

**Phosphorus Excretion in Beef Steers as Impacted by Increasing Levels of Corn Gluten  
Feed Supplementation**

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## **ABSTRACT**

Overfeeding of phosphorus (P) is a contributing factor to P levels in surface waters. The objective of this study was to determine the impact of increasing levels of corn gluten feed (CGF) as a supplemental source of P on fecal P excretions. Eight Hereford steers ( $427 \pm 79$  kg) were randomly assigned to one of four dietary treatments in a 4 x 4 replicated Latin square design. Steers were fed chopped grass hay ad libitum (0.13% P) and 0, 0.5, 1.0 or 1.5 kg/d of dried CGF pellets. All steers were supplemented with 0.91 kg/d beet pulp, 0.34 kg/d rumen-inert fat supplement and 18.14 g/d trace mineral salt. Urea was added to the respective diets at levels of 95.25, 72.57, 49.90, and 31.75 g/d to ensure equal dietary protein across treatments. Steers were fitted with total fecal collection bags and adjusted to each diet for 9-d followed by a 5-d collection period. Dietary total P increased ( $P < 0.05$ ) as CGF level increased: 8.72, 12.59, 16.75 and 20.88 g/d. Dry matter digestibility increased linearly ( $P < 0.05$ ) as dietary P increased: 50.35, 53.66, 54.25 and 55.42%. Total P excretion increased linearly ( $P < 0.05$ ) with increasing CGF level: 9.66, 11.71, 14.29, 16.96 g/day. Inorganic P excretion increased linearly ( $P < 0.05$ ) with increasing CGF level: 4.11, 5.93, 8.36 and 9.92 g/day. Total P excretion was highly related ( $P < 0.05$ ;  $r^2 = 0.79$ ) to inorganic P excretion. Management of P intake can be a strategic practice to reduce P fecal excretions in beef cattle.

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## CHAPTER 1: INTRODUCTION

Eutrophication of surface waters is a major environmental concern whereby excessive algal growth causes aquatic dead zones and impairs waters role in drinking and recreational uses. Phosphorus (P) is the limiting nutrient in aquatic plant growth, and has been found to play a key role in the eutrophication of surface waters (Carpenter et al., 1998; Parry, 1998; Smith et al., 1999). The Environmental Protection Agency (EPA) has implemented strict strategies and laws to protect our nations waterways, including reducing the amount of P inputs from both point and non-point sources. The agency has recognized P from agriculture runoff as a non-point source of P pollution, which can be hard to measure and contain (Parry, 1998). New managerial strategies must be implemented to reduce the amount of P originating from agricultural sources and its environmental impact on surface waters.

Phosphorus is the second most abundant mineral in the body (Ternouth, 1990) and is involved in many mechanisms of maintenance and growth functions. Along with calcium (Ca), P is the primary component of bone and teeth, with 80-85% of the P in the body found in the skeleton (Geisert et al., 2010). Historically, high P diets have been fed to beef cattle to ensure proper growth and reproductive performance. However, increasing dietary P above the animal's requirement increases fecal P excretions (Call et al., 1978; Wu et al., 2000). Fecal excretions from beef cattle are nutrient rich and are typically land applied and used as a replacement for commercial fertilizers. Therefore, managerial strategies, such as dietary manipulation of P, can be an important approach to reduce fecal P excretions and the amount of P available for runoff into surface waters. The following is a brief review of P and its impacts on the environment and its importance in the beef animal.

## CHAPTER 2: REVIEW OF LITERATURE

### **Environmental Impacts of Phosphorus**

#### *Water Impairment and Eutrophication*

In recent decades, the world's population has increased rapidly and with it, the demand for fresh water. Excess nutrient loading into bodies of water has prompted increased environmental and water quality concerns in lakes, streams, and estuaries in the United States. One of the major environmental concerns in United States waterways is eutrophication of surface waters. Eutrophication results in excessive algal growth (Thomann and Mueller, 1987), increased turbidity, and oxygen depletion (Boesch et al., 2001), which impairs water's role in recreation, irrigation and drinking (Sharpley et al., 1994; Carpenter et al., 1998). Smith et al. (1999) defined eutrophication as the process by which water bodies are made more nutrient rich through an increase in nutrients such as phosphates and nitrates. Eutrophication and impairment of waterways has the potential to cause water shortages, making clean water a limited and valuable resource. To unlink the chain between poor water quality and water shortage, legislation has implemented water quality control plans to reduce concerns and restore waterways to safe and clean conditions (Parry, 1998).

#### *Water Policies*

The Federal Water Pollution Control Act of 1948 was the first legislative movement to protect the nation's surface waters. Under this law, the federal government provided state and local governments with funds for pollution clean-up and pollution control research, but without any rules or guidelines to follow (Copeland, 1999). The federal government was only directly involved in cases of interstate waterways. After the 1948 law did not prove to be effective, the

amendment was revised and with its revisions, called for greater involvement of the federal government.

In 1972, the revisions of the Federal Water Pollution Control Act were put into public law and became commonly known as the Clean Water Act of 1972 (Martin, 1997). This law provided states with goals, objectives and guidelines which the law of 1948 failed to do. It required all wastes from both municipal and industrial sources to be treated before being released into waterways, forcing parties involved to develop new technology (Copeland, 1999). For this reason, the 1972 act is known as the technology-forcing statute. If water quality standards were not met after implementation of best available technology to clean up pollutants, then states were required to develop a total maximum daily load (TMDL) plan. A TMDL is used to plan further actions necessary to attain water standards and with it, provide a quantitative assessment of the problem (Copeland, 1999). This plan includes problems and pollution sources with the necessary means in which to reduce the pollutant to an acceptable level.

### *Phosphorus in Water Quality*

Total maximum daily load plans are often implemented to acknowledge water quality concerns of nitrogen (N) and phosphorus (P). Eutrophication is most commonly caused by steep inputs of essential plant nutrients N and P, with P being more of the nutrient limiting factor for aquatic plant life (Carpenter et al., 1998; Parry, 1998; Smith et al., 1999). Phosphorus can enter waterways as runoff from agricultural, industrial, or residential areas. The EPA has categorized P pollution into waterways as being from either point or nonpoint sources. Point sources of pollution are more easily controlled and defined as being from “any discernable, confined, and discrete conveyance, including, but not limited to any pipe, ditch, channel, ...concentrated animal feeding operation... from which pollutants are or may be discharged” (Section 502 of the Clean

Water Act). However, nonpoint sources of P pollution can be relatively hard to monitor and regulate (Carpenter et al., 1998) making them a potential cause as to why waterways cannot reach their water quality improvement goals. Often, nonpoint sources are linked to agricultural areas or storm water drainage systems and thus can extend over large geographical areas. Smith and Alexander (2000) speculated that depending on the watershed, up to 47% of the total P loading into water bodies results from livestock operations. Problems occur when soils are overfertilized with chemicals and/or animal wastes, making them saturated with nutrients and increasing the nutrient runoff potential.

### *Nutrient Management Plans*

Animal agriculture is an important part of the United States economy, and is both a domestic and export source of food. As the world population continues to grow, so will animal agriculture in order to keep up with the increasing need for animal products. However, the increased productivity level will need to be met with improved management practices to decrease the environmental impact on soils and waterways. Understanding manure P land application limits, reducing P overfeeding, and improving P digestibility are all possible approaches to reducing the negative environmental impact animal agriculture can have on the environment (Knowlton et al., 2004).

Concentrated animal feeding operations (CAFO's) are an effective and efficient way of producing animal products while maximizing profitability. A CAFO can be defined as any operation with 1000 or more animal units (AU) which houses animals in a confined area for 45 days or more. Nutrients from CAFO's have been found to be a significant contributor of P and other nutrient pollution into waterways (USEPA, 1998) due to the extensive amount of animal waste produced. Public concerns of CAFO practices have pressed state governments to develop

and enforce tighter environmental regulations on the disposal of such nutrient rich wastes. A common disposal method for animal wastes includes land application of manure to replace fertilizer as a source of nutrients for crops. Historically, manure has been applied at levels to meet the nitrogen (N) requirement of crops. In manure, the N:P ratio does not match that of crop requirements and therefore, has led to an increased buildup of P in soils leading to an increased susceptibility of P runoff and pollution of waterways (Knowlton et al., 2004; Harrison et al., 2012). More recently, the focus of nutrient management plans have shifted away from using manure application to meet N requirements of crops and focused more on meeting the P requirements in attempts of reducing the P buildup in soils (Knowlton et al., 2004).

#### *Overfeeding of Phosphorus*

Often, beef cattle producers feed minerals in excess of requirement to ensure reproductive performance and maximum levels of meat and milk production. Hoechst-Roussel Agri-Vet Company (1996) reported that industry averages of dietary P typically ranges between 0.35-0.39% of DM intake which is notably higher when compared to the recommended 0.20% for growing beef steers eating 2.5% of BW in DM and gaining between 0.5 and 1.0 kg/d (NRC, 2000). The cost of P in free choice minerals is not only expensive but it also results in increased concentrations of P in fecal excretions (Knowlton et al., 2004) and can contribute to the problem of P pollution in waterways. Morse et al. (1992b) reported that increasing P intake above recommended concentrations increased fecal P output by 48.6% in twelve lactating Holstein cows fed a diet containing either 0.30, 0.41 or 0.56% P on a DM basis. Similar results were found by others in both dry and lactating Holstein cows (Lomba et al., 1969; Hibbs and Conrad, 1983). Feeding P above NRC recommendations has not been found to provide any significant benefit to cattle growth performance (Brintrup et al., 1993) or to reproductive efficiency (Satter

and Wu, 1999). Feeding beef cattle according to physiological requirement is a potential useful strategy to decrease the incidence of P overfeeding and to reduce both fecal P excretions and the amount of P imported into watersheds.

