lower P treatments allowed heifers to reach

targeted goals for gain indicating that neither was deficient in P. The authors suggested that the NRC requirements for grazing cattle P intake were overestimated as cattle appeared to be able to adapt absorption efficiency to some degree in order to compensate for reduced P availability.

In most cases, cool season grasses, especially those earlier in maturity, should meet beef cattle P requirements without supplementation. Supplementation may be required when soil P is low, late in the growing season, for cattle at a high level of production, or for cattle consuming warm season forages. While warm season grasses have similar total P uptake per plant as compared to cool season grasses, they have lower concentrations of the mineral due to their higher dry matter yields. Producers grazing cattle in areas with low P soil and warm season grasses, such as those found in the southeastern and western U.S., should be especially attentive to P status of the herd. However, in many areas, well managed forage systems may reduce or eliminate the need to supplement P in areas where soil P levels are adequate or high. Also, grazing cattle may be able to adapt to forage P levels lower than NRC requirements by increasing expression for P transporters in the intestine and therefore may not require additional supplementation.

Environmental Effects of Phosphorus

Phosphorus losses into surface waters is a major contributor to eutrophication. Eutrophication can be defined as "the over-enrichment of receiving waters with mineral nutrients" (Correll, 1998). The process is characterized by rise in algae and cyanobacteria populations which subsequently use up available oxygen during respiration leading to hypoxia and death of other organisms in the ecosystem. Phosphorus is a limiting nutrient

and a main contributor to this process. In most soils, the majority of P enters the watershed through surface runoff rather than groundwater due to binding of the mineral in soil and sediment (Correll, 1998).

Reduction of P concentrations in surface water may have a greater effect on limiting eutrophication than the reduction of nitrogen (N) inputs. Many of the cyanobacteria that contribute to the eutrophication process have the ability to fix atmospheric N. By maintaining P as the most limiting nutrient in an aquatic ecosystem, cyanobacteria growth is reduced along with aquatic N fixation (Sharpley et al., 1994). In addition, in many U.S. waterways, P is more limiting for growth than N (Correll, 1998). Reduction in P runoff does not always result in an immediate decrease in algal growth and eutrophication. Long-term P pollution leads to a build-up of the mineral in bottom sediments and organic material which can continue to release P in the absence of additional sources entering the waterway (Sharpley et al., 1994).

Nutrients can enter a waterway from either point or non-point sources. In recent years, many point sources have been identified and controlled (Carpenter et al. 1998). However, most agricultural P losses are considered non-point source and therefore are much harder to control due to large drainage areas and multiple watershed entry points. Therfore, agriculture has been implicated as a major contributor to watershed pollution. In response to this, many countries and regions worldwide have developed some sort of best management practice (BMP) to combat agricultural nutrient losses (Maguire et al. 2009). These BMPs vary widely and are usually site specific. In Denmark and Northern Ireland, national regulations limit animal stock densities and fertilizer application rates. In the Chesapeake Bay watershed, nutrient management plans (NMP) and diet

modification have been utililized to reduce P losses. While initial efforts at controlling agricultural runoff through the implementation of BMPs in these areas have seen some success, work still needs to be done to reduce P surpluses in many areas (Maguire et al. 2009).

To help reduce agriculture nutrient losses, NMPs have been implemented in many countries (Beegle et al. 2000). These NMPs focus on assessment of farm nutrient inventory and crop requirements to better allot manure and fertilizer to available fields. The seperation of cropping and livestock production areas has increased the efficiency of U.S. agriculture, but have resulted in the importation of more nutrients to livestock areas than can be applied to adjacent cropping area (CAST, 2002; Dou et al., 2002). Historically, manure has been applied to soils to meet the N requirement. However, P:N ratios in animal manure are much higher than crop requirements leading to overapplication of P (Knowlton et al. 2004). As a result, soil test P levels have steadily increased in some areas and a rise in the number of soils which are high or very high in P has been observed (Sharpley et al. 1994). Carpenter et al. (1998) estimated that between 1950 and 1995, P content of agricultural soils increased ~25%. Excess soil P can result in P losses in runoff in the form of particulate P, which is more slowly released in surface waters than dissolved P (Sharpley et al., 1994).

Another form of P runoff, known as dissolved P, is comprised mostly of orthophosphate, which is water soluble, and is immediately available for uptake by aquatic organisms. A high percentage of manure P is available for transport into waterways in the form of dissolved P. Inorganic P (Pi) from manure is of the greatest concern as it has the highest runoff potential. Sharpley and Moyer (2000) found that 81%

of Pi in dairy manure was water extractable compared to only 48% of Po. The same study showed that the amount of P leached during simulated rainfall was highly correlated (R^2 = 0.98) to water extractable P. Ebeling et al. (2002) examined the effects of P feeding on manure P losses to the environment. Manure was collected from cattle fed either a low P (0.32%) of high P (0.48%) diet and observed fecal P fractions were 0.48% and 1.28% respectively. When manure from high P cattle was land applied at an equivalent P rate (36 lb P/a) as manure from low P cattle, the high P manure lost 4 times more P in runoff than the low P manure immediately after application. When the same application rate (25 wet ton/a) was used for the two treatments, runoff losses were 10 times higher from high P manure. The authors also reported that manure applied at equivalent P rates, high P manure has 4 times more runoff potential low P manure.

Dietary modification is a potentially powerful tool for reducing P losses to the environment. Most cattle diets supplement P in the form of calcium phosphate, phosphate being a water-soluble form of the mineral (Maguire et al., 2007). Removing calcium phosphate from the diet of animals already receiving adequate P from feed or forage can reduce fecal P excretion. In several long term trials, Dou et al. (2002) showed that decreasing calcium phosphate concentrations of dairy diets resulted in lower fecal P excretion without any detrimental effects on milk yields. Reducing manure P levels is a strategic option for dairy producers with limited land resources to maximize manure application rates to fields without exceeding soil P concentrations allowed by NMPs.

Current NRC P requirements may be overestimated and could be a contributing factor to excessive fecal P. In beef cattle, Call et al. (1978) conducted a long term trial that demonstrated reduced dietary P intake led to a significant decrease in fecal P

fractions. The low P diet in this study was 66% of NRC requirements and even after two years, those heifers did not show any reductions in feed efficiency, growth, reproduction, or bone composition.

In the last several decades, increasing public scrutiny of agricultural practices has resulted an many changes in operating practices aimed at reducing P losses to the environment. Over-fertilization of agricultural soils in livestock producing areas has resulted in excessive P buildup. Nutrient management plans and diet modification have shown to be viable options to increase efficiency of P utilization on the farm. While these practices have produced promising results so far, more work needs to be done to educate producers on the importance of nutrient management and reducing P losses that cause watershed degradation.

Use of Markers in Grazing Studies

Estimating forage intake and fecal output of grazing animals is inherently difficult. To overcome this, dual-marker systems have been developed which utilize both an internal and an external marker to calculate total dry matter intake and excretion. An internal marker is defined as an indigestible component which occurs naturally in the feed-stuff (Cordova et al., 1978). External markers on the other hand are indigestible substances which are exogenously administered to the animal in a known amount (Cordova et al., 1978). Indigestible NDF (iNDF), an internal marker, and chromic oxide (Cr_2O_3), an external marker, have been extensively researched to fulfill these roles in a dual marker system. A known amount of Cr_2O_3 can be administered daily and fecal concentrations measured to determine total daily fecal output (DMO). Concentrations of

iNDF can then be measured in the feces and forage and total daily DMI solved using the equation DMI x forage iNDF = DMO x fecal iNDF (Cordova et al., 1978).

Indigestible NDF

The idea of using indigestible cell wall components as an internal marker is not a new development, first being proposed by Van Soest et al. in 1966. However, methods for digestion and NDF extraction have improved over the years to the point where iNDF is a reliable digestibility marker (Cochran et al., 1987). Sample preparation is especially important for analysis involving NDF extraction. Oven drying (45º C) of clipped, fresh forage samples has been shown to decrease dry matter digestibility (DMD) by 5.3%, which in turn can affect iNDF recovery from the plants (Grant and Campbell, 1978). Heating during drying caused non-enzymatic Malliard reactions to take place which resulted in N binding to fiber. Burritt et al. (1988) reported that oven drying (40º C) of esophageal extrusa led to a significant increase in NDF content when compared to freeze drying. This effect was greatest in plants early in maturity, possibly due to the higher protein content of younger forage being available to bind to fiber. Karn (1991) also found that conventional oven drying (50º C) fecal and esophageal extrusa samples increased NDF content while freeze drying did not affect nutrient composition.

