EFFECTS OF ALUMINUM FROM WATER TREATMENT RESIDUAL APPLICATIONS TO PASTURES ON MINERAL STATUS OF GRAZING CATTLE AND MINERAL CONCENTRATIONS OF FORAGES

By

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TABLE OF CONTENTS

LIST OF TABLES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

EFFECTS OF ALUMINUM FROM WATER TREATMENT RESIDUAL APPLICATIONS TO PASTURES ON MINERAL STATUS OF GRAZING CATTLE AND MINERAL CONCENTRATIONS OF FORAGES

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Amorphous aluminum hydroxides applied to the land in the form of drinking water treatment residuals (Al-WTR) can reduce soluble P concentrations in soils and thus can reduce P contamination of the environment. Two experiments each using 36 grazing Holstein steers were conducted to determine the effects of Al-WTR pasture applications on the mineral status (principally P) and performance of cattle. A second objective was to evaluate the effects of the applied Al-WTR on bahiagrass (*Paspalum notatum*) mineral concentrations. The treatments were replicated 3 times each and were as follows: 1) control- no Al-WTR application with steers receiving free-choice mineral supplement without P, 2) control with free-choice mineral supplement plus P, 3) treatment 1 with Al-WTR, and 4) treatment 2 with Al-WTR. Total application of Al-WTR over two years was 75.8 Mg dry weight/ha on the pastures. The major minerals in Al-WTR are 7.8% Al, 0.73% S, 0.30% P and 0.30% Fe. There were no differences ($P>0.05$) in body weight gain among treatments for experiment 1, but for experiment 2, cattle subjected to the Al-WTR treatment without supplemental P had higher average final body weight ($P<0.05$) than the control treatment that received supplemental P (241 vs. 218 kg). In general,

there were few treatment effects on mineral concentrations in plasma, liver, and bone. Forage mineral concentrations for both years were generally unaffected by treatment but were affected ($P<0.05$) by collection dates (approximately every 28 days throughout the experiment). Most forage samples were deficient in Na, Cu, Se, and Co. At various collection dates, forages were deficient in Ca, P, Fe, and Zn. Forage concentrations of K and Mn were above cattle requirements and Mo concentrations were well below levels that would affect Cu metabolism. Similar quantities of the Al-WTR applied to pastures herein have previously been shown to reduce environmental P contamination to water sources. Results from the present experiment show that these Al-WTR applications had little effect on animal status of P or any other mineral analyzed. Likewise, Al-WTR had minimal effect on forage mineral concentrations. Lack of Al-WTR effects on cattle mineral status and production was likely due to low bioavailability of Al from Al-WTR. Therefore, Al-WTR is one effective method of reducing P contamination that does not adversely affect forage or cattle mineral concentrations.

CHAPTER ONE
INTRODUCTION

Waste product management has become a major concern in recent years. The amount of waste produced by the world's population far exceeds the places where it can be disposed of. Each industry has its own set of waste management problems, and the livestock industry is no different. In the United States alone, the livestock industry produces 500 million tons of manure each year (Lorentzen, 2004). Animal manure can be disposed of in several ways, but land application is the most prevalent method of disposal and has beneficial effects. Manure application to the land improves nutrient uptake because the manure acts as a fertilizer. However, excessive land application of manure can have negative environmental impacts, owing to excessive phosphorus (P) buildup in the soil. Too often, manure is over applied because of the large amounts of manure produced and the need to dispose of it. Repeated long-term manure application to the land leads to accumulation of P in soils (Novak and Watts, 2004). Once the manure and P have saturated the soil's P adsorptive capacity, additional P is subject to being washed away by heavy rains into lakes and streams, or leached downward into the groundwater system. This P lost as leaching and runoff can lead to eutrophication, causing the overgrowth of algae, and decreasing the survival of aquatic plants and animals (Novak and Watts, 2004). In addition, many coastal soils used in agriculture have been over fertilized, and much of the soil used by large industrial agricultural companies has reached its maximum capacity for P adsorption (Novak and Watts, 2004).

The drinking-water treatment industry has waste management issues as well. The major waste products of this industry are aluminum-water treatment residuals (Al-WTR). The Al-WTR are the solid sediments that result after water is coagulated, leaving behind amorphous Al oxides (Dayton and Basta, 2001). Usually these Al-WTR are placed in landfills for disposal. The Al-

WTR usually contains 5 to 8% total aluminum (Al). Because Al is highly reactive and chemically binds P, the application on manure containing soils could be one solution for P pollution of water systems by increasing soil P retention capabilities (Penn and Sims, 2002). Prior research from Florida has shown that amending soils with Al-WTR increases soil retention and reduces leaching of P (O'Connor et al., 2002). Applying Al-WTR to soils high in P could give the livestock industry another weapon for reducing water pollution as well as enhancing the perception that the industry is working towards solving the problem. Fortunately, large quantities of Al-WTR are available from holding ponds associated with drinking water treatment facilities in some areas.

Potential drawbacks to the use of WTR to address both the landfill overflow and P eutrophication, however, exist. The WTR may contain sufficient Al to constitute enhanced ecological risk for grazing animals, wildlife, surrounding floriculture, and water systems (USEPA, 2003). Grazing animals can consume up to 10 to 15% of total dry matter as soil and high dietary Al has been shown to influence the mineral status of the animals, including that of Fe, P, and Mg (Field and Purves, 1964; Healy, 1967; 1968). The objective of this study was to evaluate the effects of moderately high Al-WTR application rate on the performance and mineral status of grazing cattle and on the forage mineral content of pasture grass. The hypothesis was that, even at moderately high application rates, Al in the form of Al-WTR is of only limited availability to cattle, and does not negatively impact forage mineral concentrations.

CHAPTER 2
LITERATURE REVIEW

The History of Phosphorus

The significance of phosphorus (P) in the body and diet has been known since the 1700s. Even earlier, the disease "rickets" was common, but not yet associated with P until the 1800s. The essential nature of P in bone development was known in 1769, when bone ash was analyzed, and P was found to be one of the main components of bone material. Phosphorus deficiencies in humans were observed and described as early as 1785 (McDowell, 2003). By 1803, lime phosphate was being fed to children with rickets. The interest in P moved to advances in bone composition, which eventually led to other discoveries of the essentialities of P and calcium (Ca).

In the early 1900s, Van Niekerk (1978) used P supplementation studies to reveal the cause of bovine botulism and aphosphorosis. He showed that both conditions were the result of a severe P deficiency. Signs included sub-normal growth and reproduction and a depraved appetite or "pica" as illustrated by bone chewing in cattle. Other countries (Argentina, Brazil, and Senegal) also reported death from botulism as a result of bone chewing at different times (Dobereiner et al., 2000). Further, in Piaui, Brazil, an estimated 2 to 3% of approximately 100,000 cattle were dying annually of botulism. Botulism, rabies and plant poisoning are the three most important causes of adult cattle mortalities in Brazil today (Dobereiner et al., 2000). In the Gulf Coast area of Texas, Schmidt (1926) reported the prevention of a fatal P deficiency disease of cattle, referred to as "creeps", by bone meal and salt supplementation. Becker et al. (1933) classified the disease "stiffs" or "sweeney" in Florida and showed it to be caused by a deficiency of P.

More recently, a feed table publication in Latin America showed that 73% of all forages evaluated were P deficient (McDowell and Arthington, 2005). Even today, low P diets and diseases associated with them are problematic in most tropical regions of the world.

Phosphorus Metabolism and Requirements

Phosphorus does not occur freely in nature because it is too reactive. Therefore, all of the naturally-occurring P compounds are in bound forms, usually as phosphates. On the earth's surface, phosphates always occur in the form of orthophosphates (McDowell, 2003) Even in its bound form, P is the 2nd most abundant mineral element found in the animal body, and 80 to 85% is in bones and teeth (McDowell, 2003). The animal body could not function without P. It controls and is necessary for a wide array of functions in the animal's body, including reactions that drive glycolysis and oxidation of carbohydrates, intestinal absorption, lipid transport, and renal excretion (Georgievskii et al., 1981). Phosphorus is also a required part of the nucleic acid molecules, RNA and DNA. In fact, P and the rate of phosphorylation of the transcription factors affects the rate of nucleic acid transcription and translation, which, in turn, affects the rate of protein production (Berner, 1997). Phosphorus plays an important role in blood and body fluid buffer systems (Miller, 1985), and is necessary for normal muscle tissue synthesis and milk secretion (McDowell, 2003). It is even further involved in appetite control, and in the efficiency of feed utilization, an important facet of animal production (Ternouth and Sevilla, 1990; Underwood and Suttle, 1999).

Although P deficiencies are documented in history, P problems are easily preventable. Phosphorus is present in all common feedstuffs. Adding seeds that are typically higher in P, than are roughages, and seed by-products, such as wheat bran and oil meals, can ensure adequate P intake. Feeds containing milk and bone are also high in both P and Ca (McDowell, 2003).

Ruminants may have less of a chance of P deficiency under normal conditions considering that they secrete P naturally in their saliva. Tomas and Somers (1974) suggested, after looking at studies by Australian workers, that salivary glands also can play an important role in P homeostasis. They do this by controlling the amount of P in the gut. Sheep studies provided evidence for this function by ligation of both parotid salivary ducts, a procedure that led to a small increase in urinary P excretion and a proportional reduction in fecal P excretion (Tomas and Somers, 1974). Therefore, ruminants have a higher renal threshold for P excretion than do monogastric species, and this can be an advantage in P deficient situations.

The absorption of Ca and P occurs throughout most of the intestinal tract with the duodenum and jejunum being the most active absorptive sites (McDowell, 2003). This absorption is influenced by many factors including: source of P, intestinal pH, animal age, intestinal parasitism and dietary intakes of several other minerals including Ca, Fe, Al, Mn, K, and Mg (MacRae, 1993; McDowell and Arthington, 2005). Large intakes of Fe, Al, and Mg can interfere with the absorption of P by forming insoluble phosphates, changing the requirements for the animal entirely (McDowell, 2003).

Requirements for P also vary depending on a variety of factors including: age, sex, activity, bioavailability of P, protein and energy in the feed, stress, interactions between feed ingredients and nutrients, digestive anatomy, and reproductive status of the animal (McDowell, 2003). Therefore, any number of factors can easily affect the absorption of P and the P requirement for the animal. Ruminant animals have a lower requirement for P than monogastrics (NRC, 1985) because ruminants, unlike monogastrics, are able to use phytin P from plants. Only about 1/3 of P in most plants in the form of phytate is actually available for use by nonruminants and, for proper absorption, P must be in the available form (McDowell, 2003). Incomplete

uptake by plants can be linked to a variety of factors as well. An unavailable chemical form of P in the plant, physical barriers in the plant wall, or antagonistic substances such as oxalic acid and phytic acid which can bind P, Ca, Fe, Mn, and Zn can all affect the uptake in the plant, thereby affecting the animal's food supply (McDowell, 2003; McDowell and Arthington, 2005).

Phosphorus and Ca work together in the body, so their requirements are also linked. A dietary Ca: P ratio between 1:1 and 2:1 is assumed to be ideal for growth and bone formation as this is approximately the ratio of the two elements in bone. A close ratio is even more critical if P intake is marginal or inadequate (McDowell, 2003). Many factors influence Ca and P absorption, utilization, and metabolism, including adequate levels of one another. The status of Vitamin D is also important to maintain a desirable Ca: P ratio (McDowell, 2003). In a study of nine Ca:P ratios ranging from 0.41:1 to 14.3:1 tested by Wise et al. (1963), dietary ratios below 1:1 and over 7:1 caused a decrease in both growth and feed efficiency in animals.

Requirements can also be studied on the basis of P alone. The minimum P requirements recommended by the NRC (2005) may be too high for grazing beef cattle. In a Utah study, no difference in average weight gains (0.45 kg/day), feed efficiency, or appetite were observed in Hereford heifers fed for 2 years a diet containing 0.14% P (66% of NRC recommendation) and comparable heifers receiving the same diet supplemented with monosodium phosphate to provide a total of 0.36% P (Call et al., 1978). Williams et al. (1991) compared two levels of dietary P (0.12, 0.20%) on various chemical, physical and mechanical properties of bone in growing beef heifers. In relation to the requirement, 0.12% P was inadequate as heifers receiving 0.20% P had stronger bone properties and improved overall performance. Since there are so many factors influencing the P requirements for the body, it is difficult to place a normal

level on any given species without first looking at the different factors and interrelationships involved with P.