Understanding nutrient digestibility and implementing technologies to increase P utilization by the animal can be a useful tool in reducing the amount of P required in the diet and the amount of P excreted by livestock. Using feed additives such as the enzyme phytase to increase the P availability in feedstuffs is one specific feed management practice suggested by the National Resource Conservation Service (2003). Phytase, although mainly fed in monogastric diets, is an enzyme that is capable of breaking the phosphate groups from the inositol ring in organically bound P. Organically bound P is also known as phytate or phytic acid. Phosphorus in feedstuffs is more available to ruminants than nonruminants because ruminal microbes provide a source of endogenous phytase (Clark Jr et al., 1986; Morse et al., 1992b) capable of converting organic P ( $P_o$ ) into inorganic P ( $P_i$ ) for the animal to use. Starch fermenting bacteria are the predominant phytase-producing bacteria (Yanke et al., 1999). However, Knowlton et al. (2007) reported that fecal P content decreased by 21 percent or 11.7g/d in twenty four Holstein cows fed a diet that included phytase indicating that phytase can be effective at reducing P levels in cattle wastes. This would suggest that endogenous phytase from rumen microorganisms may not totally hydrolyze all phytate due to physical properties of the diet and the rate at which the diet passes through the rumen.

## **Phosphorus in Soils and Feedstuffs**

### *Phosphorus in Soils*

Phosphorus is an important constituent of plants and is needed for processes such as photosynthesis, energy generation, respiration, enzyme activity, and is a component of genetic material. In soils, it is present in both available and unavailable forms due to both microbial release and immobilization and the formation of P complexes with cations such as aluminum and iron (Vance et al., 2003). As much as 20-80% of soil P content can be found in the organic fraction (Schachtman et al., 1998) with the remaining inorganic portion present in over 170 different mineral forms (Holford, 1997). In unfertilized production systems, P is released from soils at a rate that is too slow to support plant growth (Schachtman et al., 1998). Although applying P to soils is common in many agricultural practices, P uptake by the plants can still be slow due to adsorption, or adhesion of the P molecule to the soil, pH, precipitation, which can enhance P runoff and pollution of surface waters, and conversion of P from the inorganic to the organic form (Holford, 1997). Fertilization practices can play a key role in maximizing herbage production because P is typically the limiting nutrient for plant growth, however, with current fertilization practices, the phosphate rock reserves are expected to be depleted in the next 60 to 80 years (Vance, 2001). Fertilizing pasture and crop lands is a management practice that can increase the amount of P available to the plant, however, a rain event immediately following fertilization can increase the amount of  $P_i$ , also known as water-soluble or water extractable P (WEP), that is leached into surface waters (Hart et al., 2004). However if a rain event does not occur,  $P_i$  will not be leached into surface waters and will be available for uptake until converted or combined with anions to form  $P_o$ .

Phosphorus is moved mainly by diffusion and is taken up by the plants in orthophosphate ( $P_i$ ) forms,  $H_2PO_4^-$  and  $HPO_4^{2-}$  (Vance et al., 2003) at a rate of  $10^{-12}$  to  $1^{-15} m^2s^{-1}$  (Schachtman et al., 1998). Soil pH below 6.0 favors uptake of the  $H_2PO_4^-$  species over the  $HPO_4^{2-}$  species (Schachtman et al., 1998) and studies have found that optimal uptake of P occurs during periods that soil pH is between 5.0 and 6.0 (Ullrich-Eberius et al., 1984; Furihata et al., 1992). Soil pH greatly impacts the availability of P to plants, such that in acidic soils, high levels of P may be unavailable to plants without the addition of lime (Woodhouse and Griffith, 1982). Diffusion through the soil happens at a slow rate, creating an area of P depletion around the root. It is important to understand the role of root geometry and morphology on P uptake. Root systems that expand vast areas and have a higher surface area compared to volume ratio will be able to burrow through more soil, allowing for a greater chance of inorganic P acquisition. Organic forms of P from both plant and animal residues must be mineralized before becoming available for uptake by the plant (Horst et al., 2001). The mineralization and immobilization of P by microorganisms in soils occurs simultaneously and the rate at which this occurs can be impacted by temperature, moisture, and aeration.

### *Phosphorus in Feedstuffs*

In 2001, the Dairy NRC proposed the concept that forages and concentrates have different P availability values, with an availability coefficient of 0.64 in forages and 0.70 in all concentrates. Phosphorus availability in feedstuffs was verified by the work of Sehested and Weisbjerg (2002) who determined P availability in forages to vary from 0.35-0.95 and in concentrates and by-product feeds 0.38 to 0.98. Therefore, to ensure the most accurate P requirement recommendations, the use of a single absorption coefficient for feedstuffs should be eliminated.

Forages can be subcategorized into grasses, legumes, and various non-legumes which can then be classified as annuals, biennials, and perennials, with all classes adapted to varying climatic areas and soil fertility levels. Phosphorus availability to plants is greatly affected by soil pH and in tropical and subtropical areas, acidic soils limit P availability to forages without the addition of lime (Pant et al., 2004). Forages of different species also differ in their ability to effectively absorb elements out of the soil. Newton et al. (2003) concluded that grasses tended to produce larger dry matter yields with higher nutrient uptakes than broadleaf forages when animal manure was applied. The authors also indicate that Bermudagrass, along with its high dry matter yields, had high tissue N and P concentrations, making Bermudagrass a potential important component, environmentally and economically, for forage based P phytoremediation systems. Other reports indicate that P removal by forages can range from 14.6 kg ha<sup>-1</sup> by a Bluegrass variety, to as much as 83 kg ha<sup>-1</sup> by a Johnsongrass variety (Pierzynski and Logan, 1993), with the difference in P removal resulting from the amount of dry matter the different species of grasses produce on a per acre basis. Increasing P fertilization rates increases P concentration in plant tissue in Bahiagrass and other rangeland grass (Burton et al., 1997; Islam and Adams, 1999) and similar results were found by Reinbott and Blevins (1997) in tall fescue pastures fertilized with P in early spring. However, Rhoads et al. (1997) found that Bahiagrass did not respond to P fertilization in terms of P tissue concentration. These results could be influenced by the amount of P in the soil prior to fertilization.

Aside from the effects of soil pH and fertilization practices, P content of forages can vary within the same species due to the effects of environmental conditions such as precipitation and temperature, stage of maturity at harvest, type of soil and possible nutrient interactions (Karn, 2001). In regions where P deficiency is known to occur, such as in south and east Texas, forage

P content does not often exceed 0.10-0.12% P (Black et al., 1949; Pinchak et al., 1989). Therefore, it is important to take into consideration the use of a forage nutrient analysis, since forage species can vary in both their level of P uptake from soil and P tissue concentration, allowing for accurate adjustments in dietary P supplementation when needed.

Phosphorus in feedstuffs can be broken into two fractions, inorganic and organic. Organic P in feedstuffs is found mainly in the form of phytate, or phytic acid, which must be hydrolyzed into the inorganic form before absorption can occur in the small intestine. Phytate is almost completely unavailable to monogastric animals due to their low intestinal phytase concentration, but is partially available to ruminants due to the capability of rumen microorganisms to hydrolyze the inositol ring. Phytate consists of myoinositol ring which is surrounded by six, covalently linked phosphate molecules. In an in vitro study, Morse et al. (1992a) reported that 90% or more of the phytate in wheat middlings, rice bran, hominy, soybean meal and distillers grains was hydrolyzed in 12 h and 24 h for cottonseed meal. Forages typically have a lower phytate content than do concentrates, where 50 to 70% of the P is in the form of phytate (Morse et al., 1992a). Playne (1976) determined that inorganic P accounted for 50% of the total P in forages with the remaining 50% largely nucleic acid P and esters of P with sugars. In straws, as much as 30% of P is bound in the phytate form.

## **Phosphorus Utilization in Beef Cattle**

### *Phosphorus Requirements for Beef Cattle*

Phosphorus requirements in beef cattle can vary due to differences between breeds (McDowell, 1996), various stages of the production cycle (Karn, 2001), P availability in feedstuffs and level of disease or parasitism in the animal (Ternouth, 1990). Phosphorus

requirements are calculated using a factorial method in the most recent NRC for beef cattle (NRC, 1996). For growing and finishing cattle, P requirements are based on body weight and rate of protein gain for which an example of this method is given in Table 1. Cows and heifers have the additional factors of milk production and fetal weight in the last 3 months of pregnancy, in their P requirement calculation. The maintenance requirement for beef cattle is suggested to be 1.6 g of P per 100 kg of live body weight, which accounts for endogenous fecal P losses by dividing the maintenance requirement, including the factorials, by the true P digestibility which is estimated to be 85% (Karn, 2001). Phosphorus requirements for protein gain are published as 3.9 g of P for every 100 g of protein gain. Dietary P requirements were calculated from a true absorption value of 68% and it is known that increasing dietary concentrations above requirement will decrease the true absorption coefficient (Karn, 2001).