Both in vitro and in vivo methods exist for iNDF determination. The first method consists of a 96-hr in vitro sample fermentation followed by a 48-hr acid-pepsin digestion and NDF extraction (Berger et al., 1979). The in vivo procedure consists of a 288-hr in sacco fermentation in ruminally cannulated cattle followed by NDF extraction (Huhtanen et al., 1994). Lippke et al. (1986) found that iNDF recovery was not different between the two methods. Huhtanen et al. (1994) disagreed with these findings and suggested total

fecal iNDF recovery was higher and closer to 100% when using the in sacco method (41 μm pore bag = 90.1%; 6 μm pore bag = 108.6) compared to the in vitro method (69.6%).

Lippke et al. (1996) concluded that a 6-8 day in vivo digestion procedure is adequate for complete disappearance of digestible NDF although samples with a larger particle size may need to be digested longer. The same authors showed fecal recovery estimates ranged from 84-108% following an 8-d in sacco digestion. Differences in recovery were attributed to particle size and it was found that even when using the same size screen during grinding, mean particle size was different between samples. Mean particle size was negatively correlated with iNDF recovery.

Using the in vitro method, Cochran et al. (1986) reported that total fecal iNDF recovery from steers was 96.6% for tall wheatgrass + soybean meal and 101.2% for alfalfa cubes. Recovery of iNDF from fresh clipped tall fescue was significantly lower at 43.2%, possibly due to handling procedures. Forage samples were oven dried (50º C) and as discussed earlier, the effects of oven-drying forage samples could have caused apparent iNDF content of oven-dried fescue to be higher than iNDF content of the forage as fed. If this was the case, total iNDF intake would have been overestimated leading to the observed discrepancy in recovery. This would not have been a problem in the other forages tested as they were air-dried prior to feeding.

Sample preparation is critical when iNDF is used as an internal marker for determining forage intake. Whenever possible, freeze drying should be utilized as this method does not appear to affect iNDF recovery compared to oven drying. Samples should be ground as uniformly as possible and small particle size is better. To help

account for differences in particle size between samples, a longer digestion time can be used to ensure complete digestion of larger particles (Lippke et al., 1986).

Chromic Oxide

Total fecal collection is difficult in the pasture setting (Cordova et al., 1978). In grazing studies, Cr_2O_3 has been recognized as a suitable external marker for estimating total daily fecal excretion. While Cr_2O_3 is one of the most used markers for digestion studies, three main problems have been noted (Titgemeyer, 1997).

The first problem associated with Cr_2O_3 use is the diurnal variation observed in fecal concentrations. Smith and Reid (1955) showed that Cr_2O_3 recovery from feces of grazing steers followed a sine-like pattern that varied between 52% and 183% throughout the day and averaged near 100% at 6 a.m. and 4 p.m. Due to this research, many subsequent experiments have relied on collecting fecal samples at 6 a.m. and 4 p.m. to overcome diurnal variation. Hopper et al. (1978), using higher animal numbers, also found that Cr_2O_3 excretion was diurnal, reaching a relative maximum at 9:00 a.m. and relative minimum at 8:00 p.m. By using paper impregnated with the marker, Nelson and Green (1969) were able to remove much of the variation, possibly due to the more even distribution of the marker through the rumen. Using paper, recovery over the whole day averaged 96% of total administered reaching a low value of 88% at 7 a.m. and peaking at 100% at 9 a.m.

The second problem noted is that recovery values often deviate from 100%. Differences between daily recoveries have been shown to vary from 92-112% (Nelson and Green, 1969) and 71-82% (Kiesling et al., 1969). Weekly recoveries have been shown to vary from 81-106% (Stevenson, 1962). Grab samples taken twice daily at 6 a.m. and 4 p.m. may underestimate Cr_2O_3 recovery when compared to representative

samples taken from a total daily collection (Stevenson, 1962). Calculated recovery of Cr_2O_3 from grab samples was equal to 92.5-92.7% of total administered while calculated recovery from representative samples was equal to 97.2-97.6% of total administered. A low recovery of Cr_2O_3 will result in an overestimate of DMO.

The third potential problem with Cr_2O_3 usage is large variation in recovery between animals. Smith and Reid (1955) found that Cr_2O_3 recovery varied from 71% to 108% of measured between animals. The 71% recovery appeared to be an outlier though as the next lowest observed recovery value was 95%. Stevenson et al. noted recoveries ranging from 90.2-98.1% between animals and Kiesling et al. (1969) observed variation between 54.4-85.5%.

Some of these errors can be avoided through the use of proper experimental procedures. Methods of Cr_2O_3 administration may affect error. Paper impregnated with the marker is thought to mix better in the rumen and allow for a more constant flow removing much diurnal variation (Nelson and Green, 1969). Administration of Cr_2O_3 in gelatin boluses can lead to regurgitation and loss of the bolus after dosing (Kiesling et al., 1969). Smith and Reid (1955) did not observe any differences in recovery when administering Cr_2O_3 either in a gelatin capsule or in a concentrate feed, but did note that the latter procedure was more difficult due to having to account for refusals. The same authors did not show any differences in recovery when administering the marker once per day or twice per day. Other possible sources of error in Cr_2O_3 estimation may occur during weighing doses and grinding fecal samples (Stevenson, 1962). Assay of chromic oxide powder found that the substance was 98.1% pure Cr_2O_3 meaning that the 10 g dose used only contained 9.81 g Cr_2O_3. During the grinding process it was suggested that the

dust lost from the machine may contain a higher proportion of Cr_2O_3 than the captured sample. A comparison of two different mills found that recovery was 2.66% lower when using the Christie and Norris model compared to a Wiley mill.

Some precautions should be taken when designing a trial using Cr_2O_3 as a marker. At least 3 days should be allowed between the beginning of dosing and the beginning to collection so that marker excretion can reach a plateau. After dosing, workers should ensure that boluses or administered material is not regurgitated and lost. Collection times should be planned to account for possible diurnal variation and the collection period should be long enough to account for daily variation in excretion. And lastly, for fecal samples, it may be appropriate to use a larger diameter screen when grinding to prevent dust loss and the associated decrease in marker recovery.

Conclusions

Phosphorus is an essential mineral for all living things. In cattle, the element is used for structural support, energy storage, the transfer of genetic information, and to supply the needs of rumen microbes. While a deficiency of the mineral will result in reduced growth and productivity, over supplementing P leads to excessive fecal excretion and unnecessary expense. Phosphorus absorption efficiency appears to be flexible and animals can adjust uptake in response to dietary surplus or shortage. Under high levels of P supplementation, absorption efficiency decreases and more of the mineral is lost to the environment. Excessive P losses into the watershed can result in eutrophication and a decrease in water quality. In some areas, forage P may be adequate to meet grazing animal needs and producers can reduce losses by determining available dietary P prior to supplementing with mineral P. However, little work has been done quantifying P balance

in grazing animals in the eastern U.S. due to the inherent difficulty in measuring this parameter in a pasture setting. The objective of this research was to examine P intake and excretion of grazing beef cattle receiving various levels of dicalcium phosphate.

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CHAPTER 3: PHOSPHORUS STATUS OF GRAZING BEEF CATTLE IN VIRGINIA

Abstract

Phosphorus (P) is an essential nutrient for beef cattle and is often supplemented in cattle mineral regardless of P available in feed and forage. In Virginia's Chesapeake Bay watershed, it is likely that forage P is adequate to supply the requirement of grazing cattle without further supplementation. A survey was conducted to characterize phosphorus (P) status of beef cattle farms in this area. Mineral tags, producer surveys, and fecal, forage, and soil samples were collected from beef cattle operations in the Bay watershed. Samples (n=166) were collected from 120 producers in 11 counties. Average farm size was 302 acres with 122 total beef cattle. Soil test P results were based on Virginia Cooperative Extension soil test guidelines. The farms sampled were characterized as low (12 %), medium (37 %), high (35 %), and very high (16 %) based on soil test P. Pasture grab samples contained 0.34 ± 0.12% P and forage P concentration increased ($P < 0.01$) across soil P categories going from low to very high. Fecal total phosphorus (TP) was lowly correlated ($R^2 = 0.15$, $P < 0.01$) to forage P concentration. Mineral supplements were categorized as nil (<1.0% P), low (1.0-<3.0% P), medium (3.0-<6.0% P), and high (>6.0% P). Fecal TP and inorganic phosphorus (Pi) concentration increased ($P < 0.01$) with mineral P levels. Fecal TP and Pi were lower ($P < 0.01$) when nil and low P mineral were supplemented as compared to medium and high P mineral. Soluble P (defined as fecal Pi/fecal TP*100) also increased ($P < 0.01$) with increasing mineral P content going from nil to high. All farms surveyed required little or no P supplementation in regard to beef cattle P requirements. The majority (82%) of producers were receptive to modifying mineral P supplementation practices based on forage P levels.