Phosphorus Deficiencies

Even with a high renal threshold and available P in common feedstuffs, lack of P is still the most prevalent mineral element deficiency for grazing animals worldwide (McDowell and Arthington, 2005). Some deficiencies can occur from antagonists alone. Certain elements found in low pH tropical soils, such as Fe and Al, can hinder P absorption in the animal. When dietary P becomes low or unavailable, an early physiological response is a decline in inorganic plasma P. Normal plasma P levels in ruminants are usually between 4.5 and 6 mg/100 ml. (McDowell and Arthington, 2005) However, when the plasma levels fall, anorexic conditions occur that decrease feed efficiency and slow energy metabolism in ruminants, such that growth and production levels decline. A significant decline in mineral P concentrations also reduces the ability of animals to properly digest fiber, protein, and carry out normal metabolic functions (McDowell, 2003).

Deficiencies of P in grazing ruminants have been reported in 46 tropical countries in Latin America, Southeast Asia, and Africa. The soil and forages in these livestock-grazing areas of tropical countries can be very low in P (McDowell, 2003). For example, younger grasses may contain 0.3% P, but mature forages may contain 0.15% P or less (McDowell, 2003; McDowell and Arthington, 2005). Williams et al. (1990) used a noninvasive dual photon absorptiometry technique to show that dietary levels of 0.12 to 0.13 % P lead to bone demineralization in Angus heifers. A heifer fed mature forages with poor mineral supplementation could easily fall below the 0.12% P requirement. When P is deficient, bones can bend and fracture even during normal activities, causing lameness or death (McDowell, 2003)

The most devastating economic result of P deficiency is reproductive failure. Failure to reproduce is associated with loss of body weight and body condition, which are the result of decreased intake of feed. The decreased intake of feed could be caused by decreased appetite, impaired locomotion, or both. More research is needed to establish whether reduced reproduction is caused by lack of P or if it is mediated through decreased body condition instead (Dunn and Moss, 1992). Animals deficient in P have been known to go two to three years without calving, a huge economic loss for the cattle industry (McDowell, 2003).

Phosphorus Impact on the Land

There is increasing public demand to reduce P transport to water bodies at risk of eutrophication from agricultural P-inputs. One major agricultural input is the land application of animal manure (Agyin-Birikorang et al., 2007). Although there are rules and regulations in place to reduce the amount of manure spread on land each year, many operations go unchecked, causing major environmental concerns. The manure does positively affect the nutrient quality of the soil in the long run, acting as a fertilizer, but the majority of P applied to the land as manure often is converted to insoluble forms in the surface horizon of the soil. The accumulated P is subject to erosion or runoff following heavy rains and transport to surface water. Regulations on manure application rates are placed in order to avoid the P pollution of surface waters (Dayton and Basta, 2001; O'Connor et al., 2002; Dayton et al., 2003). According to recent environmental research, the sheer mass of manure generated by large animal facilities poses risk to ground and surface water (Lorentzen, 2004) . The EPA suggests that farming creates 455 million metric tons of manure each year (Lorentzen, 2004).

Many states have passed more strict rules on the land application of animal manure. In Maryland, for example, all P applications must abide by the Water Quality Act of 1988, which dictates soil testing of P and determines to the degree of soil saturation with P, and if manure

application can be permitted. In Virginia, food animal practices are closely scrutinized, particularly the poultry industry. In Delaware, manure can usually be applied once every three years to comply with soil P limitations (Penn and Simms, 2002) However, in California, lax enforcement of the law has led to an overabundance in the pollutant count in several bodies of water with both P and N. Leaching and runoff from manure enriched soils is thought to be the primary cause. The reality is that this type of manure utilization is a matter of convenience, availability, and profitability rather than providing the optimal nutrients for the flora or concern for the ecosystem (Farm Press, 2004). Both N and P are constituents within animal waste products, and are harmful pollutants, yet federal, state, and county standards differ in the applicable uses and concentrations of manure for land, resulting in confusion as to how manure should be properly applied. The National Resource Conservation Service (NRCS) has attempted to control over-fertilization by implementing nutrient management plans. In most cases, though, the manure application rates for nutrient management plans are based on the N needs of the crops. When the amount of manure applied to cropland is based on crop N needs and not on the amount of P needed, over-application of P may occur. (Shirmohammadi and Ritter, 2000).

Calcium

Together, Ca and P are the most abundant minerals in the animal's body. Combined, these two minerals make up 70% of the minerals found in the body (McDowell, 2003). Calcium, by itself, is the most abundant mineral element in the animal (1-2%), and 99% of Ca occurs in bone and teeth. The remainder, constituting the physiologically active pool of free Ca, is found in the extracellular fluid and within cells (McDowell, 2003). Calcium is important to many of the processes necessary for life to function properly. For example, Ca is essential for normal blood clotting. The Ca ion must be present for prothrombin to form thrombin, which then reacts with fibrinogen to form the blood clot, fibrin (McDowell, 2000). Calcium also plays a role as a

cofactor in many enzymatic reactions, acting as an activator or stabilizer of enzymes (Peo, 1976) and is necessary for the secretion of a number of hormones and hormone-releasing factors (Arnaud and Sanchez, 1996). Dietary Ca can act as a defense mechanism by decreasing gastrointestinal lead (Pb) absorption and thereby reducing the risk for Pb poisoning in the body (Ballew and Bowman, 2001) or as a contributor to the regulation of the cell cycle (McDowell, 2003).

Regulation of Ca and P occurs as a result of the hormone 1,25-dihydroxycholecalciferol, parathyroid hormone, and calcitonin. Normal levels of all depend on factors such as bodily excretion, bone deposition, resorption, and intestinal absorption (McDowell, 2003). If Ca and P needs are not met through the diet, tetany can occur as the animal draws Ca and P from the bone in order to maintain its normal blood concentrations (McDowell and Arthington, 2005). However, animals will also absorb Ca from their gut according to need, and they can alter the efficiency of absorption to meet a change in requirement. The ARC (1980) documented that young sheep have a high Ca requirement and absorb Ca at a higher rate than do mature animals with a low requirement. In contrast to that of Ca, the percentage of P absorbed is not so closely tied to the needs of the animal (ARC, 1980). Therefore, when dietary Ca is relatively low, most of the Ca absorption is by active transport (Goodrich et al., 1985). Serum Ca concentration also varies little in spite of large changes in dietary Ca because of the body's internal method of endocrine regulation. Blood cells are almost entirely devoid of Ca, but healthy plasma contains from 9 to 12 mg per 100 ml in most species, and in all species, feces are the primary path for Ca excretion (McDowell, 2003).

Calcium is generally abundant in most forages (McDowell, 2003). Non-legume roughages such as grass hay and mature range forages have average Ca concentration (0.31-0.36%), and

legume forages such as alfalfa and clover hay contain 1.2 to 1.7% Ca (NRC, 2005). When there is insufficient dietary intake of Ca and the needs of Ca are high, as in a lactating dairy cow, a Ca deficiency called "milk fever" can occur. Milk fever in dairy cows is caused by a temporary imbalance between Ca availability and high Ca demand following the onset of lactation (Oetzel, 1996). Despite much research, milk fever incidence has remained steady in the United States at 8 to 9% (Goff, 1989).

Magnesium

Magnesium is the 2nd most plentiful cation of intracellular fluids and is widely distributed among plant and animal tissues with some 70% of total body Mg present in bones (McDowell and Arthington, 2005). As a major macromineral, Mg has a very important role as an essential ion in many fundamental reactions in intermediary metabolism and also as an "activator" of enzymes. In fact, Mg is vitally involved in the metabolism of carbohydrates and lipids as a catalyst of a wide array of enzymes which require this element for optimal activity. Magnesium is involved in at least 300 enzymatic steps in intermediary metabolism (Shils, 1996). It is also involved in certain steps of protein synthesis through its action on ribosomal aggregation, its roles in binding messenger RNA to 70S ribosomes, and in the synthesis and degradation of DNA. Furthermore, Mg plays an important role in neuromuscular transmission and activity (McDowell and Arthington, 2005).

Many dietary factors influence Mg absorption as well as Mg requirements, and include: K, Ca, P, Al, Fe, Na, protein, fat, organic acids, carbohydrate type, ionophores, Mg status, and frequency of feeding (Fontenot et al., 1989). In mature ruminants, the reticulorumen is the principal site of Mg absorption (Thomas and Potter, 1976). As a regulation mechanism, the proportion of Mg absorbed declines with increasing dietary levels (Heaton, 1960), and the Mg status of the animal alters Mg absorption (McAleese et al., 1961). Minimal requirements of Mg

for growth of grazing livestock can generally be met by pastures and diets containing 0.10 to 0.15% (McDowell, 2003). Henry and Benz (1995) reported that apparent availability of Mg in fresh grasses or grass hays varied greatly from -4% to +66%. Seemingly, most practical diets contain adequate Mg to promote optimal performance (McDowell, 2003).

The serious metabolic disorder in cows, known as lactation tetany or grass tetany, was shown to be associated with subnormal serum Mg values (McDowell, 2003). For diagnosis, the ranges in serum or plasma Mg level (mg/100 ml) for cattle and sheep are as follows: normal values, 1.8-3.2; slight hypomagnesemia 1.2-1.8; and severe hypomagnesemia 1.2 or less (NCMN, 1973). Hypomagnesemia tetany in sheep is almost exclusively a disease of the first 8 weeks of lactation with ewes nursing twins being the most susceptible. The incidence is highest 1-4 weeks after lambing (Herd, 1966). In Australia, a high incidence of hypomagnesemia tetany in breeding ewes has been correlated with periods of rapid winter growth of pastures (Underwood and Suttle, 1999). Fertilization can also be a factor in the incidence of grass tetany. In general, high N fertilization results in lower serum Mg concentration and a higher incidence of grass tetany (Kemp, 1983). Grass tetany is endemic in some countries, affecting only a small proportion of cattle (1 to 2%). However, individual herds may report incidence of tetany as high as 20% (McDowell and Arthington, 2005). The incidence of nonclinical hypomagnesemia, although not usually characterized by death, is far greater than grass tetany, and the economic consequences of lowered production can be substantial.

Aluminum

Aluminum is the third most abundant element in the earth's crust, followed by silicon and oxygen. It is also the most common metal found in the earth's crust (O'Connor et al., 2002). Aluminum and P are similar, not in their essentiality per se, but in their reactive nature. Given that Al is highly reactive, it does not normally appear in its elemental form, and instead it is

bound to other elements or compounds like P in nature (McDowell, 2003). Considered by some to be a trace element, Al is not actually needed by the body and is considered to be a toxic element in most cases. It is generally not considered to be an essential element and has no critical levels but may possibly be required in female rodents (McDowell, 2003). Yet, Al may still perform some positive functions when utilized as a cofactor in some processes. For example, Al promotes the reaction between cytochrome *c* and succinic dehydrogenase (Horecker et al., 1939) and has been shown to be a cofactor for the activation of guanine nucleotide-binding regulating protein by fluoride for the stimulation of anenylate cyclase activity (Sternweis and Gilman, 1982). However, it is not at this time known to be involved in these reactions in the human body.

Most of the time, Al causes problems for the body. Aluminum can accumulate in the body whenever uptake exceeds the disposal of this metal via urinary biliary excretion (Greger and Sutherland, 1997). It would appear that the major pathway for the elimination of any absorbed or systematically administered Al is the kidneys (Alfrey, 1986). Toxic accumulations most often result from large oral intakes, contaminated parenteral solutions, or in individuals with renal insufficiency. Aluminum toxicity has been clearly demonstrated in patients with renal failure (Flaten et al., 1996). The mechanisms by which Al exerts its toxicity are not well understood, although Al has been shown to interfere with a variety of biological and enzymatic processes (Alfrey, 1986).

Garrel et al. (2000) suggested that the deleterious effect of Al may be to decrease the level of antioxidant enzymes through an interaction with DNA, which would then alter the ability of cells to be effectively protected from oxidative stress. Regarding its skeletal toxicity, Al has been shown to be deposited in the mitochondria of osteoblasts and to inhibit formation of bone

phosphates as well (Lieberherr et al., 1982). Additionally, Al toxicity can alter the regulation of calbindin, a vitamin D-dependent protein (Cox and Dunn, 2001). Along with other toxic effects, research has shown that excess Al can form complexes with P and other minerals affecting their utilization prior to absorption, thereby affecting mineral metabolism post absorption (Valdivia et al., 1982). Other toxic effects of Al include ability to inhibit cell extension and division and interference with plant mineral nutrition (Matsumoto et al., 1977). Because Al binds to DNA in plant cell nuclei, it can limit template activity, which would further explain much of the deleterious effects of Al on plant metabolism (Matsumoto et al., 1977).