**Table 1.** Phosphorus requirements for growing and finishing steers of varying body weight and average daily gain (NRC, 1996)

ADG, kg/d	Body weight, kg					
	200	250	300	350	400	450
	Dietary P requirement, g/d					
0.5	11	11	12	12	14	15
1.0	16	16	16	16	18	18
1.5	21	21	20	20	21	21
2.0	26	25	25	24	25	24
2.2	31	30	29	27	27	26

Historically, high levels of P supplementation have been fed to ensure reproductive efficiency and with the mindset that more is better. These high levels of P have had neither beneficial nor detrimental effects on the beef animal (Morse et al., 1992b). Wise et al. (1958) fed 40 male Holstein calves (88 kg) 4 diets of increasing levels of P (0.09%, 0.12%, 0.18%, and

0.30% dietary DM, respectively), and concluded that the basal ration (0.09% P) met all nutrient requirements except that for P. Authors reported that feed intake and weight gain were favorable above 0.18% P and that the P requirement for growing calves should be set at 0.30% P to include a margin of safety. More recently, Erickson et al. (1999) fed diets of varying P content (0.14, 0.19, 0.24, 0.29, or 0.34% P, DM basis) for 105 days to 66 crossbred, finishing cattle. They observed no effect of dietary P on DMI, ADG, feed:gain ratio, and carcass weight, bone strength, meat tenderness, or marbling and concluded that no supplemental P was needed when feeding a corn-based ration to growing and finishing cattle and the NRC P recommendation of 0.22% DM may overestimate P requirements. Call et al. (1978) compared the effects of a P deficient and a high P diet on growth and reproductive performance in 96 Hereford heifers (165 kg). The P deficient diet was 66% (10.34 g/d) of the 1976 NRC recommendation for beef cattle and the high P diet was 172% (26.11 g/d) of recommendation. The authors measured no statistically significant difference in growth or reproductive efficiency between the two dietary P treatments after two years of data collection, indicating that the NRC may overestimate P requirement in beef cattle.

### *Uses in the Body*

Phosphorus is a biologically important mineral in cattle as it is the second most abundant mineral found in the body (Ternouth, 1990) with 80 to 85% of the total P found in the skeleton (Geisert et al., 2010). In the body, P is used as a structural component in bone and teeth, a factor in biochemical pathways in soft tissues, and is a vital part of the transfer of genetic material (Ternouth, 1990; Karn, 2001).

The majority of the P in the body is found in the teeth and bones where it is bound to calcium (Ca) in a 1.67:1.0 Ca:P ratio in a complex known as hydroxyapatite (Ternouth, 1990).

Hydroxyapatite in bones is tightly anchored through an association with the fibrous protein, collagen, forming a highly porous structure with an extensive surface area. When an animal's P intake is below requirement or during times of peak lactation when calcium (Ca) and P requirements are elevated, bone P is used as a P reservoir. Benzie et al. (1959) reported that cattle can withdraw up to 30% of P from their skeleton and sheep can utilize closer to 40% during times where intake is below animal requirement. Calcium and P resorption is elevated under the control of parathyroid hormone (PTH) (Sallis et al., 1963) produced from parathyroid glands (Ternouth, 1990). In the gastrointestinal tract, Ca and P absorption is increased by the conversion of 1,25-hydroxycholecalciferol to dihydroxycholecalciferol (White et al., 1964). This conversion is stimulated by PTH and ultimately encourages bone resorption (Braithwaite, 1976; Ternouth, 1990). Parathyroid hormone increases renal excretion of Ca but decreases the excretion of P. In opposition, calcitonin, a hormone secreted by the thyroid gland, triggers uptake of Ca and P from the blood to the bones and therefore increasing mineral bone density. Ternouth (1990) found that beef cows in early lactation could mobilize up to 30% of their bone mineral or 6 g/d of P when fed P-deficient diets. Similar results were found by Satter et al. (2002) using 600 kg lactating dairy cows who reported that 600 to 1,000 g of P could potentially be pulled from bone during the early lactation period.

As much as 20% of total body phosphorus can be found in soft tissues (Breves and Schröder, 1991) which play an essential role in the structural integrity of cell walls and biochemical pathways related to energy metabolism. Cell membranes are formed from structures known as phospholipids (Ternouth, 1990), which properties include a P containing hydrophobic head and a hydrophilic tail and together, create a semi-impermeable barrier. Phospholipids are also constituents of myelin sheaths where they act as insulators for nerve

impulses (NRC, 1984). In enzymatic reactions of energy metabolism, P can be found in structures adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), and creatine phosphate (Karn, 2001) which are all factors in the thermodynamics of energy transfer.

The rumen microbial population has a P requirement that is different from that of the animal's P requirement. If the microbial P requirement is not met, microbial activity can be decreased, potentially having an impact on the efficiency and productivity of the ruminant. Komisarczuk et al. (1987) reported that microbial activity can be maximized if ruminal inorganic P concentrations are kept at 75-100 mg/l. In contrast, Williams et al. (1991a) reported that after 564 days of feeding cows either a low P (0.12%) or high P (0.20%) diet, no treatment effect was detected on ruminal P concentrations (273 mg/l) but differences were measured in bone P and daily weight gains, potentially due to the mobilization of P reserves in the skeleton.

### *Phosphorus and Growth*

Phosphorus deficiency in grasslands in certain regions of the world has been a widespread problem resulting in rickets in cattle. To correct this problem, P supplementation has been recommended as a common production practice. Common symptoms of P deficiency include an unusual appetite for wood, bones and rock (Karn, 2001), lameness in limbs, infertility, and reduced growth parameters (Call et al., 1986; Morse et al., 1992b). There has been much inconsistency in the literature on P intake and its associated effects on weight gain. Karn (1995) fed Hereford and Hereford-Angus crossbred heifers (194 kg) a mixed forage and concentrate diet with or without the addition of supplemental P in the form of monosodium phosphate. The author reported that P supplementation had no effect on weight gains during a four year period. In a subsequent study, 55 Hereford-Simmental heifers (251.5 kg) were utilized

for their growth potential and heifers that were supplemented with P were found to have a 0.04 kg higher ADG than those heifers on the control diet receiving no supplemental P. Comparing the forage nutrient content of the two studies, there were no differences in chemical composition of the native pastures, indicating that the small differences in weight gain may have been due to heifer growth potential differences or breed effect, nutritional history, or dietary P availability and DM intake level. Supporting the hypothesis that P supplementation impacts growth in cattle, Williams et al. (1989) found that a diet containing 0.20% P produced heifers with 20% higher weight gains (2.68 lbs/d) than did a diet with only 0.12% P (2.16 lbs/d). It is unclear as to why there has been an inconsistent response to P supplementation in grazing beef cattle.

### *Phosphorus Digestion*

Understanding P digestion and P recycling in livestock has the potential to assist producers make better educated decisions on P feeding to meet animal requirements and reduce potential environmental impacts of overfeeding. To develop these practices, insight into both P intake and excretion must be quantified and a relationship established.

As mentioned, digestibility of P by beef cattle is assumed to be about 68% for intestinal P absorption (NRC, 1996) with the most recent NRC giving separate availability values for varying feed types such as 64% for forages and 70% for grains (NRC, 2001). The digestibility coefficient is used in calculating the nutrient requirements of beef cattle at various physiological stages, however Block et al. (2004) suggests that this coefficient may incorrectly overestimate P requirements due to feedlot trials conducted by other researchers (Erickson et al., 1999; Erickson et al., 2002). Erickson et al. (1999) fed feedlot steers a diet consisting of 1 of 5 dietary levels of P (0.14, 0.19, 0.24, 0.29, or 0.34% DM) and observed no effect of dietary P level, even though the Beef NRC recommended level is 0.22% DM.

Feed TP and  $P_i$  from salivary secretions are the major sources of P for digestion in ruminants. Inorganic P in the small intestine can be reabsorbed and re-excreted through saliva, creating a P recycling system that is important for the supply of P to the rumen microbial population (Puggaard et al., 2011). As much as 75 to 80% of salivary P is reabsorbed and recycled via the small intestine (Meyer, 2005). Valk et al. (2002) determined that cows fed at their P requirement during the lactation period secreted an average saliva P concentration of 245 mg/L and that decreasing dietary total P from 100% of requirement to 67% of requirement reduced both plasma and salivary  $P_i$  concentrations by 30%. Therefore, reducing total P intake impacts the rumen in two ways; first, the  $P_i$  supply to the rumen is directly impacted by dietary total P changes, and secondly dietary total P changes impact the concentrations of plasma and saliva  $P_i$ , with salivary secretions being imported into the rumen through the process of mastication. Similar results were found by Goff (1999) who reported that an estimated 30 to 90 g of P was secreted in saliva each day. It has been estimated that salivary P contributes as much as 50% of the total P entering the rumen and accounts for 80% of the P entering the gastrointestinal tract (Care, 1994) however, salivary P secretions can be impacted by dietary NDF content and particulate size (Khorasani et al., 1997). Higher fiber diets require a longer time of mechanical breakdown, or mastication, which results in an increase in salivary excretions to aid in the digestion process, therefore, potentially increasing the total amount of P mixed with the bolus entering the rumen. Cohen (1980) suggested that the daily amount of P in saliva is constant because P concentrations decrease as salivary secretions increase but Ternouth et al. (1985) reported that P in salivary secretions is related to flow rate since the decrease in P concentration does not equal the increase in flow rate. Length of forage can also impact salivary

P secretions with larger particles increasing chewing time and mechanical grinding of feedstuffs prior to feeding reduces salivary flow rate (Wilson and Tribe, 1963).

Phosphorus absorption mainly occurs in the small intestine, more specifically in the duodenum and the jejunum. Inorganic P is the form of P capable of absorption by both active transport and passive diffusion. Under conditions where dietary P levels are deficient, or below the animals requirement, P absorption in the small intestine occurs by actively pumping  $P_i$  across the intestinal membrane. In contrast, when P is sufficient in the diet, P is absorbed through means of passive diffusion. Phosphorus absorption and active transport of  $P_i$  can be stimulated by 1,25-dihydroxycholecalciferol (DCC) or the active form of vitamin D (Braithwaite, 1976). Ruminants receive vitamin D from two major sources. In the skin, sunlight activates the conversion of 7-dehydrocholesterol to vitamin  $D_3$  (Horst and Reinhardt, 1983). In plants, photochemical processes convert ergosterol to vitamin  $D_3$ . Once in the bloodstream, vitamin  $D_3$  is converted to 25-hydroxyvitamin  $D_3$  (25-OHD<sub>3</sub>) by the liver and from there can be converted into at least four different metabolites, including DCC. Circulating DCC levels triggers the active transport of P from the small intestine causing bone resorption.