Introduction

Phosphorus is an essential element for all living things. In cattle, the mineral is mainly stored in the bones and teeth but is also required for energy metabolism and is a component of genetic material (Underwood, 1981; Ternouth, 1990; McDowell, 2003). Rumen microbes require P and a shortage will result in a reduction in fiber digestion in the stomach (Bryant et al., 1959). Phosphorus deficiency negatively affects growth and productivity of afflicted animals (Underwood, 1981). However, overfeeding of the mineral can be expensive and also poses a threat to watershed ecosystems. Excess P runoff can contribute to waterway eutrophication causing the death of aquatic species and a reduction in water quality (Sharpley et al., 1994).

Earlier studies conducted in arid rangeland environments suggested that P supplementation had the potential to greatly improve reproductive efficiency in cattle deficient in the mineral (Black, 1943). Subsequent recommendations influenced some cattleman to supplement high levels of P to grazing cattle in order to maintain the reproductive ability of the herd (Knowlton et al., 2004). More recent work has suggested that current NRC guidelines overestimate the P requirement of cattle. Research by Ternouth and Coates (1997) found that heifers grazing low P pastures in Australia were not deficient in the mineral and demonstrated P absorption efficiency over 80% which was significantly higher than the estimate of 68% used by the NRC. Similarly, Call et al. (1978) showed that heifers receiving low P diets (66% of NRC requirement) for 2 years had growth and reproductive rates similar to heifers receiving high P diets (174% of NRC requirements). Therefore, in more intensively managed pastures of the eastern U.S., forage P levels may be sufficient to meet the requirements of grazing cattle and supplying additional P in cattle mineral can lead to P intake exceeding animal requirements.

Cattle P absorption efficiency decreases and P excretion in the feces increases as supplementation of the mineral increases beyond requirements (Coates and Ternouth, 1992; Dou et al., 2002). Inorganic phosphorus (Pi) is the most potentially harmful form of P in feces due to its high water solubility (Sharpley and Moyer, 2000). Although in many feedstuffs, organic P (Po) is the predominant form; one study showed that 70% of the Po entering the animal can be converted to Pi by the action of rumen microbes. (Feng et al., 2015). Also, phosphorus is often added to animal diets as calcium phosphate, an inorganic form of the mineral (Maguire et al., 2007). Supplementing P in excess of requirements results in increased fecal P excretion without further improvements in growth or animal performance (Brokman et al., 2008). The objective of this study was to collect data from Virginia beef cattle operations located in the Chesapeake Bay watershed to benchmark current practices and document soil, forage, and fecal P levels.

Materials and Methods

Sample Collection

Samples were collected from May 2012 to September 2013. Soil, forage, and cattle fecal samples were obtained from 120 cattle producers in 11 counties located in Virginia's Chesapeake Bay watershed (Figure 3.1). Five soil cores were collected from a depth of 10-15 cm from sampled pastures and composited into a single sample following Virginia Cooperative Extension (VCE) guidelines. Five hand-plucked, fresh forage samples were obtained from pastures that housed cattle, separated from root material and soil, and composited in a collection bag. In cases where hay was fed, hay cores were taken from three bales and composited in collection bags. Each farm could submit up to three soil and forage/hay samples for analysis as long as soil and forage samples were

from pastures currently being grazed by beef cattle. Fecal samples were collected into 100 mL Whirl-Pak bags (Nasco, Fort Atkinson, WI) from at least two cattle in each sampled pasture immediately after defecation. Mineral tags were collected from the producer for mineral supplements being supplied to cattle at the time of sample collection.

Sample Analyses

Soil samples were analyzed for pH, micro, and macro minerals at the Virginia Tech Soil Testing Lab (Blacksburg, VA). Soil test P values were characterized as low (L), medium (M), high (H), or very high (VH) based on VCE soil test P categorizations (Table 3.1). Forage samples were analyzed for minerals via wet chemistry, and protein, fiber, non-structural carbohydrates, and energy via NIR procedure at Cumberland Valley Analytical Services (Hagerstown, MD). Fecal TP was determined using the molybdovanadate yellow method (AOAC, 1984) and fecal Pi was analyzed using the molybdenum blue method following extraction with 0.5 M hydrochloric acid (Murphy and Riley, 1962). For this study, soluble P was defined as fecal Pi/fecal TP. Mineral supplement P concentration (% of DM) was categorized as nil (<1.0%), low (1.0-<3.0%), medium (3.0-<6.0%), or high (≥6.0%).

Survey Administration

A 20 question survey (IRB approval # 11-568) was administered to all producers that submitted samples. Questions were designed to collect information about farm size and producer practices (Appendix 1). Surveys were administered during farm visits by extension personnel and sixty-eight surveys were completed and returned.

Statistical Analysis

All feed and forage data and survey responses were analyzed using SAS v. 9.3 (SAS Institute Inc., Cary, NC). Linear regression of soil P vs. forage P and forage P vs. fecal TP was conducted using PROC REG. Mean separation was conducted using LSMEANS with Tukey adjustment in PROC GLM. Mineral P category was included as the main effect. Outliers were detected using residual vs. predicted plots and removed from models. Chi-square analysis was conducted using PROC FREQ to examine differences in survey responses.

Results and Discussion

Sample Data

The percentage of pasture soil samples in each VCE soil test P category were 12% (L), 37% (M), 35% (H), and 16% (VH). The state averages for soil test P over the same period were 30% (L), 35% (M), 26% (H), and 9% (VH). Due to the increased land application of manure and fertilizer, it is estimated that soil P concentrations have increased approximately 25% since 1950 (Carpenter et al., 1998). High soil P levels can contribute to environmental P losses as erosion carries soil particles into waterways (Sharpley et al., 1994).

Linear regression revealed that forage P concentrations (mean = 0.34%, SD = 0.12%) were lowly related ($R^2 = 0.18$; $P < 0.01$) to soil P concentration (Figure 3.2). Haan et al. (2007) noted that forage P content was affected by plant species, grazing pressure, and stage of maturity in addition to soil P. The low correlation observed between soil P and forage P in this study was probably due to a combination of these factors as management and environment varied greatly between sampled farms. Mean forage P concentration increased ($P < 0.01$) across soil P categories (0.23, 0.32, 0.37, and

0.42% ± 0.02% for L, M, H, and VH respectively). When compared to NRC P requirements for a 545-kg beef cow (NRC, 1996), 100% of fresh forage samples met a dry cow's requirement, 99% were sufficient through late gestation, and 92% met requirements during peak lactation (13.6 kg milk/d) (Figure 3.2).

Fecal TP was lowly related ($R^2 = 0.18$, $P < 0.0001$) to forage P (Figure 3.3). Variations in mineral P intake, may have accounted for the low correlation between fecal TP and forage P. When farms feeding medium and high P minerals were removed from the dataset, there was no improvement in the results ($y = 0.8539x + 0.0036$, $R^2 = 0.17$, $P < 0.0001$). This suggests that unaccounted differences in total forage consumption, sampling times and procedures, forage species, and feed supplementation may have also contributed to variation in P excretion. Fecal TP and Pi increased linearly ($P < 0.01$) across mineral P categories going from nil to high (Figure 3.4). This is in agreement with Brokman et al. (2008) who reported fecal P excretion increased as additional P was supplemented in the form of dicalcium phosphate to grazing Holstein steers. Soluble P excreted in the feces also increased linearly ($P < 0.01$) across mineral P levels due to the increase in fecal Pi. Dou et al. (2002) also stated that water soluble P and acid soluble P increased linearly as more calcium phosphate was added to the diet of dairy cattle. While both organic P and Pi in cattle feces are water soluble, Pi is of more concern as 81% of this form is water soluble compared to only 30% of Po (Sharpley and Moyer, 2000). Dietary modification for more efficient P feeding has the potential to reduce P losses to the environment without reducing animal production (Maguire et al., 2007).

Survey Results

Average farm size was 302 acres and ranged from 5 to 1750 acres. Herd size averaged 122 beef cattle and ranged from 6 to 650 head. The average herd consisted of

70 beef cows, 14 replacement heifers, and 38 stockers and bulls (nursing calves excluded). The farm and herd size exceeds the state average of 34 beef cows per farm (USDA-National Agricultural Statistics Service, 2012) mostly likely due to the inclusion of the Shenandoah Valley, where beef operations are larger, in the sample area.

In the past year, 51% of producers applied fertilizer to pastures and 61% applied fertilizer to crop fields. Animal manure was the most popular form of fertilizer and was used in 68% of all fields (pasture and cropland) which had received nutrient application. Cattle manure has an N:P ratio lower than most crop requirements but historically has been applied only to meet N requirements of a crop (Knowlton et al., 2004). Also, in areas with large numbers of confined animal feeding operations, manure is often over-applied as available nutrients exceed available land area and manure is expensive to transport long distances (Carpenter et al., 1998). This often results in a buildup of P in soils that consistently receive manure applications. The average soil P content of 51% of the farms sampled in this study was either high or very high.