In humans, Al has three potential toxic effects: (1) a local effect in the gastrointestinal tract, (2) the potential to cause pulmonary damage if inhaled, and (3) a capacity to exert systemic toxicity if absorbed or parenterally administered. The latter effect can also be broken down further into neural and skeletal toxicities. Aluminum can bind to brain calmodulin and change calbindin's configuration and its interaction with other proteins (Siegel and Hang, 1983). Regarding its skeletal toxicity, Al has been shown to deposit between mineralized and unmineralized bone at the calcification front preventing tetracycline labeling. This would suggest that it might locally prevent further mineralization of osteoid (Malony et al., 1982). Further, Al has been shown to inhibit bone phosphates (Lieberherr et al., 1982). Aluminum, especially in association with citrate, has been shown to inhibit crystal growth, which might also interfere with mineralization. Some suggest that inhibition of glycolysis and phosphorylation is the most toxic reaction caused by Al (Sorensen et al., 1974). Aluminum has also been shown to displace Mg from ATP with the resulting stabilization of ATP, thereby preventing phosphate transfer by Na^+, K^+ -ATPase (Harrison et al., 1972; Lai et al., 1980). All phosphate-transferring systems involving ATP and Mg may be biological targets for Al (Sorensen et al., 1974).

The maximum tolerable levels of Al for livestock include: cattle and sheep, 1000 ppm; swine, poultry, and sheep, 200 ppm (NRC, 2005). Soil Al concentrations can range from 1-30% but are typically 0.5-10% by weight (O'Connor et al., 2002). Since grazing animals can consume up to 10% of their daily intake as soil, Al toxicity is a major concern for many causative factors leading to decreasing animal health and profitability. Ingestion of soil containing Al has been implicated as a causative factor of grass tetany (Dennis, 1971; Allen and Robinson, 1980). With the increase of acid rain from pollutants in the air, Al has been rendered more soluble in surface waters and has a greater potential for causing plant toxicity as well (Johnson et al., 1984).

Interactions Between Al and P

Aluminum may interact with essential elements, namely P, Ca, Mg, and fluoride (F), and adversely affect their metabolism in animals (Allen, 1984). With the unstable, reactive nature of Al and P, as well as their abundance in most soils, interactions between these minerals may increase dietary requirements for P and cause toxic and/ or deficient implications with Al and P, respectively.

It is not uncommon to find high amounts of Al complexes in tropical sandy soils that bind soil P and make it unavailable for plant uptake (McDowell, 2003). In some cases, soil Al may be in a less available chemical form than that of the inorganic salts and thus have less effect on P availability (Robinson et al., 1984). Nevertheless, land application of highly soluble Al salts, e.g. Al chloride ($AlCl_3$) and other Al compounds are used to combat P runoff and leaching. Many researchers suggest that excess dietary Al interferes with P utilization by forming unavailable complexes in the gastrointestinal tract (Jones, 1938; Storer and Nelson, 1968). For example, Rosa et al. (1982) reported that increases in dietary P in sheep increased feed intake while it was decreased by adding Al and Fe. Increased dietary levels of Fe and Al in sheep diets

resulted in a decrease in ADG from 157 to 97 g/d in high Fe diets and from 159 to 95 g/d in high Al diets. When additional P was added to diets containing high Fe and Al, average daily gain (ADG) losses were minimized. The rationale for this response was that the diet being fed was borderline to deficient in P either at 0.17% to 0.23% (NRC, 1985). Additionally, plasma P levels increased with high Fe and decreased with high Al diets (Rosa et al., 1982). What seems to be happening in the body is that an insoluble complex of Al and P is formed in the digestive system of the animal, binding P and making it unavailable to the animal. This has been seen in sheep fed high levels of Al, and signs of P deficiency resulted (Valdivia et al., 1982). Rosa et al. (1982) concluded that excessive bioavailable dietary Al increases P requirements. Animals are not the only ones to suffer either. Crop yields could be negatively impacted if too much P is bound to Al or if increases in heavy metal contents are realized within the soil as well (Novak and Watts, 2004).

Water Treatment Residuals

Water treatment residuals (WTR) are by-products of water purification procedures. They are rich in metals like Al and Fe, though the exact composition can vary due to the process in which they are made and the age and dryness of the WTR (Dayton et al., 2003; Ippolito et al., 2003). Several authors (Elliott et al., 2002; Basta et al., 2003; Lind, 2003; Novak and Watts, 2004) have suggested that WTRs can serve as low-cost soil amendments to reduce environmental impacts of various oxyanions, notably P and As. However, these drinking-water treatment plant facilities use different water sources and different chemicals, and the WTR produced can have widely different elemental compositions and P sorption capacities (Makris and O'Connor, 2007)

During the water treatment process, a chemical, called a coagulate, is added to the water. This causes a reaction and forms a flocculent precipitate, which coats small particles, such as

clays making them more likely to be removed by sedimentation or filtration. Aluminum, iron, or calcium sulfate coagulates may be added to unfiltered water, which is then circulated with vigor to uniformly disperse the product. The product then reacts readily with alkaline products within the water and produces a hydroxide solid, which has entrapped impurities (Dayton and Basta, 2001; Brady and Weil, 2002; Ippolito et al., 2003; Water Resources, 2005). In the basic pH environment of a typical drinking-water treatment facility, Fe or Al salts added in the raw water hydrolyze to form Fe or Al hydroxides. This process of coagulation and sedimentation usually precedes filtration in a water treatment plant, and serves to reduce turbidity and increase the efficacy of bacterial removal by filtration (Dayton and Basta, 2001; Brady and Weil, 2002; Ippolito et al., 2003; Water Resources, 2005).

Poorly P-sorbing soils are abundant in southeastern United States. These sandy (coarse-textured) soils are characterized by low P-sorbing capacities, and are often accompanied by high water tables. This combination of characteristics makes such soils vulnerable to P losses and negative water quality impacts (Makris and O'Connor, 2007). Highly reactive Al could be one solution to managing the P pollution, but with possible harmful side-effects. There is a better answer. More than 2 million Mg of Al-WTR are generated from drinking-water treatment facilities in the U.S. every day (Prakash and Sengupta, 2003), making Al-WTR a suitable substitute for highly reactive Al compounds to reduce the P pollution problem. These Al-WTR can be disposed: a) directly to the receiving stream; b) to sanitary sewers; c) to a landfill, assuming that the residual contains no free-draining water and does not have toxic characteristics as defined by the Toxicity Characteristic Leaching Procedure (TCLP) test; and d) by land application (Chwirka et al., 2001). Al-WTR are specifically exempt from the 40 CFR Part 503 land disposal regulation for biosolids (USEPA, 1996) Thus, Al-WTR can be land-applied

without having to meet metal (including As) limitations of the Part 503 regulation, which will allow for easier, less-expensive application Also, the disposal cost of non-hazardous Al-WTR is low; estimated at < $50/Mg (Meng et al., 2001). Therefore, land applicaton of Al-WTR can be a cost-effective treatment for effectively sorbing excess levels of labile P (or other oxyanions, e.g., As, Mo, Se) in soils. The high amorphous Al or Fe content of the Al-WTR would be expected to increase a soil's P-sorption capacity (Elliott et al., 1990).

The physical and chemical characteristics of Al-WTR are quite similar to top soils (Haustein et al., 2000). Elliott and Dempsey (1991) tested Fe and Al-WTR and showed that mean total metal concentrations of heavy metals Cr, Ni, Pb, and Zn were within the common range for soils, implying that total metal soil concentrations will remain largely unaffected by Al-WTR application at typical loading rates. Work by O'Connor et al. (2002) showed that a Ca-WTR was much less effective in sorbing P than a Fe- and an Al-WTR. X-ray diffraction analysis of Fe- and Al-WTR suggests that amorphous Al or Fe hydroxides dominate the Al-and the Fe-WTR, respectively, with no apparent crystalline components. Oxalate-extractable Al values are typically 80-90% of total Al of Al-WTR, which supports the amorphous model of Al-WTR (Makris and O'Connor, 2007), and allows for the uptake of P. Gallimore et al. (1999) concluded that the amorphous, rather than the total Al content of Al-WTR determines their effectiveness in reducing runoff-P (Makris and O'Connor, 2007).

The Pros and Cons of Al-WTR

High dietary Al has been shown to influence status of Fe, Zn, P, Mg, and bone ash in sheep (Rosa et al., 1982). Research at the University of Florida concluded that increases in dietary Al levels reduced feed intake, gains and P plasma concentrations in sheep (Rosa et al., 1982). The Al given to these animals was in the form of $AlCl_3$. The impact of additional Al was not positive for animal gains as ADG was 105 and 148 g/d for those consuming a high Al or low Al diet,

respectively. When additional dietary P was given, ADG increased, but not to the level in animals not consuming any Al (Rosa at al., 1982). This indicates the problem with using Al in such a reactive form. In addition, other mineral plasma concentrations were also impaired with increased dietary Al. Magnesium content was depressed in the kidneys and bone of those animals receiving high dietary Al (Rosa et al., 1982). Similar results have also been documented at Rutgers University in avian species. Young chicks and mallard ducks fed high Al diets (as $AlCl_3$) had a high incidence of P binding, lowered P serum levels, depressed growth, lowered tibia weights and lower bone mineralization (Capdevielle et al., 1998).

Al-WTR may seem like the end-all solution to P pollution, but some researchers are skeptical. Also, laboratory and greenhouse data suggest that application rates as high as 25 T/A are required to control P solubility in highly P-impacted soils (O'Connor et al., 2002). Regardless, the use of Al-WTR and metal-binding by-products could be one solution to the accumulation of P on the top layer of soil, which leads to nonpoint pollution during heavy rains (Penn and Sims, 2002). Research from Florida has shown that amending soils with Al-WTR can increase soil P retention and dramatically reduce its leaching potential (Elliott et al., 2002; O'Connor et al., 2002). Al-WTR are particularly effective in controlling soluble P in acid sandy Florida soils of limited P sorption capacity (Elliott et al., 2002). In particular, Al-WTR would benefit sandy soils low in organic material. Sandy soils tend to provide little P retention capabilities and P loss is likely. Soils that are saturated with P may also benefit from Al-WTR application. Phosphorus-saturated soils are unable to hold additional P, which can result in P ground water contamination (Penn and Sims, 2002). Other research shows that added Al in the form of Al-WTR may help depress P losses by increasing soil P retention capabilities (O'Connor et al., 2002; Penn and Sims, 2002). Phosphorus adsorption capacity was increased by 20 times

with the use of Al-WTR when compared to high Al clay (Haustein et al., 2000). Gallimore et al. (1999) applied an Al-WTR to poultry litter-amended soils, and reduced soluble P in surface runoff. Haustein et al. (2000) documented decreasing P concentrations in runoff from fields excessively high in soil test P following amendment with an Al-WTR (rates up to 18 Mg/ha). Moreover, Peters and Basta (1996) significantly reduced (~50% of the initial values) soil test-extractable P concentrations of an acidic and calcareous soil incubated with high loading rates of two different Al-WTR (~60 and 200 Mg/ha). Codling et al., 2000 incorporated either Fe- or Al-based WTR into poultry litter-amended soils and significantly reduced P-leaching. Similarly, surface-applied Al-WTR had little effect on P availability to wheat in a greenhouse study, but incorporation into the entire soil significantly decreased P availability (Cox et al., 1997). Elliott et al. (2002) showed that either Fe- or Al-WTR reduced P leaching in a low P-sorbing FL sand amended with dewatered biosolids and triple superphosphate (TSP) fertilizer.

More studies are now available that compare the differences in $AlCl_3$ with Al-WTR. In a recent study at the University of Florida, lamb ADG, BW, and intakes were unaffected by dietary Al-WTR ($P>0.05$) when compared to the control. However, lambs fed 2,000 ppm Al from $AlCl_3$ had reduced growth and lower ADG ($P<0.05$) than other treatments (VanAlstyne et al., 2005). The Al-WTR did not appear to negatively affect performance of growing sheep. The apparent P absorption data strengthens the idea that Al in the form of Al-WTR is less available to the animal than the Al in $AlCl_3$. Additionally, plasma P and tissue mineral levels, with the exception of brain Al, were not altered with the administration of Al from Al-WTR. Under these experimental conditions, dietary administration of Al from Al-WTR did not cause physiological tissue damage. Overall, it was demonstrated that Al from Al-WTR does not negatively impact a

growing lamb's health or performance and could be administered at levels as high as 8,000 ppm Al without causing detrimental effects (VanAlstyne et al., 2005).