### *Phosphorus Excretion*

The major route of excretion for both endogenous and exogenous P is through the feces with a smaller portion of P exported in urine and milk. Total P excreted in urine and feces varies between animals and depends on factors such as physiological stage, dietary levels, feedstuff, vitamin D, calcium, and other factors (Church, 1979). There is a large volume of literature reporting a relationship between dietary P and fecal P excretion. Call et al. (1978) fed 96, 7 month old Hereford heifers (165 kg) gaining 0.5 kg/d either a P deficient diet (0.14% P, as fed basis) or a high P diet (0.36% P, as fed basis) using monosodium phosphate as a supplemental P

source. The authors observed that heifers receiving the P deficient diet excreted a substantial smaller amount of P in the feces than heifers on the high P diet (4.58 and 14.12 g/d; respectively). Although not the first authors to demonstrate the link between dietary P level and its impact on P excretion, Morse et al. (1992b) discovered that lactating dairy cows consuming a dietary concentration of 0.56% P increased their P excretion by 0.80 g/d for every g/d increase in P intake and also reported that 60.5% of dietary P was excreted in the feces while 0.9% was excreted in urine and 26.7% was excreted in milk. It has been demonstrated that total P excretion increases with increasing dietary P content (Scott and Buchan, 1985; Bravo et al., 2003). Similar results were observed by Wu and Satter (2000) who determined that fecal P content increased (0.51, 0.73, 0.90% DM basis) with increasing dietary P content (0.31, 0.40, and 0.49% DM basis; respectively) in 26 multiparous Holstein cows over a duration of 308 d.

Fecal TP is composed of several different P fractions (Spiekens et al., 1993) including undigested feed P, endogenous P, and salivary P. Undigested feed P is the P in the feed that is not absorbed by the animal and is the reason the NRC has created different digestion coefficients, 0.64 for forages and 0.70 for by-products and concentrates. Endogenous P includes the P from cells sloughed off in the digestive tract as well as cells originating from rumen or gut microorganisms. Salivary P, as previously mentioned, is secreted once the animal consumes feed, creating a P recycling system that is important for P homeostasis, however, if excess P is present in the digestive tract for absorption, salivary P will not be fully reabsorbed and will be excreted in the feces.

Fecal P can also be divided into organic or inorganic P fractions, with  $P_i$  being more of an environmental concern due to its solubility and runoff potential. Bromfield (1961) discovered that as total P in manure increases, the fraction of the total P that is  $P_i$  also increases, implying

that an increase in manure P increases the amount of water-soluble P that can leach into waterways if land applied. Barnett (1994) collected multiple undisturbed fecal samples from both beef and dairy cattle in several rearing facilities on multiple farms. The largest fraction of P in the total P of dairy feces was  $P_1$ , which on average accounted for 63.2% of the total fecal P. In beef cattle,  $P_1$  accounted for 47.1% of the total P in feeder cattle and 48.3% of the total P in finishing cattle. Differences in fecal P fractions between dairy and beef cattle could be due to the differences in feed TP concentrations and diet constituents since exact dietary TP intakes were not measured.

In the literature, there has been mixed reports of the impact of dietary P levels on P excretion in urine. Usually, urinary P excretion is insignificant due to efficient renal reabsorption, except in situations of high P intake (Puggaard et al., 2011). It has been shown that there is a larger amount of P excreted in urine when dietary levels of P are high in high-concentrate diets (Field, 1981; Block et al., 2004). Urinary P excretion was measured to be 0.14 or 5.5 g/d in 96, 7 month old Hereford heifers fed a P deficient diet (0.14% P, as fed) or a diet high in P (0.36% P, as fed), respectively (Call et al., 1978). Challa et al. (1989) demonstrated that P is excreted in the urine when serum P concentrations exceed 7.1 mg/dL. However, Meyer (2005) found that steers excreted 3.0 to 7.5 g/d of P in urine when fed a corn-based diet varying from 0.28 to 0.38% P of DM.

To quantify and verify the relationship between P intake and fecal P excretion, Cohen (1974) fed 18, 10-month-old steers a basal ration of mature carpet grass hay, urea, dried molasses, and non-phosphorus mineral mix and supplemented them with either 0, 2, 4, 6, 8, or 10 g of P/d using sodium dihydrogen orthophosphate. The author cited that the relationship between daily P intake (X) and daily fecal P excretion (Y) was  $Y = 0.293X + 2.361$  and that

daily P intake accounted for 91 percent of the variation in daily fecal P excretion ( $P < 0.05$ ). In a subsequent study, the author used 15, 15-month-old steers, and fed a ration consisting of different proportions by weight of mature carpet grass hay and lucerne hay. The relationship in this experiment was  $Y = 0.290X + 2.481$  ( $r^2 = 0.98$ ;  $P < 0.01$ ). Charmley and Dove (2004) used 18 crossbred sheep (37.3 kg) consuming 1 of 6 dietary levels of P (20-150 mg P/kg LW) to determine the relationship between P intake and excretion. The authors noted a linear relationship between dietary P and its effect on fecal P excretion. Dou et al. (2002) also reported that fecal TP increased with increasing dietary P content in three independent feeding trials involving dairy cattle. It was determined that 60 to 70% of P intake was excreted in the feces.

It has been evaluated that fecal P concentrations may be a better indicator of dietary P content (Moir, 1960) than animal P status (Winks et al., 1977). Moir (1966) reported that grazing cattle with fecal P concentrations less than 0.20% may show some signs of P deficiency. However, cattle with fecal P concentration of 0.30% (Winter, 1988) and 0.40% (Winks et al., 1977) also showed a response to P supplementation. Animal P status can be impacted by type of diet, DMI, physiological stage of production and length of time consuming P-deficient or P-sufficient diets, which may be the reason researchers have detected such confounding results in P supplementation and excretion.

## **Conclusions**

Phosphorus is an important mineral for the proper function of both plants and animals. It has historically been supplemented at high levels in beef cattle diets to ensure growth and reproductive efficiency. Phosphorus can be found at varying concentrations in forages due to

factors such as forage type (grasses vs legumes), soil pH, rainfall, maturity, and fertilization management practices. Supplementing cattle with P in diets that meet or exceed animal P requirements can lead to increased P excretion in urine and feces. This is of particular environmental concern in that water soluble P in feces can leach into surface waters and contribute to eutrophication. Determining dietary P levels and supplementing P only when needed can reduce the economics associated with feeding unnecessary levels of P as well as reduce excess P in feces and the impact of beef cattle on surface waters. To determine dietary P levels of beef cattle in a grazing setting, a relationship between P intake and fecal P output must be established. The objective of this study is to document P intake and excretion levels in beef steers fed increasing levels of corn gluten feed as a supplemental source of P to a forage based diet.

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## CHAPTER 3: PHOSPHORUS EXCRETION IN BEEF STEERS AS IMPACTED BY INCREASING LEVELS OF CORN GLUTEN FEED SUPPLEMENTATION

### Abstract

Overfeeding of phosphorus (P) is a contributing factor to P levels in surface waters. The objective of this study was to determine the impact of increasing levels of corn gluten feed (CGF) as a supplemental source of P on fecal P excretions. Eight Hereford steers ( $427 \pm 79$  kg) were randomly assigned to one of four dietary treatments in a 4 x 4 replicated Latin square design. Steers were fed chopped grass hay ad libitum (0.13% P) and 0, 0.5, 1.0 or 1.5 kg/d of dried CGF pellets. All steers were supplemented with 0.91 kg/d beet pulp, 0.34 kg/d rumen-inert fat supplement and 18.14 g/d trace mineral salt. Urea was added to the respective diets at levels of 95.25, 72.57, 49.90, and 31.75 g/d to ensure equal dietary protein across treatments. Steers were housed individually and fitted with total fecal collection bags. Steers were adjusted to each diet for 9-d followed by a 5-d collection period. Following the final collection of each period, a 10 ml jugular blood sample was collected and analyzed to determine serum inorganic P. Dietary total P increased ( $P < 0.05$ ) as CGF level increased: 8.72, 12.59, 16.75 and 20.88 g/d. Dry matter digestibility increased linearly ( $P < 0.05$ ) as dietary P increased: 50.35, 53.66, 54.25 and 55.42%. Total P excretion increased linearly ( $P < 0.05$ ) with increasing CGF level: 9.66, 11.71, 14.29, 16.96 g/day. Inorganic P excretion increased linearly ( $P < 0.05$ ) with increasing CGF level: 4.11, 5.93, 8.36 and 9.92 g/day. Total P excretion was highly related ( $P < 0.05$ ;  $r^2 = 0.79$ ) to inorganic P excretion. Serum inorganic P increased linearly ( $P < 0.05$ ) with increasing dietary P content: 5.61, 5.87, 6.64 and 6.80 mg/dL. Fecal P increased as CGF level increased in steers fed varying dietary levels of P from plant sources. Management of P intake can be a strategic practice to reduce P fecal excretions in beef cattle.