Fifty-five percent of surveyed producers have implemented a nutrient management plan (NMP) on their farm. Nutrient management plans focus on the assessment of farm nutrient inventory and crop requirements to better allot manure and fertilizer to available acreage (Beegle et al., 2000). The goal of NMPs is to reduce agricultural nutrient losses to the environment. Only 25% of operators sampled forage for nutrient content; however, of those that did forage test, the majority (94%; χ^2=14.5; $P <$ 0.01) also utilized a NMP.

Sixty-nine percent of participants supplemented a commercial complete mineral mix, 22% fed loose trace mineral salt, 6% used a trace mineral block, and the remaining

3% supplied a home mineral mixture. Producers were asked to rank their top three factors for selecting a cattle mineral in order of importance. Responses were weighted based on ordinal ranking (3 for primary, 2 for secondary, and 1 for tertiary criteria) and sorted by percentage of total points attained. Interpretation of response criterion suggested that the primary factor in mineral selection was price (20.6%), followed by local availability (17.8%), and trace mineral content (17.5%). Ca/P content was ranked 6th (6.7%) out of the 8 choices. While P content was not an important factor in selecting a mineral supplement, 82% of operators indicated willingness to reduce mineral P supplementation if nutrient analysis revealed that feed and forage resources were capable of meeting herd P requirements.

Implications

Phosphorus is one of the most expensive components of cattle mineral by weight. In the majority of sampled Virginia farms in the Chesapeake Bay watershed, forage P content was adequate to supply the needs of grazing cattle without additional mineral P supplementation. Exceptions may occur during peak lactation or when lower quality forage is fed although a short term P deficiency can be buffered by utilizing bone reserves. Therefore, producers should sample P content of available forages in order to avoid P over supplementation and added operating expenses. Producers could also limit expenses by adjusting fertilizer usage to reduce P application when adequate soil P is available rather than fertilizing solely to meet soil nitrogen requirements. Taking these steps could also limit P losses to the environment from soil runoff and cattle fecal P excretion and reduce the risk of P enriched runoff contributing to the P TMDL of the Chesapeake Bay watershed.

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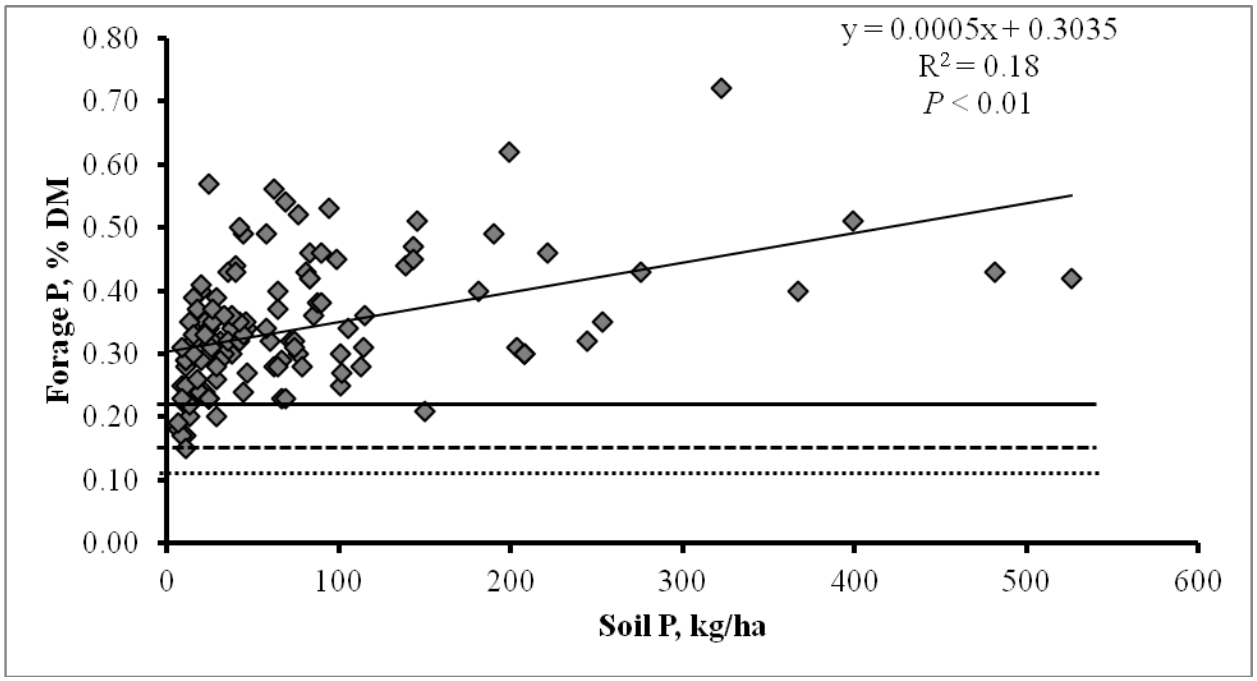
Table 3.1 Virginia Cooperative Extension soil test phosphorus (P) categories

	Low	Medium	High	Very High
Soil P (ppm)	0-5	6-17	18-55	>55

Figure 3.1 Map showing sample area and the Chesapeake Bay watershed

Figure 3.2 Relationship between soil phosphorus (P) concentration and forage P concentration in sampled pastures in Virginia's Chesapeake Bay watershed



Peak Lactation Requirement[1]

Late Gestation Requirement[1]

Dry Cow Requirement[1]

[1] Requirements based on NRC values for a 545 kg beef cow producing 13.6 kg milk/d during peak lactation (NRC, 1996)

Figure 3.3 Relationship between forage phosphorus (P) concentration and fecal P concentration in grazing beef cattle in Virginia's Chesapeake Bay watershed

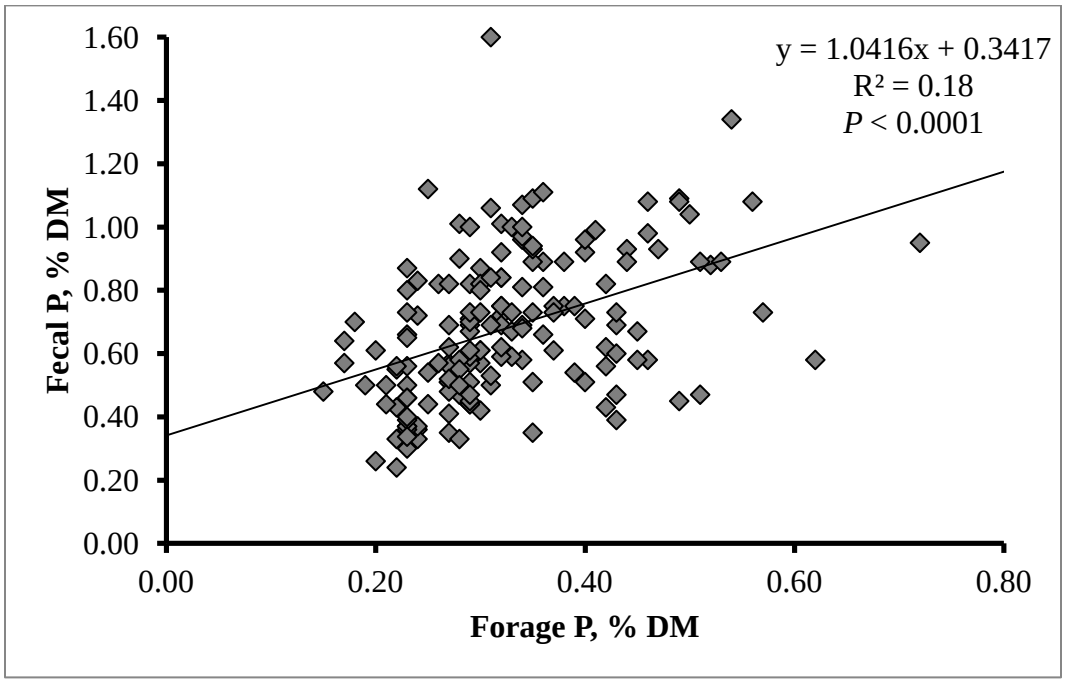
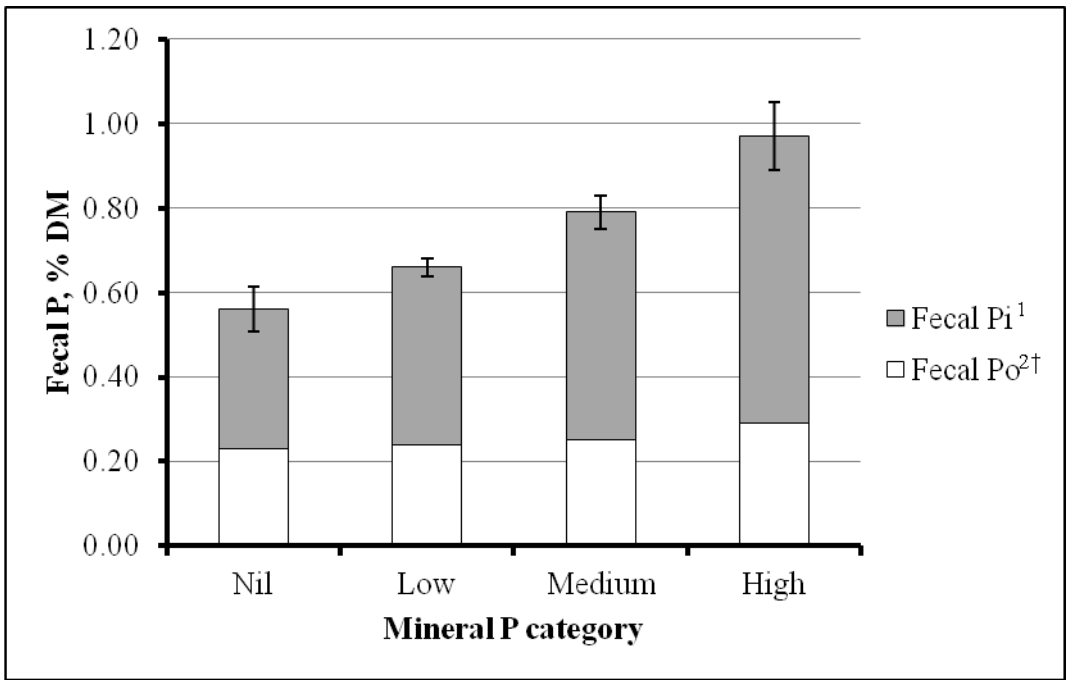