One question asked by many environmentalists is whether the fixed P will ever dissociate from the Al-WTR. In the past, insufficient data were available on the long-term stability of P retained by Al-WTR, or soils amended with Al-WTR, or metal salts, and the long-term stability of immobilized P was a major concern of state and federal regulators (Makris and O'Connor, 2007). A 6.5 year field study of Al-WTR effectiveness in reducing water extractable P in two soils with excessively high soil test P levels was conducted (Jacobs and Teppen, 2000, ; Agyin-Birikorang et al., 2007; Makris and O'Connor, 2007). Soil samples taken each year for up to 6.5 years after an initial Al-WTR application showed sustained reduction (up to 63% initial values) of water-soluble P levels in the Al-WTR-amended plots.

Potential Al-WTR particle dissolution, particularly under acidic conditions, is a concern with respect to Al-WTR field applications in humid regions. Aluminum-based Al-WTR particle dissolution in soils or aqueous suspensions might not only release significant quantities of potentially toxic Al, but allow previously immobilized P to be released to the environment (Makris and O'Connor, 2007). Long-term (80 d) equilibrations of Al-WTR were conducted in unbuffered 0.01 M KCl solutions (Makris and O'Connor, 2007). Soluble Al concentrations of untreated (no P added) Al-WTR were below the instrument's (ICP-AES) detection limit (0.03 mg Al/L). Overall, the amount of KCl-extractable Al concentrations released from Al-WTR within 80 d was minimal (<0.1% of oxalate-extractable Al) (Makris and O'Connor, 2007). Agyin-Birikorang and O'Connor (2007) also concluded in an artificial Al-WTR aging study that WTR application is capable of reducing P concentrations in P-impacted soils, doing so for a long

time, and that within the commonly encountered range of pH values for agricultural soils WTR-immobilized P should be stable.

CHAPTER THREE
EFFECTS OF ALUMINUM FROM WATER TREATMENT RESIDUAL APPLICATIONS TO PASTURES ON MINERAL STATUS OF GRAZING CATTLE AND MINERAL CONCENTRATIONS OF FORAGES

Introduction

There is an increasing public demand to reduce the amount of phosphorus (P) transported to water bodies due to the risk of eutrophication, mainly from agricultural P-inputs, including the land application of animal manure. Extensive efforts have been focused on finding ways to reduce soluble P in manure-impacted soils. Aluminum (Al) binds to P and application of Al could be one potential solution to the problem. However, application of Al to the land can also result in ingestion by livestock and potential harm to animals. Under grazing conditions, cattle typically consume 10 to 15% of their DM intake as soil (Field and Purves, 1964; Healy, 1967; 1968). However, this work was done with cool season grasses and may not directly apply to tropical grasses. Ingestion of highly available dietary Al (e.g. $AlCl_3$) by cattle and other livestock may result in a P deficiency. Notably, Al toxicity is often observed as a P deficiency (Valdivia et al., 1982). High amounts of bioavailable Al can also impact the status of Fe, Mg, and Zn in animals (Rosa et al., 1982). In sheep, for example, ingestion of soluble dietary Al suppressed voluntary feed intake, feed efficiency, plasma P, animal growth, and gains. However, when additional P was offered, the negative effects were less severe.

Aluminum water treatment residuals (Al-WTR) are the by-products of a water purification procedure. They may be one solution to the P problem, in that the Al in the product will bind with P, thus preventing leaching into groundwater. Prior research from Florida has shown that amending soils with Al-WTR increases soil retention and reduces leaching of P (O'Connor et al., 2002). Unlike $AlCl_3$, the bioavailability of Al in Al-WTR is generally low and thought to be harmless (O'Connor et al., 2002).

Two experiments were conducted to determine the effects of pasture application of Al-WTR on mineral status (primarily P) and performance of grazing cattle. A second objective was to evaluate the effects of the applied Al-WTR on forage mineral concentrations.

Materials and Methods

Two experiments were carried out in consecutive years, 2005 and 2006, to study the effects of Al-WTR on cattle mineral status and performance and forage mineral concentrations. The second experiment (2006) involved a different group of cattle and additional application of Al-WTR at the same location. All animal procedures were conducted within the guidelines of and approved by the University of Florida Institutional Animal Care and Use committee (No. E037).

Experiment 1 (2005)

Yearling Holstein steers (n=36) were utilized in a 148 d experiment at the Santa Fe Beef Research Unit, a 648 ha operation owned by the University of Florida, located in Alachua County (north central FL). The experiment began on June 1st and ended October 26th, 2005. The steers weighed 306.7 ± 34.5 kg at d 0. The cattle were not dewormed before the trial, but received a free-choice complete mineral salt prior to the beginning of the trial so that they would not be "salt-starved" and thus, be accustomed to eating free-choice minerals early in the study.

Steers were allotted (three/pasture) to one of twelve 0.81 ha bahiagrass (*Paspalum notatum*) pastures on d 0 and provided *ad libitum* water and grazing access. The chemical composition of forages can be found in Appendix A. Soil series that exist at this location are Millhopper sand, Bonneau fine sand and Gainesville sand. Experimental pastures were randomly allotted to one of four treatments with three replications per treatment. Half of the twelve pastures received Al-WTR at a rate of 22.8 Mg dry weight/ha from the Bradenton, FL, water treatment plant. Bradenton, FL water treatment plant was chosen based on the reactivity of the Al-WTR at this location in previous studies, and this rate of Al-WTR is also consistent with

previous studies (Makris and O'Connor, 2007). The Al-WTR product contained 0.30% Fe, 7.8% Al, 0.11% Ca, 0.024% Mg, 0.30% P, 0.004% Mn, 0.73% S, 0.006% Cu, 0.002% Zn, and approximately 70% solids. The treatments were 1) control-no Al-WTR application with steers receiving commercial free-choice mineral supplement but no P, 2) control with free-choice mineral supplement plus P, 3) treatment 1 with Al-WTR and 4) treatment 2 with Al-WTR. The mineral supplement was provided in covered mineral feeders located in each pasture. Mineral supplement was offered every 28 days in 11.34 kg increments starting on d 0 and continuing to d148. Pastures were clipped to a height of 0.01 m and Al-WTR was surface applied to the pastures from one to two days prior to grazing using a spreader truck. All pastures were fertilized with 190 kg N/ha as ammonium nitrate before Al-WTR application.

Weights, blood, and liver biopsies were taken at d 0, d 84, and d 148. Bone biopsies were obtained on d 148. Blood samples (jugular venipuncture) were collected (10 mL) with a 20 x 1 vacutainer needle (Vacutainer; Becton Dickinson, Franklin Lakes, NJ) into evacuated tubes containing sodium heparin. Immediately after collection, blood was centrifuged for 20 min. at 700 x g, and plasma was then extracted. Plasma was kept on ice at the collection site, and subsequently transported to the University of Florida for further preparation and analysis. Plasma was frozen at -20º C upon arrival at the laboratory. Stored plasma samples were thawed and deproteinated using 10% trichloroacetic acid (Miles et al., 2001). Plasma was analyzed for Al, Ca, Cu, Mg, P, and Zn.

Liver biopsies were obtained *in vivo* using the aspiration liver biopsy technique (Miles et al., 2001). Liver samples (0.1-0.8g DM) were placed on filter paper to remove the excess blood, placed in a sterile plastic bag, and sealed. Bags were cooled on ice until transportation to the laboratory where they were frozen at -20º C until time of analysis. Liver samples were thawed,

dried, weighed, ashed, and solubilized in HNO_3 (Miles et al; 2001). Liver samples were analyzed for Al, Cu, and P.

Bone samples were collected using an electric drill and trephine (1.5 cm) as described by Miles et al. (2001). Bone samples were sealed in sterile plastic bags and kept on ice, then frozen until the time of analysis. Bone samples were thawed, washed with saline, cleaned of all soft tissue, dried, and fat extracted with petroleum ether before ashing. Bone samples were then analyzed for Al, Ca, P, and Mg as described in Miles et al. (2001).

Forage samples were taken on d 0 and approximately every 28 d thereafter for five mo. Two composite forage samples were taken from each 0.8 ha pasture using a transect technique. Subsamples of each composite sample were taken from the beginning, middle, and end of the pastures, no closer than 10 m from the fences. The subsamples were cut to a height of 3-5 cm to simulate observed grazing height and were collected at different areas of the pasture to more closely mimic what the animals appeared to consume. The forage samples were clipped using a stainless steel knife, and placed into clean paper bags on location. Samples were cut with stainless steel scissors and placed into clean paper bags on location. Samples were transported to the laboratory where they were dried in an oven at 60° C for 48 hr and subsequently ground, using a Wiley Mill, with a 1-mm stainless steel sieve. Ground samples were stored in air-tight plastic bags until analysis. Samples were prepared and digested according to Miles et al. (2001). Forage samples were analyzed for Al, Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn. Cobalt and Mo were analyzed on samples collected in three of the five months (May, August, and November). Selenium was analyzed for one-third of the samples collected each month.

For all samples, Ca, Cu, Fe, K, Mg, Mn, Na, and Zn were determined by atomic absorption spectrophotometry (Perkin-Elmer Model 5000, Perkin-Elmer Corp., Norwalk, CT). To ensure

the quality of data, the calibration standards were prepared simultaneously and the standard curves were recalibrated in the middle and at the end of each set of samples analyzed. In addition, to ensure the overall reliability of the analytical methods, certified National Bureau of Standards (NBS) reference materials (citrus leaves SRM-1572; bovine liver SRM-1577a) were acquired from the National Institute of Standards and Technology (NIST; Gaithersburg, MD) and included as standards. Colorimetric determination of P was accomplished using a method described by Harris and Popat (1954). Selenium was determined using fluorometric procedures (Whetter and Ullrey, 1978). Aluminum concentrations were analyzed by inductively coupled plasma-atomic emissions spectroscopy (ICP-AES), (Perkin-Elmer Plasma 3200, Perkin-Elmer, Wellesley, MA). Cobalt and Mo were determined at a private lab (Advanced Environmental Laboratories, Inc., Gainesville, FL) using ICP-AES. Data were analyzed for treatment effects using PROC MIXED og SAS (SAS for Windows v9; SAS Inst., Inc. Cary, NC) for a completely randomized design with a 2x2 arrangement of treatments. A repeated measures statement was included. Post hoc testing was done to determine sampling date effects with the PDIFF statement of SAS. Contrasts (control vs. Al-WTR, no P vs. P, and the interaction) were used for mean separation. Significance was declared at $P < 0.05$.

Experiment 2 (2006)

Yearling Holstein steers (n=36) were utilized in a second experiment (145 d) at the same location used for Experiment 1. The experiment began on May 23rd and ended October 15th, 2006. Steers weighed 169 ± 8.8 kg at d 0. Steers were dewormed with Dectomax® (Pfizer Animal Health, Cambridge, MA) and fed mineral salts before the start of the trial.

The same treatments used in experiment 1 were examined. An additional 53 Mg dry weight/ha from the Bradenton, FL water treatment plant was applied to the same pastures that received Al-WTR in 2005. The total 2-year load of WTR was 75.8 Mg dry weight/ha on the

pastures. The second application of Al-WTR was applied to the pastures 5 to 19 d prior to initiation of grazing. All pastures were fertilized with 190 kg N/ha as ammonium nitrate before Al-WTR application.

Weights, blood, and liver biopsies were taken at d 0, d 70, and d 145. Bone samples were taken on d 145. Forage samples were taken at d 0 and approximately every 28 d thereafter for six mo. Blood, liver, bone, and forage samples were analyzed for the same minerals, minus Co and Mo, and utilized the same experimental procedures as Experiment 1. All other animal management, sample preparations, and statistical evaluations were the same as those reported for Experiment 1.

Results and Discussion

There were no interactions between P and Al-WTR during Experiments 1 and 2.

Performance Results (Experiment 1)

The experimental pastures provided adequate forage throughout the experiment. The steers consumed the free-choice mineral supplement throughout the experiment and the average consumption varied from 34 to 55 g/animal/d among treatments. Increases in body weight were observed as the experiment progressed for all treatments except in treatment 2, when the body weight decreased from d0 to d84 (Table 3-1). The greatest increases ($P<0.05$) occurred from d 84 to d 148 in all treatments. Average daily gains were generally low for all treatments, ranging from 0.17 to 0.23 kg/d, with no treatment differences ($P>0.05$).

Performance Results (Experiment 2)

There were no treatment differences ($P>0.05$) in BW of the steers, except at experiment termination, where in treatment 2, cattle had lower weights ($P<0.05$) than treatment 3 (Table 3-2). Average daily gains (ADG) ranged from 0.39 to 0.48 kg/d. The experimental pastures provided

Table 3-1. Effect of Aluminum-water treatment residuals (Al-WTR) and P supplementation on Experiment 1 Steer Body Weights (kg) [1-3]

	Day				
Trt	0	70	145*	Mean	SD
1	307[a]	309[a]	343[b]	320	20.2
2	312[a]	307[a]	340[b]	320	17.8
3	307[a]	310[a]	332[b]	316	13.7
4	302[a]	302[a]	335[b]	313	19.1
SD	4.14	3.69	4.95	3.40	

[a,b] Means with same letters within rows are not different (P>0.05).