## Introduction

Phosphorus (P) is an essential mineral for both plants and animals. In beef cattle, the majority of P is used in the body as a structural component for bone and teeth formation. It is also needed for energy metabolism and is an important component of genetic material. Dietary P deficiencies can negatively impact growth performance and cause reproductive distress (Hignett and Hignett, 1951; Short and Bellows, 1971). Many forages, especially in regions where soil P levels are low, can contain less P than the requirement of a lactating beef cow and cattlemen typically address this by including P in a free choice mineral. Phosphorus is not only the most expensive mineral to supplement, but it is also a major environmental concern, causing eutrophication or algae blooms in watersheds such as the Chesapeake Bay.

Historically, high phosphorus diets fed to confined livestock and poultry has received the most attention and scrutiny as possible sources of P runoff into surface waters. The contribution of grazing beef cattle to P loading into waterways has received little if any attention. Excess dietary P is excreted mainly in the feces of cattle. The total amount of P excreted has been found to increase with increasing levels of dietary P (Scott and Buchan, 1985; Bravo et al., 2003). Fecal total P (TP) is composed of inorganic P ( $P_i$ ) and organic P ( $P_o$ ) fractions, with  $P_i$  being more of an environmental concern due to its water solubility and runoff potential (Barnett, 1994; Knowlton et al., 2004). The quantity of P provided in feed and mineral supplements to beef cattle consuming fresh forage or hay should impact the amount and form of P excreted. Supplementing P only when needed has the potential to reduce P imports into watersheds and potentially reduce mineral costs therefore providing an economic incentive to decrease unnecessary P in diets.

The objective of this study was to evaluate P excretion in beef steers as affected by increasing levels of corn gluten feed supplementation. Our hypothesis was that by increasing the amount of corn gluten feed supplementation fed to beef steers, total P and the fraction of P<sub>i</sub> would both increase linearly.

## **Materials and Methods**

### *Experimental design*

The Virginia Tech Institutional Animal Care and Use Committee approved all procedures for this experiment. Eight Hereford, large frame steers (initial BW = 427±79 kg) were used in a replicated 4 x 4 Latin square design to evaluate the impacts of dried, pelleted, corn gluten feed (CGF) on fecal P parameters. Steers were housed and fed individually in a covered barn. Pens were assigned randomly and consisted of concrete floors that were bedded with wood fiber bedding. Each steer was allotted 18.61 m<sup>2</sup> of pen space. Steers were randomly assigned to 1 of 4 dietary treatments and were fed twice daily at 0600 and 1700 h. Steers were allotted a nine day adjustment period followed by a five day collection period for each diet.

### *Dietary Treatments*

Dietary treatments of CGF supplementation were 0, 0.5, 1.0, and 1.5 kg/d resulting in a daily P intake of 8.72, 12.59, 16.75 and 20.88 g/d, DM to represent increasing levels of dietary P that were below, met or exceeded NRC recommended P requirements for a 450 kg beef steer gaining 0.5 kg/d (NRC, 1996). The increasing dietary concentrations of P were achieved by adding varying levels of dried CGF pellets (0, 0.50, 1.00, and 1.50 kg/d) to a basal diet of chopped, grass hay fed ad libitum (0.13% P, DM) (Table 2). All steers were supplemented with 0.91 kg/d beet pulp (BP) containing molasses (Midwest Agri-Commodities, San Rafael, CA) for

palatability and energy needs, 0.34 kg/d rumen-inert fat supplement (Energy Booster 100, Milk Specialties Global), and 18.14 g/d P-free trace mineral (TM) salt (Champion's Choice Selenium 90, Cargill) (Table 2). Urea (Eastern Mineral Inc., Henderson, NC), which is equivalent to 287% protein, was added to the diet at decreasing levels with increasing levels of dietary P to ensure equal dietary protein intake across treatments at levels of 95.25, 72.57, 49.90, and 31.75 g/d, respectively. Total diet ingredient and nutrient composition can be found in Table 3. Chopped grass hay was fed twice daily (4.54 and 3.63 kg as-fed) while supplementation of CGF, BP, fat, TM salt, and urea was fed once daily at 1700h and fed separately from the hay to insure no feed refusals. Beet pulp was allowed to soak in water for 30 minutes before the other supplements were incorporated and fed.

### *Experimental Sampling*

Before the initiation of the study, steers were halter broken and fitted with total fecal collection bags as described by Tolleson and Erlinger (1989). Total fecal collection bags were placed on the steers on d 10 of each collection period and removed on d 14, prior to start of the next dietary treatment. Fecal samples were collected and weighed during each day of the collection period at 1700 and 0600h. Total fecal collection bags were changed, rinsed, and allowed to dry twice daily. A daily representative fecal sample was collected at 0600 h and immediately placed in a -18° C freezer for further analysis. Samples were then thawed, weighed and dried in a 60° C forced-air oven (Thermo Scientific Precision 645, Danville, IN) for 7 d to remove water from the sample. Dried samples were ground using a 2 mm screen in a Wiley Mill (Arthur H. Thomas, Philadelphia, PA) and then pooled by period for each steer.

Hay, CGF, BP and hay refusals were collected, weighed, and sampled daily during the 5 d collection period at 0600 h. All feed and refusal samples were immediately stored in a -18° C

freezer for further analysis. Samples were then weighed and placed into a 60° C forced-air oven (Thermo Scientific Precision 645, Danville, IN) to dry to a common weight. Once dried, samples were ground through a 2 mm screen in a Wiley Mill (Arthur H. Thomas, Philadelphia, PA) and then pooled by period for each steer.

Blood samples were collected on day 5 of each collection period at 0800 h via jugular venipuncture using sodium heparin-containing vacutainers. Samples were placed on ice after collection and centrifuged at 30,000 x g for 10 min at 4° C. Serum supernatant was removed and placed into a -18° C freezer for further analysis. Steers were weighed on day 5 of each collection period during blood collection.

#### *Laboratory Analyses*

Dried and ground feed offerings, feed refusals, and fecal samples were analyzed in duplicate for total P using the molybdovanadate yellow method described by (AOAC, 1984; Eaton et al., 1998. 20th ed.). All samples were analyzed in duplicate for P<sub>i</sub> by extraction with 0.5 M HCl and then molybdenum blue method described by (Murphy and Riley, 1962). Serum supernatant was analyzed for P<sub>i</sub> using the colorimetric method (Inorganic Phosphorus Reagent Set, Pointe Scientific, Canton, MI).

To determine micro dry matter content, all samples were placed in a 100° C forced air oven overnight to dry. Once dry, samples were placed in a dessicator for approximately 20 min and then a final weight was taken (AOAC, 2000). Samples were then placed in 500° C cool muffle furnace for 2 hours with determination of inorganic content calculated by difference of weight (AOAC, 2000). Crude protein content of all samples were determined using a Carbon/Nitrogen (CN) analyzer (CHNOS Elemental Analyzer, Hanau, Germany) (AOAC,

2000). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) of the manure, feed, and feed refusal samples were determined using  $\alpha$ -amylase and the ANKOM<sup>200</sup> fiber analyzer (AOAC, 1984; Van Soest et al., 1991). Calcium content was determined by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP) (Franson, 1998, 20th Ed.) using the Spectro ARCOS ICP Model FHS16 with CETAC Auto Sampler (Spectro Analytical Instruments, Inc., Mahwah, NJ).

### *Statistical Analysis*

All data were analyzed using the PROC GLIMMIX procedure of SAS (Cary, NC) with a model that included diet and period. Linear, quadratic and cubic treatment effects were evaluated using orthogonal contrasts. Significance was concluded at  $P < 0.05$ . Data from the current study were combined with data from Riley et al. (2014) to develop a regression model of P intake (g/d) from percent fecal TP, Pi, Ash and calcium (Ca) excretions. An initial factor screening was used to find variables for a full four-way factorial regression models using the Fit Model procedure in JMP Pro (SAS, Cary, NC). A refinement of the full four-way factorial model using a reverse stepwise regression was used to support the use of the full factorial design.

## **Results and Discussion**

### *Effect of CGF on Nutrient Digestibility*

Dry matter intake increased linearly as corn gluten feed supplementation increased ( $P < 0.01$ ; Table 4). Total hay intake was not affected by diet ( $P > 0.05$ ) and the increase in DMI may be caused by the addition of increasing levels of CGF across treatment levels. Geisert et al. (2010) reported that DMI was decreased in heifers fed a diet containing 0.10% P compared to diets of 0.17, 0.24 and 0.31% P, relating the lowered DMI directly to the low P content of the

diet. Similarly, Shupe et al. (1988) found that beef cows had decreased appetites when dietary P levels did not meet P requirements. Dry matter digestibility increased linearly with increasing levels of corn gluten feed supplementation ( $P < 0.05$ ; Table 3), likely due to the inclusion level and digestibility of corn gluten feed over the lower quality and digestibility of the grass hay. Fleck et al. (1988) also reported that inclusion of CGF at 1.57 kg/d to a low quality forage diet increased dry matter digestibility from 47 to 54% in 32 non-lactating cows.

Crude protein digestibility was significantly depressed with increasing levels of CGF supplementation ( $P < 0.01$ ; Table 4). Diets were originally calculated to be isonitrogenous and nutrient composition of the diet (Table 3) verifies those efforts. However, increasing the amount of urea added to the diet at decreasing levels of CGF may have changed the digestibility of nitrogen between diets, with urea being more digestible than the other dietary ingredients. Urea is rapidly converted to ammonia in the rumen which is then either utilized by rumen microbes in the production of microbial protein or absorbed through the rumen wall and transported to the liver (Chalupa, 1968). Ernst et al. (1975) fed diets consisting of ad lib hay and variations of inclusion of 227 g molasses and/or 57 g urea. Authors concluded that CP digestibility increased in diets containing urea when compared to diets where urea was removed.