Figure 3.4 Effect of mineral P content (0.0 to <0.9% = nil; 1.0 to <3.0% = low; 3.0 to <6.0% = medium; >6.0% = high) on fecal phosphorus (P) fractions of grazing beef cattle in Virginia's Chesapeake Bay watershed



Fecal TP and Pi increased linearly ($P < 0.01$) as mineral P category increased from nil to high

[1]Inorganic phosphorus

[2]Organic phosphorus

†Fecal Po = Fecal TP - Fecal Pi

Appendix 3.1 Virginia beef cattle phosphorus survey with averages of all responses

1. How many head of beef cattle are at your farm today for each of the following categories?

Beef cows (that have calved during the past year)	70 head
Replacement heifers (weaned-pregnant)	14 head
Other beef cattle (stockers, bulls)	38 head

2. Acreage committed to: (estimates)

Pasture only	205 acres
Hay only	41 acres
Crop/grazing	2 acres
Hay + Pasture	48 acres
Silage	6 acres

3. What is your calving season?

Fall (Sep- Dec)	21%
Spring (Jan- Apr)	31%
Fall & spring	31%
No defined season	17%

4. What is the primary forage for the cow herd in the winter months?

Hay	73%
Silage/baleage	10%
Pasture	14%
Other	3%

5. On average, how many days annually do you plan to feed harvested feeds to your cows?

127 days/year

6. Do you sample your harvested feeds for a forage analysis?

Yes	75%
No	25%

7. Do you purchase any harvested feed for the cow herd?

Yes	76%
No	24%
If yes, what% of herd's need is met with purchased forage	40%

8. Have you fertilized any pastures during the past twelve months?

Yes	51%
No	49%
If yes; to what % of pastures were nutrients applied	48%

What was the nutrient source?

19%	Commercial fertilizer	164 lbs/acre
68%	Manure application	2985 lbs/acre
13%	Biosolid application	8000 lbs/acre

9. Have you fertilized any hayfields or croplands from which you harvest forage for winter feed during the past twelve months?

Yes	61%
No	39%

If yes; to what % of hay/crop fields were nutrients applied 89%

What was the nutrient source?

26%	Commercial fertilizer	171 lbs/acre
61%	Manure application	2100 lbs/acre
13%	Biosolid application	8000 lbs/acre

10. Which supplement feeding practice best describes your management?

No supplemental feeding	27%
Corn or other grain	12%
Corn gluten feed	5%
Soyhulls	2%
Protein block	16%
Liquid feed	3%
Commercial supplement	15%
Commodity pellet	16%
Other supplement/home mix	4%

11. How much supplement do you typically provide to your cow herd (lbs/cow) on an annual basis?

388 lbs/cow/year

12. What is your mineral supplement for the cow herd?

Plain salt block	0%
Loose white salt	0%
Home mineral mixture	6%
Trace mineral salt block	3%
Loose trace mineral salt	15%
Commercial complete mineral mix	67%

13. Do you provide a high magnesium (>10%) mineral mix to the cow herd at any time?

Yes	80%
No	20%
If yes # months fed	9.5

14. How do you store purchased feed?

Commodity shed	10%
Bags	57%
Grain bin	17%
Other	16%

15. Who is your primary source of nutrition advice?

Neighbors/other cattlemen	9%
NRCS personnel	2%
Extension agent/specialist	42%
Local cooperative/feed store	25%
Veterinarian	8%
Other	14%

16. Please rank the **top three** factors in selecting a free-choice mineral

	Ranking by importance		
Factor	1st	2nd	3rd
Local availability	14	8	6
Past experience	12	6	13
Palatability	7	6	7
Price	8	20	10
Ca/P content	5	2	5
Trace mineral content	13	9	6
Vitamin content	0	7	10
Ionophore/antibiotic inclusion	1	2	3

17. Source of purchased mineral supplements.

Local cooperative/feed store	77%
Dealer/company representative	18%
Local Cattlemen's association	2%
Other	3%

18. Does your farm have a formal nutrient management plan?

Yes	55%
No	45%
If yes, how many years?	9.5 years

19. Have you implemented any of the following management practices? (Note: Producers were asked to select all that applied, percentages are not cumulative.)

Stream exclusion	52%
Alternate water sources	61%
Stream crossing	38%
Unroll hay when feeding	75%
Tarp or barn storage rolls of hay	66%

Rotational grazing	62%
Stockpiling fescue	49%
Winter feeding areas	49%
Stream/riparian installation	41%
Addition of clovers to pastures	64%

20. If forage analysis revealed that feed and forage adequately met the cow phosphorus requirement, would you feel comfortable in reducing the P content of the minerals you provide?

Yes	82%
No	3%
Maybe	15%

CHAPTER 4: EFFECT OF DICALCIUM PHOSPHATE SUPPLEMENTATION ON PHOSPHORUS EXCRETION IN GRAZING BEEF CATTLE

Abstract

Reducing phosphorus (P) supplementation to grazing beef cattle has the potential to reduce fecal P losses to the environment. A 56-d study was conducted with eight yearling Hereford steers (261±30 kg) grazing cool-season fall re-growth to determine the effects of varying levels of P supplementation on fecal P excretion. Treatments consisted of dicalcium phosphate supplemented at 0 (D1), 10.0 (D2), 20.0 (D3), or 30.0 (D4) g/d in a randomized 4x4 replicated Latin square design. These treatments provided an additional 0, 1.9, 3.7, and 5.6 g/d of P respectively. Two esophageally cannulated steers were used to collect forage samples for nutrient analysis. Forage P content was analyzed from hand collected samples. Forage P concentrations averaged 0.49% DM across all periods. Chromic oxide (Cr_2O_3) was administered twice daily via gelatin capsule at 0630 and 1830 to serve as an external marker for determination of fecal dry matter excretion (DME). Indigestible NDF (iNDF) was used as an internal marker to determine DMI. Due to the high forage P content, average P intake was in excess of NRC requirements for all diets (D1 = 203%; D2 = 221%; D3 = 223%; D4 =244%). Orthogonal contrasts were performed to assess the relationship between treatment and P excretion. A linear response ($P < 0.0001$) in daily inorganic P (Pi) excretion (15.5, 15.3, 18.0, and 18.4 g/d ± 0.69 for D1, D2, D3 and D4, respectively) was observed across treatments. Daily total P (TP) excretion increased linearly ($P < 0.01$) across treatments (23.1, 23.2, 26.8 and 27.2 g/d ± 0.89 for D1, D2, D3 and D4, respectively. There was no difference ($P > 0.20$) in dry matter digestibility (DMD), CP digestibility, NDF digestibility, or ADF digestibility between treatments. When forage P content is sufficient to meet the requirement of

grazing cattle, increasing P supplementation results in greater P excretion without additional benefits to growth or nutrient digestibility.