[1] Data represent treatment least square means and standard deviations.

[2] Treatments were as follows: 1) control-no Al-WTR application with steers receiving commercial free-choice mineral supplement but no P, 2) control with free-choice mineral supplement plus P, 3) treatment 1 with Al-WTR and 4) treatment 2 with Al-WTR.

[3] Animals received free-choice mineral supplement with the following minerals: Ca, 16-18%; P, 8% (treatments 2 and 4 only); NaCl, 23-27%; Mg, 2%; Fe, 1%; Co, 50 mg/kg; Cu, 500 mg/kg; I, 50 mg/kg; Mn, 2000 mg/kg; Se, 26 mg/kg; and Zn, 4000 mg/kg.

[4] Average mineral consumption for each treatment was as follows: 1) 48 g/animal/d; 2) 67 g/animal/d; 3) 53 g/animal/d; 4) 62 g/animal/d.

adequate forage and the steers consumed free-choice mineral supplement in amounts varying from 48 to 67 g/animal/d throughout the experiment. As the experiment continued, increases in body weight ($P<0.05$) were observed for all treatments.

Performance Discussion

In general, differences in animal performance among treatments were limited throughout the experiment in both years. The study showed few losses in weight, which seems to be attributed to the proper amounts of dietary P supplied, as well as other nutrients. In experiment 2, steers receiving the complete mineral supplement, including P, had lower BW gains than steers grazing Al-WTR pastures and receiving a P-free mineral supplement. Cattle from experiment 2 had higher ADG values ranging from 0.39 to 0.48 kg/d, compared with ADG values for steers in experiment 1 ranging from 0.17 to 0.23 kg/d. Possible explanations for the higher gains in experiment 2 include lower starting weights, deworming before the experiment, and higher daily consumption of supplemental minerals. In both experiments, application of Al-WTR to pastures of grazing ruminants to control environmental P was not detrimental to the animal when considering BW alone.

Plasma Mineral Results (Experiment 1)

Plasma Ca and Mg were the only macromineral concentrations affected by collection date of sampling ($P<0.05$), and no plasma macrominerals showed a difference among treatments (Table 3-3). Plasma Ca decreased ($P<0.05$) from d84 to the end of the experiment in treatment 1, but were in the normal range (>8mg/dL) during the entire experiment. The other treatments had no collection date differences in plasma Ca. In plasma Mg, a collection date effect was found only for treatment 1 ($P<0.05$). Magnesium levels increased ($P<0.05$) from d0 to d84 and then decreased ($P<0.05$) at the end of the experiment (Table 3-3). However, no treatment effects were

Table 3-2. Effect of Aluminum-water treatment residuals (Al-WTR) and P supplementation on Experiment 2 Steer Body Weights (kg) [1-3]

	Day				
Trt	0	70	145[*]	Mean	SD
1	175[a]	182[b]	232[c]	196	31.1
2	159[a]	170[b]	218[c]	182	31.4
3	178[a]	186[b]	241[c]	202	34.3
4	165[a]	176[b]	235[c]	192	37.6
SD	8.81	7.00	9.75	8.41	

[a,b] Means with same letters within rows are not different (P>0.05).

[*] At experiment termination, treatment 2 was lower (P<0.05) than treatment 3.

[1] Data represent treatment least square means and standard deviations.

[2] Treatments were as follows: 1) control-no Al-WTR application with steers receiving commercial free-choice mineral supplement but no P, 2) control with free-choice mineral supplement plus P, 3) treatment 1 with Al-WTR and 4) treatment 2 with Al-WTR.

[3] Animals received free-choice mineral supplement with the following minerals: Ca, 16-18%; P, 8% (treatments 2 and 4 only); NaCl, 23-27%; Mg, 2%; Fe, 1%; Co, 50 mg/kg; Cu, 500 mg/kg; I, 50 mg/kg; Mn, 2000 mg/kg; Se, 26 mg/kg; and Zn, 4000 mg/kg.

[4] Average mineral consumption for each treatment was as follows: 1) 48 g/animal/d; 2) 67 g/animal/d; 3) 53 g/animal/d; 4) 62 g/animal/d.

shown at any time and no other treatments showed collection date effects. Magnesium plasma concentrations ranged from 2.30 mg/dL to 2.70 mg/dL and were considered to be in the normal range (>1.7 mg/dL). There were no treatment or collection date differences in plasma P concentrations (P>0.05). Phosphorus levels ranged from 5.42 mg/dL to 6.68 mg/dL and were in the normal range above the critical level of 4.5 mg/dL throughout experiment 1 (Table 3-3). Zinc was the only micromineral to show differences (P<0.05) in date of collection throughout experiment 1, but there were no treatment differences (P>0.05) (Table 3-3). In treatments one and three, Zn levels increased gradually from the start of the treatment until the end of the experiment. In treatment four, Zn levels increased (P<0.05) from d84 until d148 (1.84 to 2.10 µg/mL, repectively). Zinc plasma concentrations never reached a level near that suggested as a deficiency (<0.60 µg/mL). Plasma Cu concentrations were not affected by treatment (P>0.05) or date (P>0.05) throughout the experiment (Table 3-3). Across treatments, mean plasma Cu levels ranged from 0.77-0.99 µg/ml and were above the critical concentration of 0.65 µg/mL. There were no treatment or collection date differences (P>0.05) among treatments in plasma Al concentrations (Table 3-3). Most of the samples analyzed for Al were 0.02 µg/mL, with a detection limit of 0.02 µg/mL.

Plasma Mineral Results (Experiment 2)

Calcium and Mg plasma concentrations were again the only macrominerals to show an effect by collection date (P<0.05), and no plasma macrominerals had a difference (P>0.05) among treatments (Table 3-4). Calcium increased significantly (P<0.05) from d0 to d70 in all 4 treatments and remained higher than at d0 until the end of experiment 2. Plasma Ca never reached a deficient concentration (<8 mg/dL).

Table 3-3. Plasma mineral concentrations as affected by Al-WTR and P supplementation (Experiment 1)[1,2]

	Trt	Day 0	Day 84	Day 148	Means	SD
Ca, mg/dL	1	12.0[a]	12.5[a]	10.5[b]	11.7	1.05
	2	12.1	11.8	11.4	11.8	0.37
	3	12.2	11.7	11.7	11.9	0.30
	4	12.4	11.7	11.6	11.9	0.45
	SD	0.17	0.39	0.55	0.10	
Mg, mg/dL	1	2.30[a]	2.70[b]	2.07[a]	2.36	0.32
	2	2.47	2.50	2.44	2.47	0.03
	3	2.40	2.42	2.33	2.38	0.05
	4	2.53	2.65	2.38	2.52	0.14
	SD	0.10	0.13	0.16	0.08	
P, mg/dL	1	6.65	6.11	5.42	6.06	0.62
	2	6.44	6.27	5.49	6.07	0.51
	3	5.89	6.02	6.68	6.20	0.42
	4	6.07	5.61	5.51	5.73	0.30
	SD	0.35	0.28	0.60	0.20	
Al, µg/mL	1	0.02	0.02	0.02	0.02	0.00
	2	0.01	0.02	0.02	0.02	0.00
	3	0.02	0.02	0.02	0.02	0.00
	4	0.02	0.02	0.02	0.02	0.00
	SD	0.01	0.00	0.00	0.00	
Cu, µg/mL	1	0.89	0.81	0.77	0.82	0.06
	2	0.94	0.82	0.77	0.84	0.09
	3	0.99	0.82	0.84	0.88	0.09
	4	0.93	0.80	0.89	0.87	0.07
	SD	0.04	0.01	0.06	0.03	
Zn, µg/mL	1	1.79[a]	1.85[ab]	2.04[b]	1.89	0.13
	2	1.65	1.82	1.75	1.74	0.09
	3	1.73[a]	1.89[ab]	2.02[b]	1.88	0.15
	4	1.84[a]	1.84[a]	2.10[b]	1.93	0.15
	SD	0.08	0.03	0.16	0.08	

[a-c] Means with same letters within rows are not different ($P<0.05$).

[1] Data represent treatment means and overall standard deviations.

[2] Treatments were as follows: 1) control-no Al-WTR application with steers receiving commercial free-choice mineral supplement but no P, 2) control with free-choice mineral supplement plus P, 3) treatment 1 with Al-WTR and 4) treatment 2 with Al-WTR.

Magnesium concentrations increased from d0 to d70 in the first three treatments and then continued at high levels to the end of experiment 2 (Table 3-4). No collection date effects were shown in Mg concentrations in treatment 4. No treatment effects were shown ($P>0.05$) at any time in plasma Mg. Plasma Mg concentrations ranged from 1.73 to 2.34 mg/dL and some values were considered to be slightly below the normal range of 1.8-3.2 mg/dL. Serum P was not affected ($P>0.05$) by treatment or collection date and concentrations were above critical levels (>4.5mg/dL) throughout the experiment. Phosphorus levels ranged from 4.66 to 5.67 mg/dL (Table 3-4).

Plasma Zn did not show differences ($P>0.05$) among treatments, but was lower ($P<0.05$) for the final date of collection for all treatments than the previous two collections (Table 3-4). In treatment 3, plasma Zn was higher ($P<0.05$) on d70 than on d0 or d145. Plasma Cu concentrations did not show any treatment differences ($P>0.05$) throughout the experiment (Table 3-4). Across treatments, plasma Cu levels ranged from 0.72-0.97 µg/ml. In treatments 1 and 2, without Al-WTR, Cu levels did increase at d70, and then decreased at d145 ($P<0.05$). Plasma Cu was in the normal range (>0.65 µg/mL) throughout the experiment. There were no treatment or collection date differences for plasma Al concentrations; with all samples analyzing 0.02-0.03 µg/mL.

Plasma Mineral Discussion

Plasma macrominerals (Ca, Mg, and P) were higher, in general, in experiment 1. Yet, the microminerals (Al, Cu, and Zn) were generally higher in experiment 2. Currently, there is no evidence to explain this finding.

Cunha et al. (1964) reported that values of 10-12 mg/dL of Ca in plasma are normal for healthy cattle, with deficiency occurring at levels below 8 mg/dL. Later, the NRC (1996)

Table 3-4: Plasma mineral concentrations as affected by Al-WTR and P supplementation (Experiment 2)[1,2]

	Trt	Day 0	Day 70	Day 145	Means	SD
Ca, mg/dL	1	9.03[a]	11.1[b]	10.4[b]	10.2	1.06
	2	8.44[a]	9.74[b]	10.2[b]	9.45	0.90
	3	8.39[a]	10.5[b]	10.2[b]	9.71	1.15
	4	9.19[a]	10.7[b]	10.5[b]	10.1	0.81
	SD	0.41	0.57	0.15	0.35	
Mg, mg/dL	1	2.01[a]	2.33[b]	2.34[b]	2.23	0.15
	2	1.73[a]	2.15[b]	2.29[b]	2.06	0.24
	3	1.80[a]	2.25[b]	2.24[b]	2.10	0.21
	4	2.14	2.20	2.26	2.20	0.05
	SD	0.19	0.08	0.04	0.08	
P, mg/dL	1	5.26	5.08	4.99	5.11	0.14
	2	4.66	4.96	5.06	4.89	0.21
	3	5.17	5.67	5.01	5.28	0.34
	4	4.94	5.75	5.60	5.43	0.43
	SD	0.27	0.40	0.29	0.23	
Al, µg/mL	1	0.02	0.02	0.02	0.02	0.00
	2	0.03	0.03	0.03	0.03	0.00
	3	0.02	0.02	0.02	0.02	0.00
	4	0.02	0.02	0.02	0.02	0.00
	SD	0.01	0.01	0.01	0.01	
Cu, µg/mL	1	0.72[a]	0.90[b]	0.81[ab]	0.81	0.09
	2	0.76[a]	0.97[b]	0.74[a]	0.82	0.13
	3	0.78	0.90	0.83	0.84	0.06
	4	0.77	0.76	0.84	0.79	0.04
	SD	0.03	0.09	0.05	0.02	
Zn, µg/mL	1	1.88[a]	2.13[a]	1.47[b]	1.83	0.33
	2	2.10[a]	2.21[a]	1.58[b]	1.96	0.34
	3	1.97[a]	2.42[b]	1.45[c]	1.95	0.49
	4	2.01[a]	2.19[a]	1.48[b]	1.89	0.37
	SD	0.09	0.13	0.06	0.06	

[a-c] Means with same letters within rows are not different (P<0.05).