#### *Effect of CGF on P Intake and Excretion*

Diet means of P intake and excretion are presented in Table 5. As expected, total P intake increased with higher levels of CGF supplementation ( $P < 0.01$ ). Likewise, there was a corresponding linear increase in  $P_i$  and  $P_o$  intake ( $P < 0.01$ ; Table 5). Diets were formulated to provide 4.7, 11.7, 17.4, and 23.2 g/d total P which would represent 31, 78, 116 and 155 % of the P requirement for a 450 kg growing beef steer gaining 0.5 kg/d. However, variations in hay intake among animals prevented target P intake levels from being achieved and were

approximately 58, 84, 112 and 139% of requirement. There was a significant ( $P < 0.01$ ) period effect in P intake levels (13.72, 14.84, 15.65 and 14.74; respectively), however, this effect may be explained by variation in hay intake and P content, which may have been impacted by ambient temperature as the trial ran into colder months of the fall. No period by diet interactions were detected. Serum  $P_i$  responses were proportional ( $P < 0.02$ ) to dietary P content and is considered a reflection of P intake in ruminants as supported by the work of others (Winks et al., 1977; Winter, 1988; Williams et al., 1991b; Karn, 1997). However, there have been reports that serum  $P_i$  may be normal or elevated during P and Ca deficient diets, when bone resorption may be stimulated (Ternouth, 1990) indicating that serum  $P_i$  may not be a precise indicator of dietary P intake.

There was a corresponding linear increase in fecal total P excretion with increasing levels of dietary P ( $P < 0.01$ ; Table 5). Similar results have been reported (Scott and Buchan, 1985; Wu et al., 2000; Bravo et al., 2003), and is likely due to both increasing dietary P above recommended P requirements and increasing the amount of unavailable or undigested P. Call et al. (1978) reported that Hereford heifers (165 kg) gaining 0.5 kg/d and fed a high P diet excreted more fecal P than heifers fed a P deficient diet (0.36 and 0.14%P, as fed basis; respectively) (14.12 and 4.58 g/d; respectively). Linear regression models indicate a strong positive relationship between total P intake and fecal total P excretions, with dietary P accounting for 81% of the variation in fecal total P (Figure 2.). Extrapolation would suggest that at 0 g/d of P intake, beef steers fed a forage based diet will excrete 4.46 g of endogenous total P and for every 1 g/d of dietary P intake, 0.59 g/d of total P will be excreted in the feces, resulting in an absorption coefficient of 0.41 (Figure 2). The absorption coefficient calculated in this study is lower than the 0.68 absorption coefficient described by NRC (1996). However, Charmley and

Dove (2004) also found a linear relationship between P intake and excretion in 18 crossbred sheep consuming monosodium phosphate at 1 of 6 dietary levels of increasing P (10-150 mg P/kg LW). The authors concluded that crossbred sheep would excrete 0.59 mg/d per kg LW for every 1 mg/d per kg LW consumed. Similar work (Nennich et al., 2005) suggests lactating Holstein cows will excrete 0.560 g/d of total P for every 1 g/d intake and calves, ranging in BW from 86 to 205 kg, will excrete 0.622 g/d for every 1 g/d of P intake.

Apparent P digestibility was calculated by subtracting total P excretions, which includes endogenous P losses, from P intake and then dividing the sum by P intake. Apparent P digestibility increased quadratically with increasing levels of P supplementation ( $P < 0.01$ ; Table 5) as has been reported by others in both beef and dairy cattle (Wu et al., 2000; Geisert et al., 2010). However, there have been reports of a decrease in P digestibility with increasing levels of dietary P (Knowlton and Herbein, 2002; Chantiratikul et al., 2009). Phosphorus digestibility was negative in diet 0, fed at 58% of P requirement, indicating more P was excreted than ingested which is most likely contributed by endogenous sources of P. As dietary P increased, P digestibility exhibited a quadratic response and plateaued at 139% of P requirement, indicating a limit to the amount of dietary P used in the kinetics of the P recycling system in cattle ( $P < 0.01$ ; Figure 5).

Inorganic P excretion was linearly related to dietary P intake ( $P < 0.01$ ; Table 5). Linear regression models indicate a strong positive relationship between total P intake and  $P_i$  excretion, with total P intake explaining 74% of the variation in fecal  $P_i$ . During periods of no dietary P intake, forage fed beef steers excreted 0.34 g/d of endogenous  $P_i$  and for every 1 g/d increase in dietary P an additional 0.46 g/d of  $P_i$  will be excreted. When comparing  $P_i$  as a percent of total P or P solubility (Table 5), determined by dividing fecal  $P_i$  excretion by fecal total P excretion,

there was a significant linear relationship between dietary total P and the percent of the total P excreted in the inorganic fraction ( $P < 0.01$ ; Figure 1). Therefore, by feeding greater amounts of P over the recommended requirement level, not only does the total amount of fecal P increase, but the portion of the total P that is  $P_i$  or water soluble also increases. Other reports have suggested that  $P_i$  or water extractable P is the largest single fraction in animal feces (Dou et al., 2000a; Dou et al., 2000b; Sharpley and Moyer, 2000) and Bromfield (1961) reported that P uptake and plant yield are directly related to  $P_i$  in feces. Dou et al. (2002) used 3 independent feeding trials to determine the impact of increasing dietary P concentrations on fecal P excretions and observed an increase in total fecal P with a larger portion of the P in the water-soluble fraction.

Net increase in both fecal total P and  $P_i$  was observed with increasing dietary P. There appears to be a strong relationship between fecal total P and  $P_i$  ( $r^2 = 0.79$ ; Figure 4), with every g/d of total P excreted, there is 0.72 g/d of  $P_i$  excreted. Bromfield (1961) reported that in sheep, an increase in fecal total P from 1.8 to 17 mg P/g of feces resulted in an increase in the proportion of  $P_i$ , 22 to 90%, in the feces.

### *Modeling*

Many times, simple fecal samples can be collected in the field with no context of dry matter intake or fecal output. Data from this experiment were evaluated together with data from a previous experiment (Riley et al., 2014) to determine a model capable of predicting P intake in a grazing setting. An initial factor screening of variables was used to determine that percent fecal total P,  $P_i$ , ash, and Ca, were statistically significant ( $P < 0.05$ ) predictors of total P and  $P_i$  intake (Table 6). These variables along with their factorial interactions were capable of explaining a substantial portion of the variation in total P and  $P_i$  intake for beef cattle in a forage

based production system and were used in the development of the prediction model (Table 7). Full factorial models were capable of explaining 84% of the variation in total P intake and 94% in  $P_i$  intake (Figures 6 and 8, respectively). Plots of residuals by predicted values were used to check the assumption that the distribution of residuals in a population is normal at all levels of predicted TP and  $P_i$  (Figure 7 and 8, respectively). The regression models indicate that by determining the concentration of TP,  $P_i$ , Ca and ash of a fecal grab sample, we can accurately predict, to a high degree, the amount of P in a beef steers diet. The P intake prediction can then be compared to NRC P recommendations for a growing beef steer and a decision can be made on the level of P supplementation needed. Efforts are currently underway to validate the predictive capability of the model and its utility as a field diagnostic tool using 3 independent groups of cattle of vary age, breed and production level.

## **Conclusions**

An increase in dietary total P and  $P_i$  directly impacted the amount of fecal total P and  $P_i$  excreted, with an associated increase in fecal P solubility. Increasing level of CGF supplementation has a beneficial effect on DM digestibility when forage quality is low for a growing beef steers. However, decreasing supplemental P from diets can reduce the amount of total P and water-soluble P in feces, reducing the potential for P runoff in surface waters from grazing cattle. Through accurate prediction of dietary P intake, P supplementation could be more strategically managed in order to more precisely meet the P requirement of grazing beef cattle.

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**Table 2.** Ingredient composition of diet, as fed

	<b>Diet</b>			
	<b>0</b>	<b>1</b>	<b>2</b>	<b>3</b>
Grass hay, kg	6.87	6.85	6.76	6.92
Beet pulp, kg	0.91	0.91	0.91	0.91
Corn gluten feed, kg	0.00	0.50	1.00	1.50
Fat, kg	0.34	0.34	0.34	0.34
Urea, g	95.25	72.57	49.90	31.75
Trace mineral salt <sup>1</sup> , g	18.14	18.14	18.14	18.14

<sup>1</sup>Champions Choice Selenium 90 trace mineral salt, Cargill, contained 57% Cl, 37% Na, 0.35% Zn, 0.2% Mn, 0.2% Fe, 0.03% Cu, 0.009% Se, 0.007% I, and 0.005% Co.

**Table 3.** Ingredient and nutrient composition of diets, % of DM

		Diet			
		0	1	2	3
Ingredient	Grass hay	81.02	76.50	72.22	68.98
	Beet pulp	11.35	10.71	10.22	9.55
	Corn gluten feed	0.00	5.90	11.26	15.78
	Fat	6.08	5.73	5.47	5.11
	Urea	1.30	0.94	0.61	0.37
	Trace mineral salt <sup>1</sup>	0.25	0.23	0.22	0.21
Nutrient	NDF	51.71	52.07	52.15	52.40
	ADF	31.18	31.00	30.46	30.17
	CP	10.47	10.24	10.39	10.36
	Ash	5.88	5.93	6.19	6.26
	Ca	0.64	0.61	0.60	0.57
	P	0.11	0.16	0.20	0.24

<sup>1</sup>Champions Choice Selenium 90 trace mineral salt, Cargill, contained 57% Cl, 37% Na, 0.35% Zn, 0.2% Mn, 0.2% Fe, 0.03% Cu, 0.009% Se, 0.007% I, and 0.005% Co.