Introduction

Phosphorus (P) is an essential element for both plants and animals (Haan et al., 2007). In cattle, the majority of the mineral is stored in the bones and teeth. Phosphorus also supports cell membranes, is a component of genetic material and plays a role in energy metabolism (Underwood, 1981; Ternouth, 1990; McDowell, 2003). Rumen microbes require P and a shortage will result in a reduction in fiber digestion in the stomach (Bryant et al., 1959). Phosphorus deficiency negatively effects growth and productivity of afflicted animals (Underwood, 1981). However, overfeeding of the mineral can be expensive and also poses a threat to watershed ecosystems. Excess P runoff enters into the watershed, contributing to a rise in algae growth. In turn, algal blooms consume oxygen needed by other organisms leading to death and a reduction in water quality through a process known as eutrophication. This process results in the death of aquatic organisms and a decrease in water quality (Sharpley et al., 1994).

In cattle, studies have shown that P absorption efficiency decreases and P excretion in the feces increases as supplementation of the mineral exceeds animal requirements. (Coates and Ternouth, 1992; Dou et al., 2002). However, little work has been conducted in a pasture setting due to the associated difficulties in sample collection. Inorganic phosphorus (Pi) is the most potentially harmful form of P in manure because it is highly water soluble (Sharpley and Moyer, 2000). Phosphorus is often added to cattle diets in the form of calcium phosphate, an inorganic form of the mineral (Maguire et al., 2007). It is likely that supplementing P beyond requirements will result in the increased

excretion of water soluble P in the feces without further increasing growth or animal performance (Feng et al., 2015). The goal of this study was to quantify P losses from beef cattle grazing pasture and receiving varying levels of a mineral supplement consisting of dicalcium phosphate.

Materials and Methods

Experimental Design

All animal handling and care procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee (# 14-096 and # 14-131). Eight yearling Hereford steers (initial BW=261±30 kg) were used in a 4x4 replicated Latin square design to evaluate the effects of supplementing dicalcium phosphate on fecal P parameters and nutrient digestibility in growing beef cattle consuming fall cool-season forage re-growth. The 56-d trial consisted of four 14-d periods divided into 9-d adaptation period followed by a 5-d collection period (Feng et al., 2015). Eight grams of chromic oxide ($Cr_2O_{3)}$ was administered twice daily via gelatin capsule at 0630 and 1830 h to serve as an external marker and iNDF was utilized as an internal marker. Steers were housed in a 3.2 ha pasture with soil containing 55 ppm P. Two esophageally cannulated steers were used to estimate nutrient content of grazed forage. Due to the high P content of saliva, forage P content was determined from hand-plucked forage samples. Fecal samples were collected at 12-h intervals throughout the collection period that were staggered 2.5-h daily to account for diurnal variation in marker flow.

Treatments

Treatment supplements consisted of one of four levels of dicalcium phosphate; 0.0 (D1), 10.0 (D2), 20.0 (D3) and 30.0 (D4) g/d, which contained 0, 1.9, 3.7, and 5.6 g/d

of P respectively. Supplements were offered with 0.25 kg/d of dried beet pulp (Midwest Agri-Commodities, San Rafael, CA) and fed at 0630 and 1830 h daily. All steers were allowed to graze cool-season pasture consisting primarily of fescue and orchardgrass. A trace mineral supplement (Champion's Choice Selenium 90, 57% Cl, 37% Na, 0.35% Zn, 0.2% Mn, 0.2% Fe, 0.03% Cu, 0.009% Se, 0.007% I, 0.005% Co, Cargill Inc., Minneapolis, MN) containing no P was provided ad libitum throughout the trial. Forage nutrient composition for each period can be found in Table 4.1.

Sample Collection

Prior to the initiation of the study, all steers were halter broken to facilitate handling during sample collection. Fecal grab samples were collected twice per 24-h period. Collections occurred at 0030, 0300, 0530, 0730, 1000, 1230, 1500, 1730, 1930, and 2200 h across the 5 d period. Samples were weighed and placed in a 55º C forced draft oven (Thermo Scientific Precision 645, Danville, IN) within 2-h of collection and allowed to dry for 5 d to determine percent DM. Dried fecal samples were ground through a 2-mm screen using a Wiley Mill (Arthur H. Thomas, Philadelphia, PA) and then composited by pooling an equal weight of ground sample from each collection time within a period. A single urine sample was collected from each steer during the last 2 d of each period and stored at -20º C until analysis.

Three forage samples were taken on alternate days during the collection period at 0900 h and immediately placed on ice. One allotment of samples were weighed and placed in a 55º C forced draft oven (Thermo Scientific Precision 645, Danville, IN) within 2-h of collection and allowed to dry for 5 d to determine percent DM. Forage samples were ground through a 2-mm screen using a Wiley Mill (Arthur H. Thomas, Philadelphia, PA) and then composited by pooling an equal weight of ground sample

from each collection time within a period. The second allotment of forage samples were freeze dried for fiber analysis. A 25 g aliquot of beet pulp was collected each day of the collection period and composited. The composited sample was then dried and ground in the same manner as forage samples.

Blood was collected via jugular venipuncture using 10 mL vacutainers (Becton, Dickinson and Company, Franklin Lakes, New Jersey) containing sodium heparin. Samples were placed on ice after collection and centrifuged at 1,850 x g for 15 min at 4º C. Plasma was collected and stored at -20º C for later analysis. Steers were weighed on day 5 of each collection period prior to blood collection.

Laboratory Analyses

Dried and ground forage, beet pulp, and fecal samples were analyzed in duplicate for total P using the molybdovanadate yellow method (AOAC, 1984). All samples were analyzed in duplicate for Pi by extraction with 0.5 M hydrochloric acid followed by the molybdenum blue method (Murphy and Riley, 1962). Blood plasma samples were deproteinated and analyzed for Pi using the molybdate blue method (Miles et al., 2001).

All samples were weighed into crucibles and dried overnight in a 100º C oven. Dried samples were placed in a desiccator for 20 min and weighed back to determine micro dry matter content (AOAC, 2000). Samples were then placed in a 500º C muffle furnace for 2 h to determine ash content (AOAC, 2000). Crude protein content of forage and fecal samples was determined using a carbon/nitrogen analyzer (CHNOS Elemental Analyzer, Hanau, Germany) (AOAC, 2000). Forage and fecal samples were weighed into filter bags (ANKOM Technology, Macedon, NY) and neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using an ANKOM^{200} fiber analyzer (AOAC, 2000; Van Soest et al., 1991).

Marker Analysis

Forage and fecal samples were prepared for Cr_2O_3 analysis using the method of Divakaran et al. (2002). Digested samples were diluted to 35 mL and analyzed for Cr by inductively coupled plasma-atomic emission spectroscopy (Franson, 1998) using the Spectro ARCOS ICP Model FHS16 with CETAC Auto Sampler (Spectro Analytical Instuments Inc., Mahwah, NJ). Indigestible NDF was determined by weighing 5.0 g of each forage and fecal sample into 5x10 cm Dacron bags (ANKOM Technology, Macedon, NY). Duplicate samples were incubated in the rumen of two cannulated cows for 288 h. At the end of rumen incubation, samples were rinsed in cold water (30 min) in a household washing machine, dried at 55ºC in a forced draft oven (Thermo Scientific Precision 645, Danville, IN) for 48-h and then weighed. Residues were analyzed for NDF.

Calculations

Daily DME was estimated using the equation:

DME (kg) = Cr_2O_3 dose (g/d) / fecal Cr_2O_3 concentration

Indigestible NDF was estimated using the equation:

iNDF = post incubation NDF recovery / pre-incubation DM

Daily DMI was estimated using the equation:

DMI (kg) = [fecal iNDF (%) * DME (kg)] / forage iNDF (%)

Statistical Analysis

Data analysis was performed using SAS v. 9.3 (SAS Institute Inc., Cary, NC). Means separation was conducted using the MIXED procedure. The statistical model was:

$$Y_{ijkl} = \mu + Sq_i + Pd_j + A_k(Sq_i) + T_l + \varepsilon_{ijkl}$$

where

Y_{ijkl} = dependent variable

μ = overall mean

Sq_i = fixed effect of Latin Square

Pd_j = fixed effect of period

$A_k(Sq_i)$ = fixed effect of steer nested within Latin Square

T_l = treatment effect

ε_{ijkl} = residual error

Pre-planned contrasts were used to evaluate linear, cubic, and quadratic treatment effects. Outliers, determined as being greater than 1.5 times the interquartile range, were removed. Results are reported as least squares means, with the largest SEM reported where unequal observations were analyzed. Effects were considered significant at $P < 0.05$. Regression analysis was also conducted in PROC MIXED to examine the effects of estimated P intake on the estimated excretion of P. The results from the regression analysis were not different from the mixed model and therefore the regression model was only used to determine the slopes of examined parameters. However, the regression model accounted for period effects that were significant in the mixed model due to P intake being used as the dependent variable as opposed to treatment. Treatment by period interactions were tested in the mixed model and were found to not be significant. Regression analysis was performed using the REG procedure. Residuals plotted against predicted values were used to check the model for homoscedasticity and remove outliers.