[1] Data represent treatment means and overall standard deviations.

[2] Treatments were as follows: 1) control-no Al-WTR application with steers receiving commercial free-choice mineral supplement but no P, 2) control with free-choice mineral supplement plus P, 3) treatment 1 with Al-WTR and 4) treatment 2 with Al-WTR.

mentioned that blood Ca concentration is not a good indicator of Ca status, because levels are maintained between 9-11 mg/dL by homeostatic mechanisms (parathyroid hormone, calcitonin, and the active form of vitamin D), which regulates Ca absorption, reabsorption, and resorption from bone. Plasma Ca levels stayed above the deficient range in both experiments.

Plasma P concentrations were higher in experiment 1 than experiment 2 (6.02 vs. 5.18 mg/dL). Plasma P concentrations consistently below 4.5 mg/dL indicate a P deficiency (McDowell, 2003). In both experiments, P levels were normal to low, but never reached a level of deficiency at any point. Therefore, the Al in the Al-WTR did not complex with P enough to cause a deficiency in the cattle in either experiment.

Serum Mg values are often used to assess the Mg status of animals (Miller et al., 1972). Underwood (1981) considered the normal range of serum Mg in cattle to be 1.8-3.2 mg/dL, and found serum Mg concentrations below 1.7 mg/dL in cattle suffering from hypomagnesemic tetany. McDowell and Arthington (2005) consider the critical level in serum to be 1 to 2 mg/dL. Magnesium levels ranged from 1.73 to 2.70 mg/dL for both experiments, and never reached a deficient range.

Plasma Zn results showed no treatment effects in either years. It has been suggested that deficiency occurs when plasma levels are below 0.6-0.8 µg/mL (McDowell, 2003). All of the plasma samples remained well above the Zn critical range for both experiments.

The Cu concentrations in various fractions of blood are regularly used when evaluating the Cu status in ruminants. Plasma Cu concentrations were not affected by collection date in any of the treatments for both experiments. The critical level for plasma is estimated to be 0.65 µg/mL (McDowell and Arthington, 2005). In both experiments, the Cu concentrations stayed in the normal range.

For both experimental years there were no treatment or date differences (P>0.05) in plasma Al concentrations. In both experiments, the Al plasma concentrations were very low (0.02 µg/mL, on average), indicating that the Al in Al-WTR may be unavailable to the animal and safe to use on pastures to reduce the P environmental problem.

Liver Mineral Results (Experiment 1)

In experiment 1, Cu ranged from 171.1-393.5 mg/kg DMB (Table 3-5). There were also changes (P<0.05) in liver Cu as the experiment progressed, and differences among treatments were shown pre-experiment. However, there was no treatment effect (P>0.05) in liver Cu. In treatments two and four, liver Cu increased from d0 to d84 and then decreased to concentrations not different than d0. During experiment 1, liver Cu never fell below the critical level (>25 to 75 mg/kg DMB). Liver Al values ranged from 67.3 to 185 mg/kg (Table 3-5). For treatments 1, 3 and 4, liver Al was higher (P<0.05) at d84 compared to d0 and d148. Aluminum concentrations were higher (P<0.05) for treatment 2 than treatment 4 at d0 (67.3 vs. 126 mg/kg, respectively). There were no other treatment differences in liver Al.

Liver P (P>0.05) was not affected by treatment during the experiment. In treatment 4, liver P concentrations increased from d0 to d84. There were no collection date effects in treatments 1-3. Phosphorus concentrations ranged from 0.82 to 0.95% in experiment 1 (Table 3-5).

Liver Mineral Results (Experiment 2)

Similar to experiment 1, liver mineral concentrations of Cu were in the normal range (Table 3-6). There were no treatment or collection date differences (P<0.05) for these liver Cu or Al concentrations. In experiment 2, Cu liver concentrations ranged from 303.9-378.9 mg/kg DMB (Table 3-6). Liver Al concentrations ranged from 138 to 162 mg/kg. There were no other treatment or collection date effects seen during the experiment for liver Al.

Table 3-5. Liver Al, Cu, and P concentrations (DMB) as affected by Al-WTR application to pastures and P supplementation (Experiment 1)[1,2]

	Trt	Day 0	Day 84	Day 148	Means	SD
P, %	1	0.82	0.95	0.89	0.89	0.07
	2	0.79	0.93	0.93	0.88	0.08
	3	0.78	0.91	0.90	0.86	0.07
	4	0.67[a]	0.95[b]	0.92[b]	0.85	0.15
	SD	0.07	0.02	0.02	0.02	
Al, mg/kg[*]	1	100[a]	138[b]	114[a]	117	19.2
	2	126[a]	141[a]	81.3[b]	116	31.1
	3	115[a]	185[b]	99.8[a]	133	45.4
	4	67.3[a]	141[b]	104[a]	104	36.9
	SD	25.5	22.5	13.7	11.9	
Cu, mg/kg[*]	1	246	314	327	295	43.3
	2	240[a]	393[b]	281[a]	305	79.6
	3	342	316	238	299	54.4
	4	171[a]	376[b]	264[a]	270	103
	SD	70.3	40.7	37.4	15.4	

[a,b] Means with same letters within rows are not different ($P<0.05$).

[*] Pre-experiment differences ($P<0.05$) were: 1) Al was different for treatments 2 and 4; 2) Cu was different for treatments 3 and 4.

[1] Data represent treatment means and overall standard deviations.

[2] Treatments were as follows: 1) control-no Al-WTR application with steers receiving commercial free-choice mineral supplement but no P, 2) control with free-choice mineral supplement plus P, 3) treatment 1 with Al-WTR and 4) treatment 2 with Al-WTR.

Liver P was lower (P<0.05) at d70 in treatment 3 than in treatments 1, 2, and 4. Liver P also increased (P<0.05) throughout the experiment for treatment two only. Phosphorus concentrations ranged from 0.77 to 1.06% in experiment 2 (Table 3-6).

Liver Mineral Discussion

On average, mineral concentrations (P, Al, and Cu) in experiment 2 were higher than experiment 1. There were fewer collection date effects seen in year 2 for all minerals analyzed.

In healthy ruminants, liver Cu concentrations should be between 100-400 mg/kg DMB (McDowell, 2003); the critical levels in liver are estimated to be 25-75 mg/kg DMB (McDowell and Arthington, 2005). In both experiments, liver Cu concentrations were in the normal range, with year 1 concentrations remaining slightly lower than year 2.

Although liver concentrations of Al and P are not good indicators of mineral status, the minerals were assessed mainly to look at possible treatment differences. As with liver Cu, differences were seen in Al and P during the experiment, usually reflecting the change in season. Liver Al levels were too low to cause any toxic effects in both experiments. Liver Al in both experiments was slightly above 4.1 mg/kg; this is based on limited analysis reported for several species (Alfrey, 1986). The differences in liver Al during the experiment did not give any significant conclusions about any long-term treatment effect.

Bone Mineral Results (Experiment 1)

There were no treatment differences (P>0.05) or collection date trends in bone P, Mg, Ca, and Al mineral concentrations at experiment termination (Table 3-7). Bone P was slightly below the average concentration of 17% (ash basis) for all treatments, but not low enough to be considered a deficiency (Table 3-7). Bone Mg was normal throughout the experiment, ranging from 0.63% to 0.75%. Bone Ca was normal (>37.6%), ranging from 42.41% to 47.73%, with no treatment effects shown. Bone Al concentrations ranged from 87.0 to 130 mg/kg.

Table 3-6. Liver Al, Cu, and P concentrations (DMB) as affected by Al-WTR application to pastures and P supplementation (Experiment 2)[1,2]

	Trt	Day 0	Day 70	Day 145	Means	SD
P, %[*]	1	0.93	0.99	0.97	0.96	0.03
	2	0.85[a]	1.01[ab]	1.06[b]	0.97	0.11
	3	1.01	0.77	1.00	0.93	0.14
	4	0.95	0.99	0.88	0.94	0.06
	SD	0.07	0.11	0.08	0.02	
Al, mg/kg	1	144	138	157	146	9.71
	2	145	148	156	150	5.69
	3	148	157	157	154	5.20
	4	151	142	162	152	10.0
	SD	3.16	8.26	2.71	3.42	
Cu, mg/kg	1	364	379	364	369	8.67
	2	312	309	409	343	56.5
	3	341	328	304	342	18.8
	4	351	327	327	335	13.9
	SD	22.1	30.1	45.9	14.9	

[a,b] Means with same letters within rows are not different ($P<0.05$).

[*] On d70 for liver P, treatment 3 was lower ($P<0.05$) than the other treatments.

[1] Data represent treatment means and overall standard deviations.

[2] Treatments were as follows: 1) control-no Al-WTR application with steers receiving commercial free-choice mineral supplement but no P, 2) control with free-choice mineral supplement plus P, 3) treatment 1 with Al-WTR and 4) treatment 2 with Al-WTR.

Bone Mineral Results (Experiment 2)

No treatment or collection date differences (P>0.05) were seen during the course of the experiment in any of the measured Ca, P, Mg and Al concentrations. Bone P ranged from 12.11 to 12.53%, below the critical range of 17% (Table 3-8). On average, bone Ca was lower than experiment 1, ranging from 38.53% to 41.68%. Bone Mg was normal throughout the experiment, ranging from 0.60% to 0.64%. Bone Al means ranged from 65.2 to 83.3 mg/kg.

Bone Mineral Discussion

All bone mineral concentrations were higher in experiment 1. The Al-WTR treatments with and without supplemental P had no effect (P>0.05) on bone Ca, P, Mg, and Al concentrations for both experiment. The critical level for bone Ca (ash-basis) is 37.6% (McDowell and Arthington, 2005). Bone Ca concentrations were normal to low in both experiments and never reached a deficient range.

McDowell and Arthington (2005) suggest that the critical level for P on an ash basis is 17.6%. In experiment 1, the bone P was slightly below the critical level. In experiment 2, the bone P fell below the critical level throughout the experiment. It could be assumed that the low concentrations of P are due to the Al in the Al-WTR complexing the P and making it unavailable. However, low levels were seen pre-experiment as well, and there were no differences in the treatments without Al-WTR that also contained P in the mineral supplement. Therefore, it can be concluded that unknown factors caused the low bone P concentrations and not the Al in the Al-WTR .

Forage Mineral Results (Experiment 1)

Mean forage Ca varied from 0.26% to 0.42% throughout the experiment (Table 3-9). All treatment and control means of forage Ca were below the critical level (0.35%) for the non Al-WTR group until November. Forage Ca concentrations were below the critical level after May

Table 3-7. Effects of Al-WTR and P supplementation on bone mineral concentrations (ash basis) of Ca, P, Mg, and Al (Experiment 1)[a-c]

	Treatment Means					
	1	2	3	4	Means	SD
Ca, %	42.4	47.7	42.9	43.5	44.2	2.43
Mg, %	0.67	0.75	0.64	0.63	0.67	0.05
P, %	16.2	16.9	16.5	16.1	16.4	0.38
Al, mg/kg	125	90.9	130	87.0	108	22.4

[a] Data represent least squared means and overall standard deviations; n = 9 per treatment.
[b] No differences (P>0.05) among treatments or collection periods.
[c] Treatments were as follows: 1) control-no Al-WTR application with steers receiving commercial free-choice mineral supplement but no P, 2) control with free-choice mineral supplement plus P, 3) treatment 1 with Al-WTR and 4) treatment 2 with Al-WTR.

Table 3-8. Effects of Al-WTR and P supplementation on bone mineral concentrations (ash basis) of Ca, P, Mg, and Al (Experiment 2)[a-c]

	Treatment Means					
	1	2	3	4	Means	SD
Ca, %	41.7	38.5	41.2	41.3	40.7	1.45
Mg, %	0.64	0.60	0.61	0.64	0.63	0.02
P, %	12.5	12.3	12.2	12.1	12.3	0.19
Al, mg/kg	65.2	83.3	65.2	68.2	70.5	8.67

[a] Data represent least squared means and overall standard deviations; n = 9 per treatment.
[b] No differences (P>0.05) among treatments or collection periods.
[c] Treatments were as follows: 1) control-no Al-WTR application with steers receiving commercial free-choice mineral supplement but no P, 2) control with free-choice mineral supplement plus P, 3) treatment 1 with Al-WTR and 4) treatment 2 with Al-WTR.

for the Al-WTR group. Mean forage Ca concentrations did not vary (P>0.05) between the no Al-WTR group and the Al-WTR group except in November. In November, forage where Al-WTR had been applied was lower (P<0.05) in Ca than the non-WTR treated forages.