**Table 4.** Diet DMI and digestibility (%) of beef steers fed increasing levels of corn gluten feed

	Diet				SEM	<i>P</i> -value		
	0	1	2	3		Linear	Quadratic	Cubic
DMI, kg	7.39	7.81	8.21	8.75	0.30	<0.01	0.58	0.77
DM, %	50.35	53.66	54.25	55.42	1.35	0.01	0.31	0.49
NDF, %	41.59	45.13	44.98	45.82	1.83	0.07	0.37	0.49
ADF, %	36.26	39.86	39.17	40.84	2.26	0.13	0.61	0.44
CP, %	57.55	55.56	53.94	53.26	1.19	0.01	0.51	0.88
Ash, %	30.09	30.65	36.15	32.76	2.62	0.23	0.43	0.22

**Table 5.** Phosphorus intake and excretion of beef steers fed increasing levels of corn gluten feed

	Diet				SEM	<i>P</i> -value		
	0	1	2	3		Linear	Quadratic	Cubic
TP from Hay, g/d	8.06	7.76	7.73	7.69	0.47	0.20	0.49	0.73
TP intake, g/d	8.72	12.59	16.75	20.88	0.37	<0.01	0.45	0.54
TP excretion, g/d	9.66	11.71	14.29	16.96	0.55	<0.01	0.44	0.76
Pi intake, g/d	5.16	7.09	9.07	11.16	0.20	<0.01	0.38	0.94
Pi excretion, g/d	4.11	5.93	8.36	9.92	0.34	<0.01	0.63	0.22
Po intake, g/d	3.56	5.51	7.68	9.73	0.23	<0.01	0.65	0.38
Po excretion, g/d	5.55	5.78	5.93	7.04	0.55	0.04	0.33	0.62
P solubility, %	42.67	50.52	58.54	58.83	2.43	<0.01	0.11	0.43
Serum Pi, mg/dL	5.61	5.87	6.64	6.80	0.36	0.02	0.89	0.50

<sup>1</sup>Fecal P solubility = (Pi excreted / TP excreted) \* 100

**Table 6.** Factor screening of variables used in P intake and excretion models<sup>1</sup>

Fecal Parameter <sup>2</sup>	TP Intake	Pi Intake
	<i>P</i> -value	
TP	<0.01	<0.01
Pi	<0.01	<0.01
Ca	<0.01	0.02
Ash	<0.01	0.08
CP	0.22	0.87
NDF	0.48	0.46
ADF	0.29	0.36

<sup>1</sup>Factor screening using backward elimination

<sup>2</sup>Parameters were expressed as a percentage of fecal DM

**Table 7.** Prediction variables used in P intake and excretion model

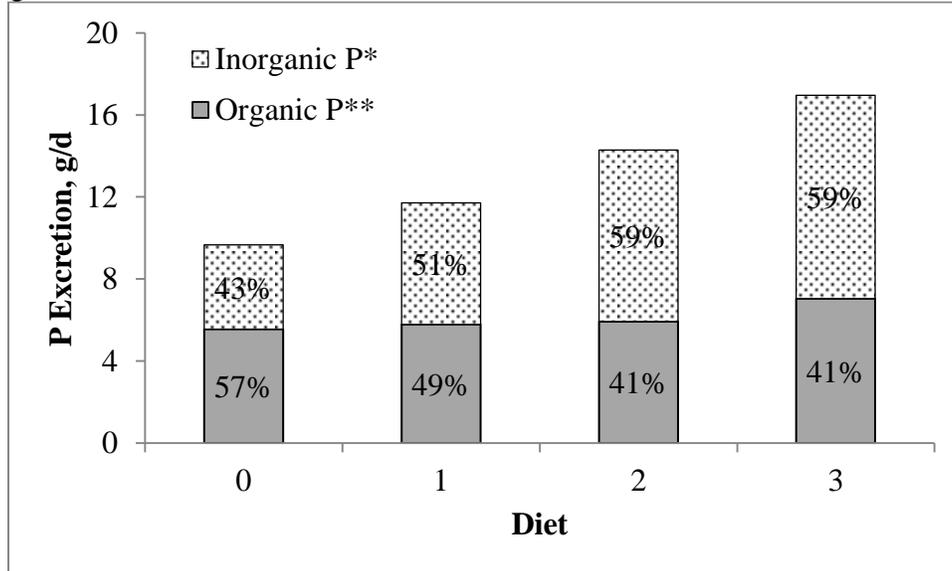
Fecal Parameter <sup>1</sup>	TP Intake <sup>2</sup>			Pi Intake <sup>3</sup>		
	Coefficient	SE	<i>P</i> -value	Coefficient	SE	<i>P</i> -value
TP	18.01	9.63	0.07	17.83	5.99	<0.01
Pi	18.27	11.74	0.13	12.97	7.31	0.08
Ca	18.64	3.36	<0.01	2.67	2.09	0.21
Ash	-1.12	0.42	0.01	0.16	0.26	0.55
TP x Pi	-87.38	25.20	<0.01	29.50	15.68	0.07
TP x Ash	-2.94	8.93	0.74	-14.50	5.55	0.01
TP x Ca	142.02	79.00	0.08	83.01	49.17	0.10
Pi x Ca	-109.63	83.77	0.20	-119.69	52.14	0.03
Pi x Ash	-3.19	9.83	0.75	11.85	6.12	0.06
Ca x Ash	-4.49	2.78	0.11	-3.26	1.73	0.07
TP x Pi x Ca	-140.99	16.95	0.37	-20.89	97.43	0.83
TP x Pi x Ash	47.90	16.94	<0.01	8.73	10.55	0.41
TP x Ca x Ash	137.32	64.68	0.04	119.21	40.25	<0.01
Pi x Ca x Ash	-197.59	80.13	0.01	-162.13	49.87	<0.01
TP x Pi x Ca x Ash	29.94	83.11	0.72	100.29	51.72	0.06

<sup>1</sup>Parameters were expressed as a percentage of fecal DM

<sup>2</sup>TP intake regression intercept = -6.49

<sup>3</sup>P<sub>i</sub> intake regression intercept = -5.32

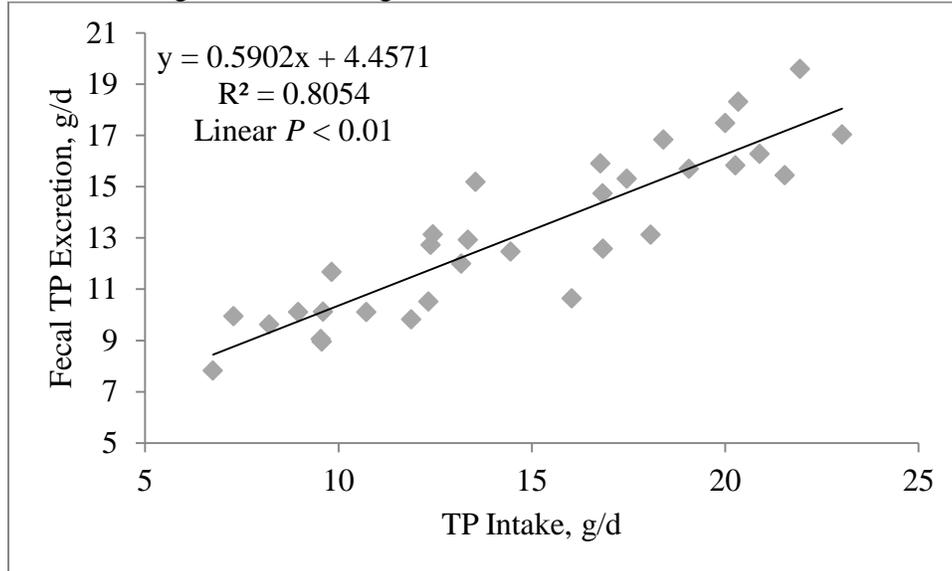
**Figure 1.** Fecal total P fractions of beef steers fed increasing levels of corn gluten feed



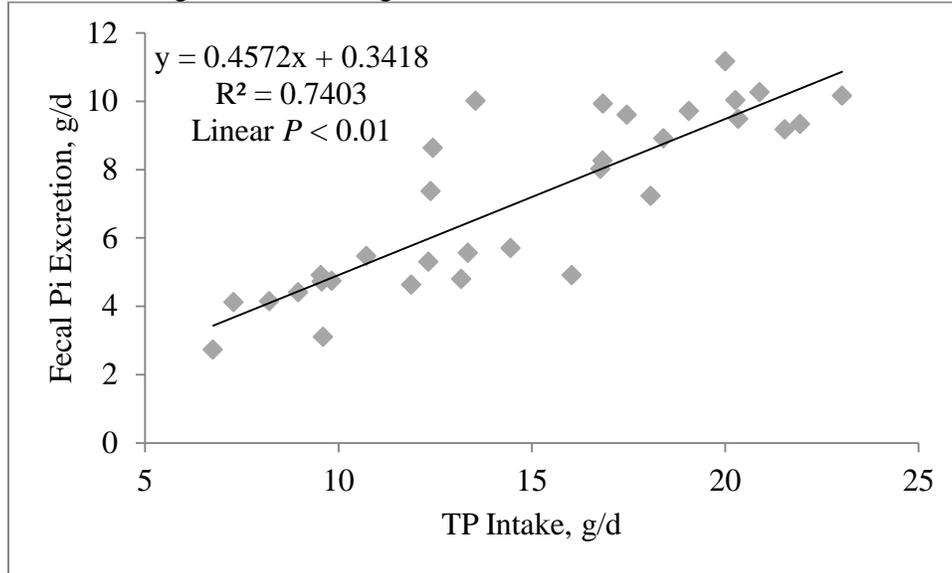
\*Linear ( $P < 0.01$ )

\*\*Linear ( $P < 0.05$ )