Results and Discussion

Diurnal variation in chromic oxide recovery

Previous work examining the usage of Cr_2O_3 as an external marker has noted significant diurnal variation in marker recovery (Smith and Reid, 1955; Hopper et al., 1988). To overcome this problem, fecal sample collection times were delayed 2.5 h each day to provide representative 24 h sampling. Smith and Reid (1955) suggested that sampling at 0600 h and 1600 h would provide samples which represented average daily marker output. To save time and expense, two steers which had fecal samples for every collection time point through all four periods were selected and Cr_2O_3 analysis was conducted on the non-composited samples from those animals. Average estimated fecal DME from the two steers based on marker recovery was 2.07 kg/d with a maximum observed value of 2.66 kg/d at 1000 h and a minimum observed value of 1.92 kg/d at 1730 h. Estimated values from all time points are shown in Figure 4.1. Fecal DME estimates observed at 0530 and 1500, nearest the sampling time points suggested by Smith and Reid, were 2.05 kg/d and 2.11 kg/d, and the average of the two points was almost identical to the observed average from all points (2.08 kg/d vs. 2.07 kg/d). Based on this, future research using chromic oxide as an external marker could utilize sampling at or near 0600 and 1600 to reduce time and labor inputs.

Nutrient digestibility

Digestibility of DM, CP, NDF, and ADF were not different ($P > 0.20$) among treatments. Ash digestibility tended ($P < 0.10$) to be different between treatments D2 and D3, leading to a cubic effect ($P < 0.05$) of treatment to be observed (Table 4.2). Due to the high SEM it is unlikely that this result had any biological significance and probably varied due to soil ingestion during grazing. Komisarczuk et al. (1987) found that rumen microbial growth and nutrient digestibility was normal at rumen P concentrations above

100 mg/L. Since none of the diets in this study were deficient in P, a reduction in nutrient digestibility was not expected.

Phosphorus intake

Dry matter intake and forage P intake (Table 4.3) were not different (P > 0.20) between treatments. Studies have indicated that DMI is depressed on low P diets (Call et al., 1986, Morse et al., 1992). In this study, total P intake across treatments equaled 203%, 221%, 223%, and 244% of the requirement for a 300 kg steer gaining 1.0 kg/d (NRC, 1996) and no reduction in DMI was observed. Total P intakes were 32.4, 35.4, 35.6, and 39.1 g/d and increased linearly ($P < 0.0001$) across treatments. Inorganic P intakes (Figure 4.2) were 16.0, 18.1, 19.1, and 21.7 g/d and also increased linearly ($P < 0.0001$) due to the addition of dicalcium phosphate.

Phosphorus digestibility and excretion

True P digestibility is difficult to measure due to the addition of endogenous salivary P to the diet (Bravo et al., 2003a; McDowell, 2003). Apparent digestibility, which does not account for endogenous losses, has been shown to decrease as dietary P intake increases (Knowlton and Herbein, 2002). In this study apparent P digestibility was not different between treatments and averaged 32%. Phosphorus intakes in this study were only 21% higher on D4 than D1while in the Knowlton and Herbein trial there was a 91% increase in P intake from the low to the high diet. The low range of observed P intakes most likely played a role in the lack of response in apparent P digestibility. In cattle, Pi is the only form available for absorption and rumen microbes convert Po to Pi (Bravo et al., 2003a). Therefore, measuring Po digestibility should indicate the efficiency at which rumen microbes are able to convert Po to Pi. In this study, organic P digestibility

(Table 4.4) was not different ($P > 0.20$) between treatments. Once again this lack of response may have been due to the low range of P intakes observed in this study.

Diet means for fecal P excretion are presented in Table 4.4. Fecal total phosphorus (TP) and Pi excretion increased linearly ($P < 0.01$) across treatments. Linear regression suggests a strong positive relationship ($R^2 = 0.77$, $P < 0.0001$) between TP intake and fecal TP excretion (Figure 4.3). Based on the slope of this regression, apparent digestibility of TP was 65%. This is similar to the assumption of 68% used by the NRC for its calculations. However, Coates and Ternouth (1992) found that at higher P intakes, P digestibility was reduced. Phosphorus intake in this trial was over twice the NRC requirement for all treatments. There was a similar trend ($R^2 = 0.67$, $P < 0.0001$) between TP intake and fecal Pi excretion (Figure 4.4). This is in agreement with other studies which reported increasing P intake resulted in increased fecal P (Call et al., 1978; Morse et al., 1992; Knowlton et al., 2004).

In the current study, blood plasma P level was unrelated to treatment (Table 4.4). While other authors have demonstrated a direct link between P supply and blood P concentration (Challa et al., 1989; Ternouth and Coates, 1997), the high P intakes observed in this study resulted in elevated plasma P (2.2 mmol/L). Challa et al. (1989) reported that the renal threshold for P re-absorption is near 2.3 mmol/L. A subsequent study suggested that this threshold was closer to 2.7 mmol/L (Care et al., 1994). In the current trial, plasma P approached or exceeded the stated threshold for P re-absorption and it is highly likely that as a result, excess P was lost in the urine. Urinary P concentrations were measured but total urinary P excretion was not due to the difficulty of performing total urine collection in a pasture setting. Urinary P concentration (Table

4.4) was not affected by diet ($P > 0.20$) but steer nested within square had a significant effect ($P < 0.05$) on this parameter indicating there was more variation in urinary P between steers than between treatments. Betteridge and Andrewes (1986) noted considerable variation between urinary P excretion in individual steers and also observed diurnal variation in urinary P concentration. Therefore, it is likely that spot urine samples have limited value as an estimator of total urinary P excretion.

Conclusions

In many cases, forage P may be adequate to supply the needs of grazing cattle without additional P supplementation. When requirements are met by available dietary P, supplementation of dicalcium phosphate results in increased excretion of fecal TP and Pi and raises the risk of P runoff entering the watershed. Producers should sample forage and feed to determine available P before supplementing minerals containing the element.

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Table 4.1 Nutrient composition of pasture consumed by grazing steers supplemented with increasing levels of dicalcium phosphate

	Period 1	Period 2	Period 3	Period 4
DM, %	16.0	16.0	18.1	17.1
TDN†, %	62.4	60.8	59.8	61.2
NDF, %	52.7	55.2	60.0	55.0
ADF, %	33.4	36.1	38.8	35.4
CP, %	25.9	24.5	22.8	22.7
Ash, %	12.6	12.3	12.8	12.8
Ca, %	0.52	0.55	0.57	0.59
P, %	0.50	0.50	0.50	0.47

†TDN calculated by the equation: CP + nonfiber carbohydrates (NFC) + ether extract (EE) + NDF – metabolic fecal TDN

Table 4.2 Nutrient digestibility of grazing steers supplemented dicalcium phosphate at 0 g/d (D1), 1.9 g/d (D2), 3.7 g/d (D3), or 5.6 g/d (D4)

	D1	D2	D3	D4	SEM	Linear	Quadratic	Cubic
DM, %	67.0	68.9	67.0	67.4	0.84	0.8329	0.3308	0.0638
NDF, %	73.1	73.7	72.4	73.4	0.55	0.9249	0.6940	0.1130
ADF, %	73.1	73.5	72.0	72.7	0.66	0.3166	0.8222	0.1271
CP, %	70.0	71.3	70.2	70.6	0.73	0.8362	0.5557	0.2335
Ash, %	19.2	26.1	15.0	22.3	3.07	0.9059	0.9534	0.0160

Table 4.3 Dry matter, forage phosphorus (P), total phosphorus (TP), inorganic phosphorus (Pi), and organic phosphorus (Po) intake of grazing steers fed dicalcium phosphate at 0 g/d (D1), 1.9 g/d (D2), 3.7 g/d (D3), or 5.6 g/d (D4)

	Treatment					*P*-values		
	D1	D2	D3	D4	SEM	Linear	Quadratic	Cubic
DMI, kg/d	6.8	7.0	6.7	7.0	0.24	0.7509	0.8294	0.2602
Forage P intake, g/d	32.1	33.4	31.7	33.6	1.18	0.6036	0.7836	0.2288
Pi intake, g/d	16.0*	18.1	19.1	21.7	0.59	<0.0001	0.6470	0.2907
Po† intake, g/d	16.7	17.3	16.5	17.4	0.61	0.6974	0.7977	0.2653
TP intake, g/d	32.4	35.4	35.6	39.1	1.15	0.0010	0.8458	0.2522