Forage K concentrations (Table 3-9) were high and adequate for both Al-WTR and no Al-WTR treatments, exceeding the 0.60% critical level at all sampling times, except in the month of December, where they fell dramatically (P<0.05). There were no treatment effects (P>0.05) evident throughout the grazing season. All treatment means for forage Na (Table 3-9) concentrations were well below the critical limit of 0.06%. Although there were treatment effects (P<0.05) in November, they were small and did not persist with time.

Forage Mg concentrations ranged from 0.13 to 0.20% in experiment 1 and were all above the critical level (0.10%). There were no collection date or treatment effects (Table 3-9).

Both treatment groups produced adequate P concentrations (>0.18%) until August, and various Al-WTR and no Al-WTR treatments were borderline adequate to deficient during the remainder of the experiment. Phosphorus concentrations ranged from 0.06 to 0.23% and decreased (P<0.05) steadily as the experiment progressed in both treatments.

Forage Al concentrations were different (P<0.05) depending on collection date, however, there was no discernable pattern (Table 3-9). There was a treatment difference (P<0.05) in July, the second month after Al-WTR application, where the Al-WTR treatment resulted in a higher Al mean concentration than the control (65.3 vs. 45.9 mg/kg).

Forage Cu means were low for both treatment groups (with or without Al-WTR application) at each of the samplings; forage Cu was below beef cattle requirements (10 mg/kg). None of the forage samplings were different (P>0.05) in forage Cu as a result of treatment (Table 3-9). Copper concentrations were generally higher in the early season, with treatment means for

Table 3-9. Forage minerals as affected by water treatment residuals (Experiment 1)[1-5]

	Trt	May	Jul	Aug	Sep	Oct	Nov	Dec	Means	SD
Ca, %[*]	1	0.38[a]	0.30[b]	0.27[b]	0.27[b]	0.28[b]	0.32[b]	0.31[b]	0.30	0.04
	2	0.33[ad]	0.27[ac]	0.31[acd]	0.27[ac]	0.26[c]	0.42[b]	0.35[d]	0.32	0.06
	SD	0.04	0.02	0.03	0.00	0.01	0.07	0.03	0.01	
K, %	1	1.38[a]	1.43[a]	1.33[ad]	0.82[bd]	1.09[ad]	2.14[c]	0.43[b]	1.23	0.50
	2	1.51[a]	1.36[a]	1.18[a]	1.02[a]	1.09[a]	2.09[b]	0.44[c]	1.24	0.47
	SD	0.09	0.05	0.11	0.14	0.00	0.04	0.01	0.01	
Mg, %	1	0.18	0.19	0.16	0.16	0.18	0.16	0.13	0.17	0.02
	2	0.17	0.20	0.19	0.15	0.17	0.19	0.15	0.17	0.02
	SD	0.01	0.01	0.02	0.01	0.01	0.02	0.01	0.00	
Na, %	1	0.02[bc]	0.02[acd]	0.02[a]	0.02[a]	0.03[c]	0.03[b*]	0.01[d]	0.02	0.01
	2	0.02[c]	0.02[ac]	0.02[c]	0.01[ad]	0.02[c]	0.04[b*]	0.01[d]	0.02	0.01
	SD	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	
P, %	1	0.23[a]	0.23[a]	0.15[b]	0.14[b]	0.14[b]	0.14[b]	0.06[c]	0.16	0.05
	2	0.22[a]	0.21[a]	0.17[b]	0.12[b]	0.14[b]	0.15[b]	0.06[c]	0.15	0.05
	SD	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.01	
Al, mg/kg[*]	1	35.0[b]	65.3[a]	17.3[b]	36.1[b]	18.7[b]	26.2[b]	17.7[b]	30.9	15.8
	2	25.1[ab]	31.9[ab]	15.8[b]	28.9[ab]	39.2[a]	37.4[a]	20.1[ab]	28.3	8.00
	SD	7.00	23.6	1.06	5.09	14.5	7.92	1.70	1.84	
Cu, mg/kg	1	9.69[a]	8.17[ac]	8.75[a]	5.76[b]	6.18[b]	6.87[bc]	7.95[ac]	7.66	1.31
	2	8.41[a]	8.23[a]	7.52[a]	5.32[b]	5.55[b]	8.48[a]	8.29[a]	7.39	1.27
	SD	0.91	0.04	0.87	0.31	0.45	1.14	0.24	0.19	
Fe, mg/kg	1	66.3[a]	59.1[ac]	33.3[bd]	34.3[bd]	35.4[bd]	54.9[ac]	42.1[cd]	44.5	12.48
	2	43.9[ac]	43.1[ac]	33.7[a]	36.7[ac]	36.4[ac]	66.6[b]	52.4[bc]	44.7	10.66
	SD	15.8	11.3	0.28	1.70	0.71	8.27	7.28	0.14	
Mn, mg/kg	1	78.3	83.8	60.5	56.5	90.7	96.1	95.1	80.1	14.89
	2	48.9	49.7	48.6	48.3	63.2	70.0	79.6	58.3	11.78
	SD	20.8	24.1	8.41	5.80	19.4	18.5	11.0	15.4	
Zn, mg/kg	1	34.4[a]	28.8[ab]	28.7[ab]	25.9[abc]	23.3[bc]	23.0[bc]	18.6[c]	26.1	4.73
	2	43.9[ace]	43.1[ad]	33.7[ace]	36.7[bce]	36.4[ac]	66.6[de]	52.4[ace]	44.7	10.66
	SD	6.72	10.1	3.54	7.64	9.26	30.8	23.9	13.2	

[a-d] Means with same letter within rows are not different (P>0.05).

[*] In November for forage Ca, treatment with Al-WTR was lower (P<0.05) than the control. In July for forage Al, control treatment was lower (P<0.05) than treatment with Al-WTR.

[1] Treatments were as follows: 1)Al-WTR; 2) Control- no Al-WTR.

[2] Means represent 12 samples per month per treatment.

[3] Critical concentrations are as follows: Ca, 0.35%; P, 0.18%; Mg 0.10%; K 0.60%; Na, 0.06%; Cu, 10.0 mg/kg; Fe, 50.0 mg/kg; Mn, 20.0 mg/kg; Zn, 30.0 mg/kg (NRC, 1986; McDowell and Arthington, 2005).

[4]Water treatment residual contained 0.30% Fe, 7.8% Al, 0.11% Ca, 0.024% Mg, 0.30% P, 0.004% Mn, 0.73% S, 0.006% Cu, and 0.002% Zn.

[5]Dry Matter Basis.

both groups at their highest in May, after which there was a trend for Cu concentrations to decrease to very low levels from September through November.

The range of forage Fe (Table 3-9) concentrations across all treatments was 33.3-66.6 mg/kg, with more than half of the means providing less than the requirement of 50 mg/kg. There was a trend for all pastures, with or without Al-WTR, to have lower Fe concentrations at the mid-season samplings, a condition that improved by season's end. Forage Fe concentrations varied and were affected ($P<0.05$) by time.

Forage Mn concentrations (Table 3-9) for the control and the treatment pastures far exceeded the minimum 20 mg/kg of diet at all sampling times. They ranged from 48.3-96.1 mg/kg throughout the experiment and there were no treatment effects ($P>0.05$). With the exception of the lowest Mn concentrations found in September, individual treatment means fluctuated moderately from month to month, with little apparent discernable pattern. During the last months (November and December), forage Mn was at its highest concentration.

Cobalt and Mo were analyzed for only three sampling periods (May, August, and November), while one-third of samples collected each month were analyzed for Se. For those three elements, there were no treatment or sampling date differences ($P>0.05$). Cobalt concentrations averaged 0.04 ± 0.04 mg/kg, ranging from 0.01 to 0.20 mg/kg. Over 99% of all the samples analyzed had Co concentrations less than the 0.1 mg/kg requirement. There was a trend for all treatment forages to increase in Co from May through August, and then decrease to November. All samples analyzed for Se were below the requirement of 0.1 mg/kg, and ranged from 0.02 to 0.08 mg/kg. Forage Mo concentrations ranged from 0.09 to 2.45 mg/kg and averaged 0.69 ± 0.60 mg/kg and over half of the samples analyzed were below 1.5 mg/kg.

Forage Mineral Results (Experiment 2)

There were no treatment effects (P>0.05) from Al-WTR application to pastures for any of the minerals analyzed in experiment 2 (Table 3-10). Mean forage Ca concentrations varied from 0.29% to 0.42% throughout the experiment, which is similar to year one data. All treatment and control means were at or slightly above the critical level (0.35%) for both treatments, except in June, where they were slightly below the requirement.

All treatments produced low to adequate P concentrations except for treatment one in September, which fell to 0.15 % DM (<0.18%). Forage K (Table 3-10) was high and adequate for treatments, exceeding the 0.60% critical level at all sampling times. There were no treatment effects (P>0.05) for forage K throughout the grazing season. All treatment means for forage Na (Table 10) concentrations were well below the critical limit of 0.06%, just as in year one. There were no treatment or collection date effects (P>0.05) in year 2 for Na.

Forage Mg levels ranged 0.17 to 0.21% in experiment 2. At all times, Mg concentrations were above the critical level of 0.10%. There were no collection date or treatment effects seen in the second experiment.

There were no treatment differences (P>0.05) in experiment 2 for forage Al. However, there were collection date differences (P<0.05) in treatment 2 (with no Al-WTR). There was no discernable pattern as the experiment progressed. Forage Al concentrations ranged from 21.3 to 37.7 mg/kg.

There were no differences (P<0.05) among treatments in forage Cu during the six collection dates. Copper means were low for both treatments at each of the samplings, and below levels considered adequate for beef cattle (10 mg/kg). Copper concentrations ranged from 7.4 to 9.5 mg/kg across treatments. None of the collection samplings were different (P<0.05) as a result of treatment (Table 3-10). Copper concentrations were generally higher in the early

season, with treatment means for all groups at their highest in May, after which there was a trend for Cu concentrations to decrease to very low levels throughout the rest of the experiment.

The range of forage Fe (Table 3-10) concentrations across all treatments was 39.5 to 58.5 mg/kg, with half of the means providing less than the 50 mg/kg, similar to experiment 1. Forage Fe concentrations varied and were affected ($P<0.05$) by the changing season.

Forage Mn concentrations for the control and the treatment pastures far exceeded the minimum 20 mg/kg of diet at all sampling times (Table 3-10). No forage sample was deficient in Mn, with month means ranging from 55.7-142.4 mg/kg throughout the experiment and there were no month effects ($P>0.05$). Individual treatment means fluctuated moderately from month to month, with no apparent discernable pattern.

Thirty-two forage samples were analyzed for Se, and there were no treatment or sampling date differences ($P>0.05$). Forage Se concentrations averaged 0.05 ± 0.02 mg/kg. As in experiment 1, of all samples analyzed for Se were below the critical level of 0.1 mg/kg. Forage Se ranged from 0.01 to 0.03 mg/kg and they were lower than in experiment 1 on average.

Forage Mineral Discussion

Mean forage Ca concentrations were very similar in both experiments. In November of experiment 1, forage from the Al-WTR treatment 1 had lower ($P<0.05$) Ca concentrations than the control (treatment 2). This same effect was seen in September of experiment 2. Similar forage Ca concentrations were reported in Florida bahiagrass by Cuesta et al. (1993). In a central Florida study, Espinoza et al. (1991a) found Ca and P concentrations in bahiagrass to be adequate for modest growth for beef cattle during most months. Flores et al. (1993) also found Ca in north Florida bahiagrass to be adequate for modest cattle gains.