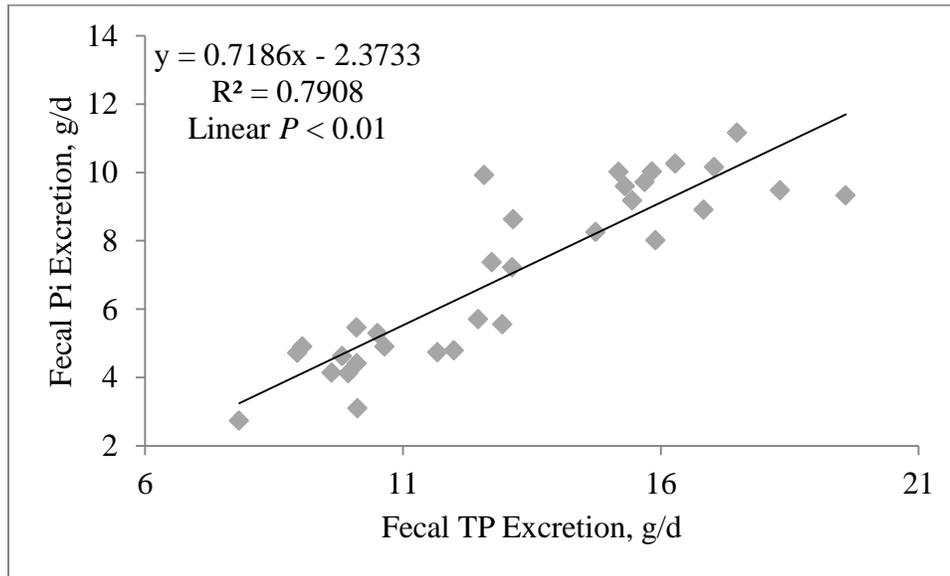
**Figure 2.** Relationship between total P intake and excretion in beef steers fed increasing levels of corn gluten feed



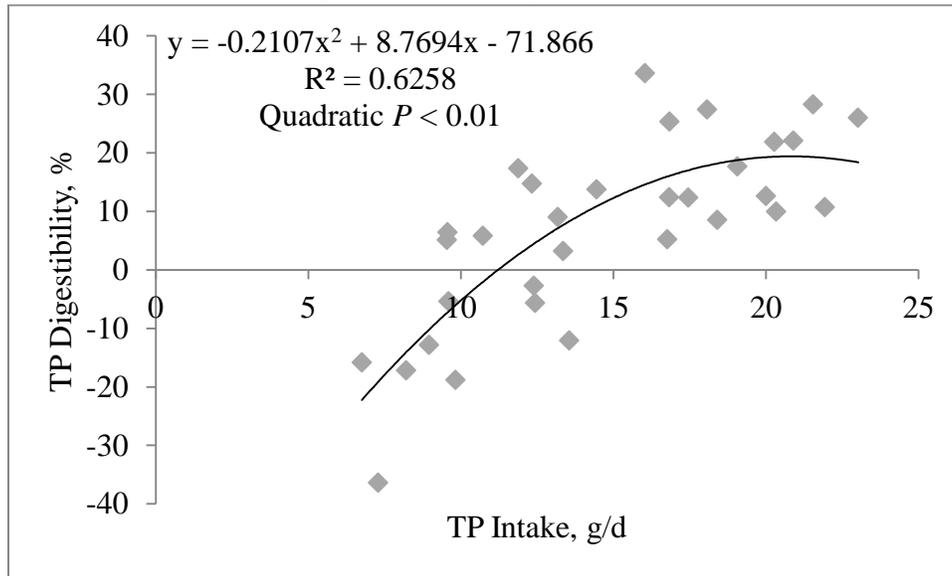
**Figure 3.** Relationship between TP intake and P<sub>i</sub> excretion in beef steers fed increasing levels of corn gluten feed



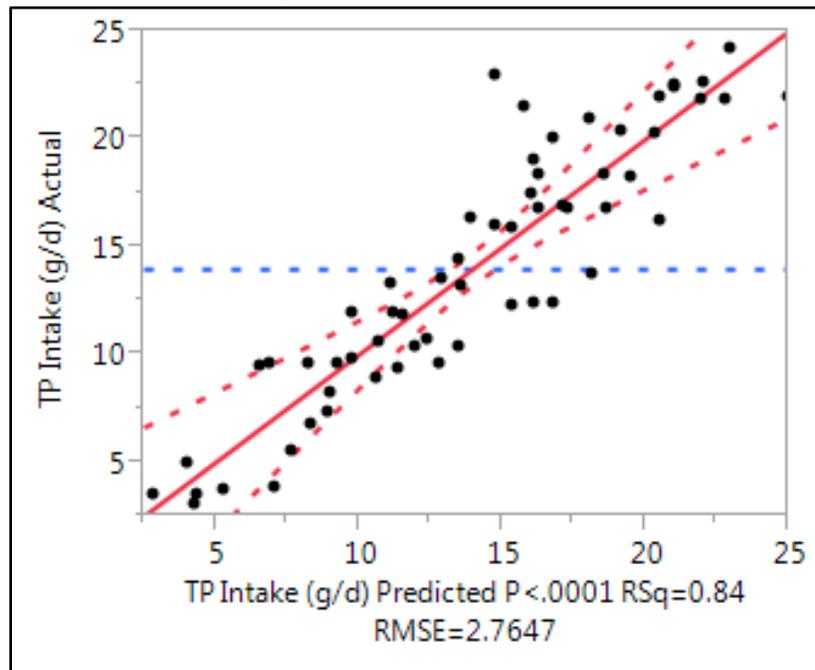
**Figure 4.** Relationship between total P and P<sub>i</sub> excretion in beef steers fed increasing levels of corn gluten feed



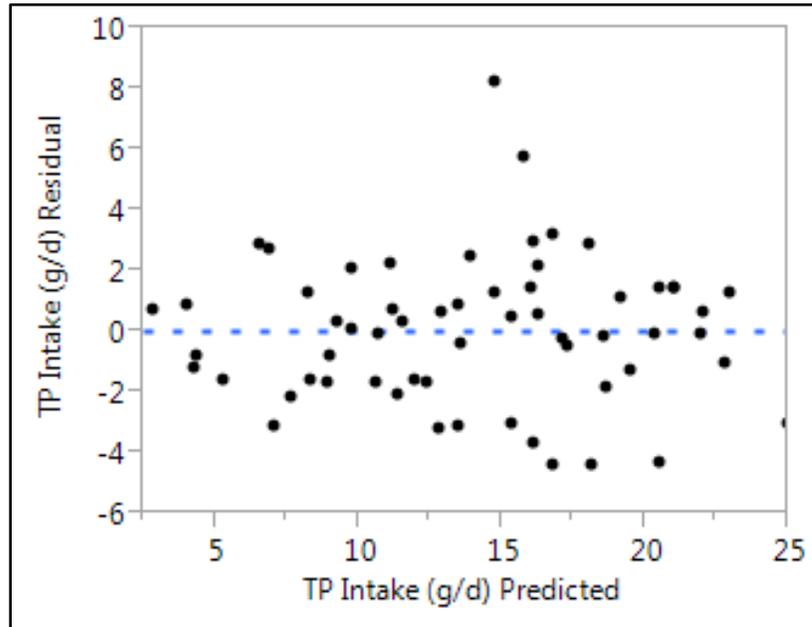
**Figure 5.** Relationship between total P intake and total P digestibility in beef steers fed increasing levels of corn gluten feed



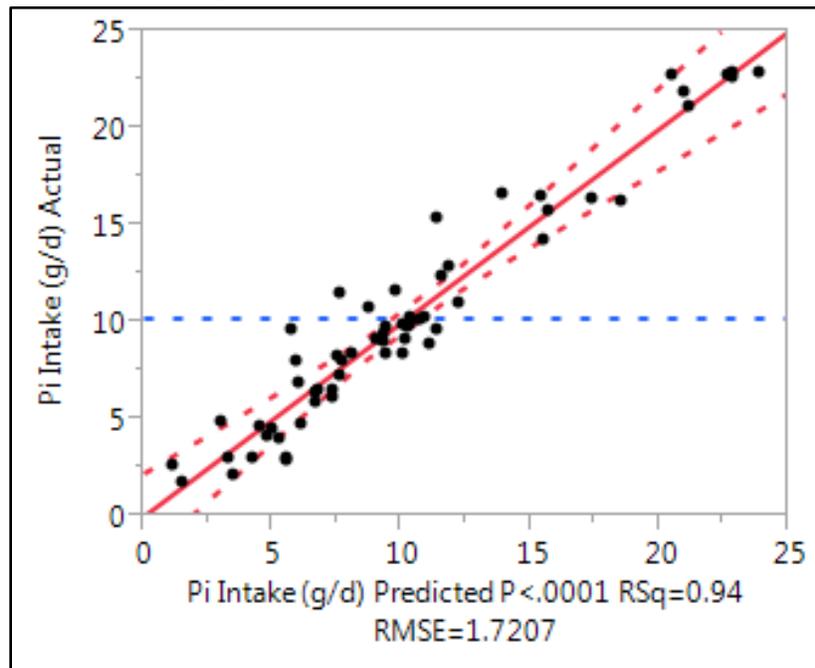
**Figure 6.** Regression relationship predicting total P intake in beef steers fed a forage based diet



**Figure 7.** Relationship of residual TP intake by predicted TP intake in beef steers fed a forage based diet



**Figure 8.** Regression relationship predicting P<sub>i</sub> intake in beef steers fed a forage based diet



**Figure 9.** Relationship of residual  $P_i$  intake by predicted  $P_i$  intake in beef steers fed a forage based diet