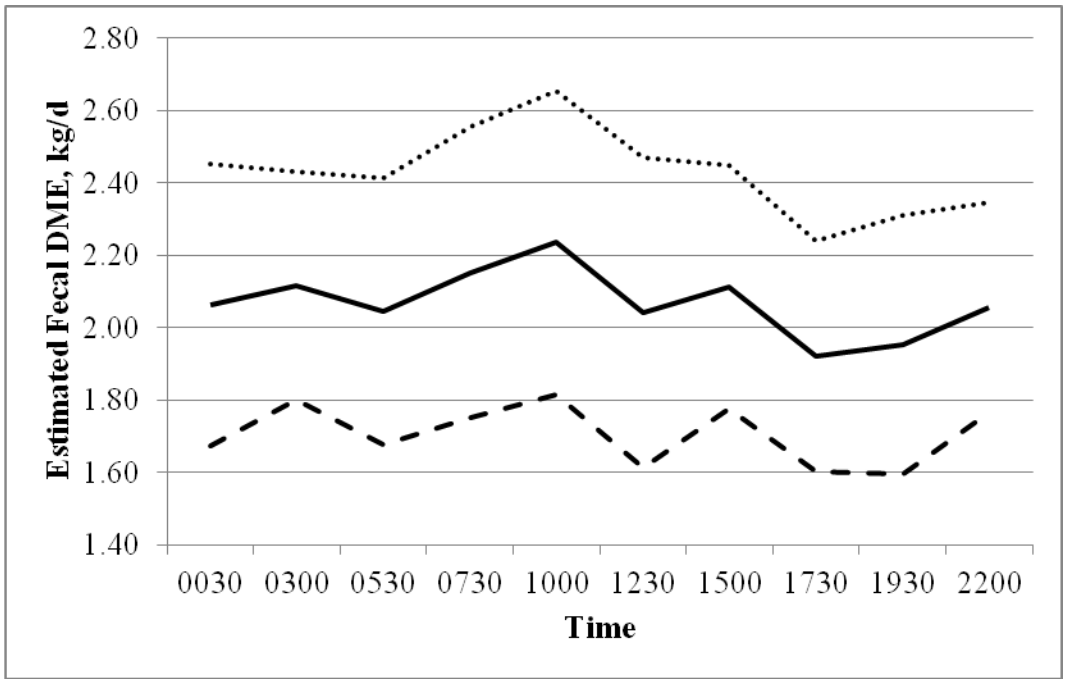
*outlier removed
†Po = TP – Pi

Table 4.4 Least squared treatment means for phosphorus (P) digestibility, excretion, and blood plasma P of grazing steers fed dicalcium phosphate at 0 g/d (D1), 1.9 g/d (D2), 3.7 g/d (D3), or 5.6 g/d (D4)

	Treatment					*P*-values		
	D1	D2	D3	D4	SEM	Linear	Quadratic	Cubic
Apparent P digestibility, %	38.1	34.8	34.3	37.0	3.43	0.8170	0.3930	0.9663
Po[1†] digestibility, %	51.7	54.4	49.3[*]	54.1	2.29	0.8138	0.6251	0.0900
Pi[2] excretion, g/d	15.5	15.3	18.0	18.4	0.69	0.0018	0.6954	0.1120
Po excretion, g/d	7.6	7.9	8.8	8.8	0.40	0.0189	0.7108	0.4085
TP[3] excretion, g/d	23.1	23.2	26.8	27.2	0.89	0.0008	0.8893	0.1111
Plasma Pi, mg/dL	6.3	7.0	7.2	6.8	0.41	0.3122	0.2035	0.8836
Urine P, mg/L	31.1	53.5	35.2	108.5	28.9	0.1157	0.3906	0.3197

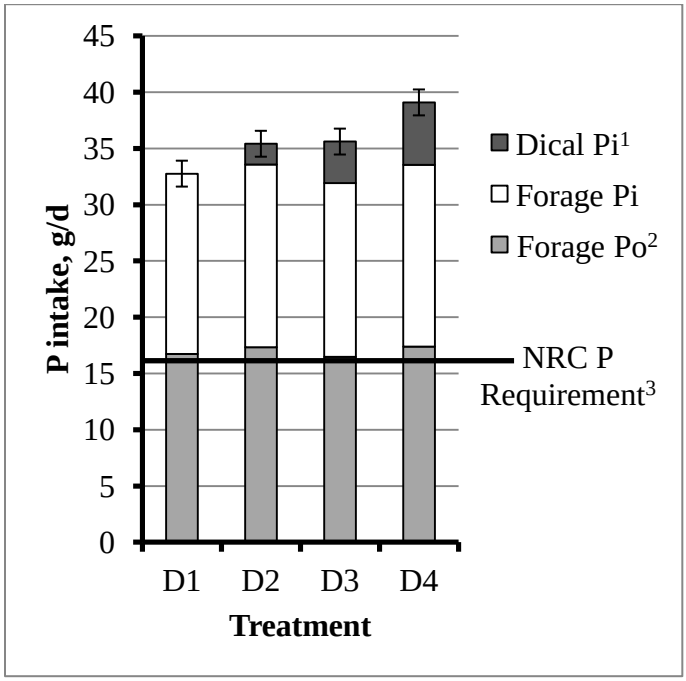
[1]Organic phosphorus
[2]Inorganic phosphorus
[3]Total phosphorus
[*]outlier removed
†Po = TP - Pi

Figure 4.1 Estimated fecal dry matter excretion (DME) of grazing steers based on chromic oxide recovery at selected time points



Steer 1
Steer 2
Average of steers 1 and 2

Figure 4.2 Phosphorus (P) intake and fractions of grazing steers supplemented with dicalcium phosphate at 0 g/d (D1), 1.9 g/d (D2), 3.7 g/d (D3), or 5.6 g/d (D4) relative to NRC requirements



Total P intake was linear ($P < 0.0001$) across treatments
Inorganic P intake was linear ($P < 0.0001$) across treatments
[1]Inorganic phosphorus
[2]Organic phosphorus
[3]300 kg steer gaining 1.0 kg/d (NRC 1996)
†Forage Po = Forage TP – Forage Pi

Figure 4.3 Relationship between total phosphorus (TP) intake and fecal TP excretion in grazing steers fed increasing levels of dicalcium phosphate

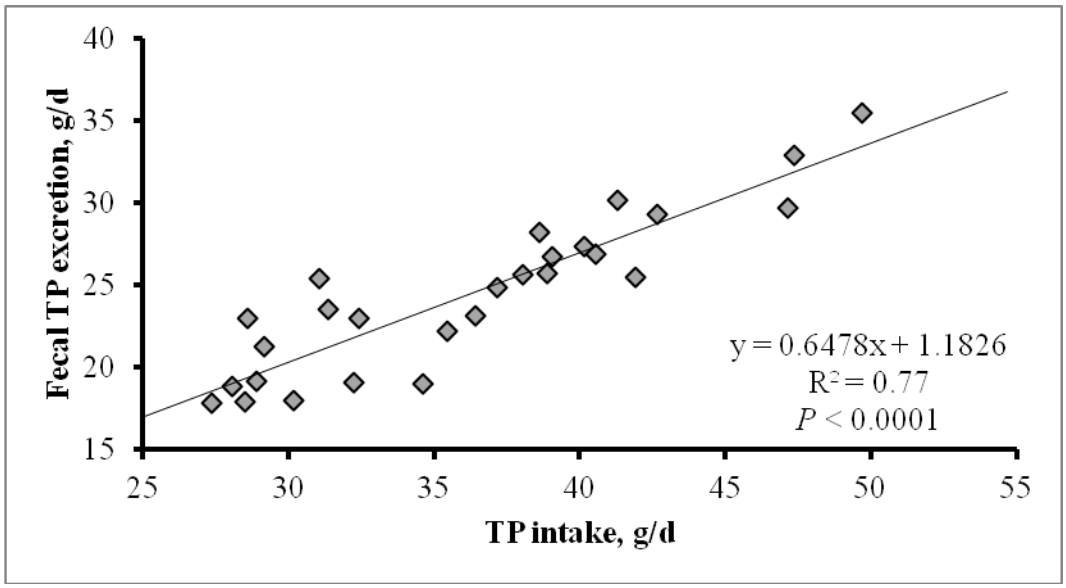


Figure 4.4 Relationship between total phosphorus (TP) intake and fecal inorganic phosphorus (Pi) excretion in grazing steers fed increasing levels of dicalcium phosphate