Similar to Ca requirements, dietary P requirements are based on the level of production for a given animal. Dietary P requirements for 300 kg growing and finishing beef cattle expected to

Table 3-10. Forage minerals as affected by water treatment residuals (Experiment 2)[1-5]

	Trt	May	Jun	Jul	Aug	Sep	Oct	Means	SD
Ca, %	1	0.42	0.32	0.38	0.38	0.33	0.37	0.37	0.04
	2	0.37	0.29	0.31	0.36	0.39	0.35	0.33	0.05
	SD	0.04	0.02	0.05	0.01	0.04	0.01	0.03	
K, %	1	1.49	1.39	1.33	1.31	1.23	1.21	1.33	0.10
	2	1.42	0.96	1.14	1.40	1.58	1.45	1.33	0.23
	SD	0.05	0.30	0.13	0.06	0.25	0.17	0.00	
Mg, %	1	0.18	0.20	0.19	0.21	0.17	0.19	0.19	0.01
	2	0.18	0.20	0.18	0.19	0.19	0.18	0.19	0.01
	SD	0.00	0.00	0.01	0.01	0.01	0.01	0.00	
Na, %	1	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.00
	2	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.00
	SD	0.00	0.01	0.01	0.00	0.00	0.00	0.00	
P, %	1	0.20	0.19	0.19	0.19	0.15	0.17	0.18	0.19
	2	0.21	0.21	0.19	0.19	0.18	0.17	0.19	0.02
	SD	0.01	0.01	0.00	0.00	0.02	0.00	0.01	
Al, mg/kg	1	23.4	24.7	26.3	21.3	33.7	30.3	26.6	4.61
	2	26.2^{abc}	30.8^{bce}	23.2^{cd}	27.5^{bdf}	40.5^{ef}	37.7^{be}	31.0	6.80
	SD	1.98	4.31	2.19	4.38	4.81	5.23	3.11	
Cu, mg/kg	1	9.54	8.33	8.67	8.41	8.78	7.63	8.54	0.62
	2	9.16	9.27	8.13	7.47	8.39	7.88	8.35	0.71
	SD	0.27	0.66	0.38	0.66	0.28	0.18	0.13	
Fe, mg/kg	1	50.8^{ab}	47.2^{ab}	54.5^{a}	55.2^{a}	48.4^{ab}	39.5^{b}	49.3	5.75
	2	58.5^{a}	53.8^{ac}	44.8^{bc}	42.2^{bc}	43.8^{bc}	41.0^{b}	47.4	7.10
	SD	5.44	4.67	6.86	9.19	3.25	1.06	1.34	
Mn, mg/kg	1	92.0	69.1	84.7	64.8	88.2	142.4	90.2	27.76
	2	55.7	58.9	40.6	74.1	90.1	93.3	68.7	20.77
	SD	25.7	7.21	31.2	6.58	1.34	34.7	15.2	
Zn, mg/kg	1	37.4^{acde}	26.3^{be}	31.5^{bc}	19.8^{b}	20.5^{bd}	44.4^{c}	30.0	9.72
	2	25.5^{ab}	24.9^{ab}	21.0^{ab}	29.1^{ab}	15.7^{a}	32.4^{b}	24.8	5.90
	SD	8.41	1.00	7.42	6.58	3.39	8.49	3.68	

[a-d] Means with same letter within rows are not different (P>0.05).

[1] Treatments were as follows: 1)Al-WTR; 2) Control- no Al-WTR.

[2] Means represent 12 samples per month per treatment.

[3] Critical concentrations are as follows: Ca, 0.35%; P, 0.18%; Mg 0.10%; K 0.60%; Na, 0.06%; Cu, 10.0 mg/kg; Fe 50.0 mg/kg; Mn, 20.0 mg/kg; Zn, 30.0 mg/kg (NRC, 1986; McDowell and Arthington, 2005).

[4]Water treatment residual contained 0.30% Fe, 7.8% Al, 0.11% Ca, 0.024% Mg, 0.3% P, 0.004% Mn, 0.73% S, 0.006% Cu, and 0.002% Zn.

[5]Dry Matter Basis.

gain 0.89 kg/d are 0.18% DM. (NRC, 1996). Using this as a guideline, both treatment groups produced adequate P concentrations until August/September for both experiments. These concentrations, regardless of treatment, were similar to earlier reports (Espinoza et al., 1991a; Cuesta et al., 1993). Flores et al. (1993) also found that P levels in grass were low and possibly limiting to cattle growth. Since P concentrations decreased as the experiment continued in both years, it is questionable as to whether or not the complexing Al in Al-WTR is the culprit. Since P levels dropped more in experiment 1 when there as less WTR applied to the pastures, it can be inferred that the Al-WTR was not the primary cause of the drop in forage P.

Forage K levels did not drop in December like experiment 1 K levels, which fell dramatically ($P<0.05$). Cuesta et al., (1993) showed K varying throughout the season as both experiments did. However, experiment 1 showed significant differences ($P<0.05$) in forage K concentrations throughout the year, unlike experiment 2.

Forage Mg levels were higher in experiment 2 than in experiment 1 (0.19 and 0.17%, respectively). Magnesium concentrations were well above the critical level of 0.10% (NRC, 1996) for all treatments throughout the grazing season. Forage Mg concentrations were similar to those reported previously by Cuesta et al. (1993) in north Florida during both experiments.

All forage Na concentrations were below the critical level of 0.06% during both experiments. Sodium deficiency in Florida is well-documented, and the concentrations determined by these experiments are similar to earlier reports by Salih et al. (1988) and Cuesta et al. (1993) in central Florida. Espinoza et al. (1991b) also found that Na concentrations in forage generally provided less than half of the requirements.

All forage Cu concentrations were below the critical level of 10 mg/kg in both experiments and were lower, on average, in experiment 1. Low forage Cu concentrations were also reported

by Merkel et al. (1990) and Espinoza et al. (1991b) in north and central Florida. Low forage Cu is of particular concern to cattle producers in this region, as inadequate Cu in the diet can cause depressed growth, anemia, and a variety of nervous disorders (McDowell, 2003).

There was a trend for all pastures, regardless of treatment, to have lower Fe concentrations towards the experiment termination, a condition that did not improve by season's end like experiment 1 did. Forage Fe concentrations varied throughout the experiments. Espinoza et al. (1991b) found variation in forage Fe concentration and a higher percentage of Fe deficient samples in a study conducted in central Florida.

Experiment 2 forage Mn means were considerably higher than for the previous experiment. All forage Mn concentrations were well above the critical level of 20 mg/kg in both experiments. High dietary levels of Mn are not viewed as a problem for grazing ruminants, as they can tolerate dietary levels as high as 1,000 mg/kg (McDowell and Arthington, 2005).

Aluminum levels were similar in experiments 1 and 2 and varied by date ($P<0.05$) in all but treatment 1 of experiment 2. There is very little forage Al analysis data; however Underwood and Suttle (1999) suggest uncontaminated forage Al to range from 50 to 100 mg/kg. For most samples reported for the two years, forage Al was less than 50mg/kg.

Zinc levels also varied by date in both experiments and were similar between treatments ($P>0.05$). Low levels of Zn in soils, plants, and animal tissues have been reported through many tropical regions of the world (McDowell and Arthington, 2005). Most Zn levels were below the critical level of 30 mg/kg in both experiments.

Only a limited number of samples were analyzed for Co, Mo, and Se. Forage Mo varied, depending on factors such as soil Mo, soil pH, and season. Molybdenum is an essential micronutrient required for plant growth. Forage Mo means were not variable between

treatments, and generally low throughout all sampling periods. Forage Mo concentrations ranged from 0.09 to 2.45 mg/kg and averaged 0.69 ± 0.60 mg/kg. Forage Mo concentrations were similar to those found by Espinoza et al. (1991b) in previous Florida studies. For ruminants, the effects of excess Mo are largely those of Cu deficiency (McDowell and Arthington, 2005). In the present study, forage Mo concentrations were too low to influence Cu deficiency. Over 99% of all Co samples taken were below the critical concentration of 0.1 mg/kg. Forage Co and Mo data were not taken in experiment 2. Forage Se can range from less than 0.05 to considerably over 100 mg/kg, but the samples analyzed in this study were extremely deficient and were all less than the requirement of 0.1 mg/kg.

Summary and Conclusions

Aluminum applied to the land has been shown to reduce soluble P concentrations and P losses from soils and thus can be used to reduce environmental P contamination. Under grazing conditions, ruminants typically consume 10 to 15% of their DM intake as soil. The main question to be answered is whether Al-WTR applied to pastures will be detrimental to grazing ruminants. Two experiments using grazing Holstein steers were conducted to determine the effects of an Al water treatment residual (Al-WTR) as pasture applications on mineral status (principally P) and cattle performance. A second objective was to evaluate the effects of these applied Al-WTR on forage mineral concentrations.

The experiments were in late spring of consecutive years, 2005 and 2006, and were 148 and 145d, respectively. For each experiment, 36 steers were allotted (3/pasture) to 12 bahiagrass pastures of 12.0 ha and were provided mineral supplement. In experiment 1, one-half of the pastures received Al-WTR at a rate of 22.8 Mg/ha. In experiment 2, 53 Mg dry weight/ha additional Al-WTR was applied to the same pastures as in experiment 1. The treatments were in replicate and as follows: 1) control- no Al-WTR application with steers receiving free-choice

mineral supplement without P, 2) control with free-choice mineral supplement plus P, 3) treatment 1 with Al-WTR, and 4) treatment 2 with Al-WTR. Forage samples were taken on d0 and approximately every 28 d thereafter for 5 or 6 mo. Weights, blood, and liver biopsy were taken initially, at midpoint, and at termination, while bone biopsies were taken only at termination. The following minerals were analyzed: plasma- Ca, P, Mg, Al, Cu, and Zn; liver- Cu, Al, and P; bone- Ca, P, Mg, and Al; and forage- Ca, P, K, Mg, Na, Al, Co, Cu, Fe, Mn, Se, and Zn.

As the experiment progressed, there were increases in body weight for all treatments ($P<0.05$) in both experiments. There were no differences ($P<0.05$) in body weight gains among treatments for experiment 1, but for experiment 2, cattle with the Al-WTR treatment without supplemental P had a higher average final body weight ($P<0.05$) than the control treatment receiving supplemental P (241 vs. 218 kg).

There were collection date plasma differences for Ca, Cu, Mg, and Zn, but no differences among treatments ($P>0.05$) for both experiments. There were only collection date and treatment differences for liver P during experiment 2. Liver P increased ($P<0.05$) for treatment 2 as the experiment progressed and treatment 3 (Al-WTR plus P supplementation) was lower ($P<0.05$) than the other treatments. There were no treatment differences ($P<0.05$) in bone minerals for both experiments.

All forage mineral concentrations in experiment 1 except Mg and Mn and only forage Al, Fe, and Zn in experiment 2 were affected by collection date. During both experiments, there were no treatment effects ($P<0.05$) from Al-WTR applications on forage mineral concentrations except in July, 2005 for Al and November, 2005 for Na. In July 2005, the second month after Al-WTR application, Al-WTR pastures were higher ($P<0.05$) in forage Al than not treated

pastures (65.3 vs. 31.9 ppm), but this trend did not continue. The high value could be attributed to soil contamination on the forages during the collection.

All plasma, bone, and liver minerals were above critical levels for grazing cattle for both years, with the exception of bone P, which was under the critical level of 17%. Manganese was the only forage that was above cattle requirements at all collections. A few Ca samples were below 0.35%, and approximately 50% of forage P concentrations were below 18%. Forage K was below the critical level of 0.6% only in December of experiment 1. Most forage Zn fell below 30 mg/kg. All forage Cu fell below the 10 mg/kg requirement, and more than 50% of forage Fe fell below 50 mg/kg during the course of the experiments. All Na forage concentrations were below the critical level of 0.06%. Over 99% of Co and Se samples fell below the critical level of 0.1 mg/kg.

The quantities of Al-WTR applied to pastures in this study have previously been shown to reduce environmental P contamination to water sources. Results from the present experiment demonstrate that the Al-WTR applications had little or no effect on animal status of P or any other mineral analyzed. Likewise, Al-WTR had minimal effect on forage mineral concentrations. Lack of Al-WTR application effects on cattle mineral status and production was likely due to low bioavailability and amorphous nature of Al from Al-WTR. In these two experiments, it has been shown that Al-WTR from the Bradenton, FL water treatment plant and similar Al-WTR is safe to use on pastures in low to moderately high levels to help alleviate the environmental P problem.

APPENDIX
CHEMICAL COMPOSITION OF FORAGES

Table A-1. Chemical composition of bahiagrass without Al-WTR application for Experiments 1 and 2[a]

	Experiment 1		Experiment 2	
Component	May	September	May	September
DM,%	91.0	91.2	91.6	91.3
CP,%	16.1	10.3	13.9	10.3
ADF,%	30.2	39.1	30.0	38.7
NDF,%	60.2	68.9	60.8	65.0
NFC,%	11.8	8.4	13.2	11.9
TDN,%	55.5	54.0	56.0	54.5

[a] Data means shown for dry matter (DM), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), non-fiber carbohydrate (NFC), and total digestible nutrients (TDN).

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BIOGRAPHICAL SKETCH

Rachel Kristin Pratt was born in Weymouth, Massachusetts. She attended some grade school in Massachusetts and then moved to Florida at the age of 8. Rachel finished grade school at the Canterbury School of Florida and attended high school at St. Petersburg Catholic High School in Florida until 2001. She earned her bachelor's degree in animal biology at the University of Florida in 2005 and her master's degree in 2007.