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Phosphorus utilization in domestic animals

by

YaHsin Joan Cheng

A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Physiology

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Jesse P. Goff, Major Professor
Donald C. Beitz
Richard L. Engen
Ronald L. Horst
Timothy A. Reinhardt
Jerry L. Sell

Iowa State University

Ames, Iowa

2001

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Major Professor

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CHAPTER 1. GENERAL INTRODUCTION

Statement of the Problems

P pollution from livestock speeds up eutrophication

Eutrophication is a natural process by which lakes eventually become land. Unfortunately this process can be speeded up by certain actions associated with intensive animal production. More than 500 million tons of animal manure are added to our environment every year. It often contains high amounts of phosphorus (P) because animals do not efficiently use the organically bound phosphorus in their feed. In areas of intensive livestock production, large amounts of P from animal waste can saturate the soil beyond the P levels required for high crop yields. The excessive P can then dissolve in groundwater and surface water, and eventually, flow to the lakes, rivers, and oceans. In water, high P concentration stimulates water plants to grow rapidly. When these plants die and are decomposed by bacteria, the oxygen is consumed in the process. Aquatic creatures, especially fish, are suffocated by the low oxygen content of the water. If the high P content in animal feces could be decreased, we could decrease the amount of P flowing into the environment and the eutrophication process could be slowed.

Much of the P in grains such as soybeans and corn is bound to an organic compound called phytic acid, which renders it unavailable for absorption. Phytate P can only be absorbed when the phosphoester bonds are hydrolyzed. Unfortunately, there is little phytase activity in the intestinal tracts of birds and swine, which results in large amount of undigested phosphorus in manure. Ruminants can digest 98% of the phytate-P in their feed as a result of microbial production of phytase in their rumen. However, in dairy cows the gastrointestinal motility is increased during lactation due to high feed intake. As a result, the retention time for nutrients in the rumen is shortened and phytate may not be completely broken down. The result is decreased P absorption. In addition, dairy and beef cattle farmers are prone to add supplemental inorganic P at higher than the NRC requirements in the mistaken belief that

they will observe improved growth performance and milk production. This practice also contributes to the high P in ruminant manure.

It is known that vitamin D is the hormone that controls the active transport of P in the small intestine. In several of these studies, we are trying to use *Solanum glaucophyllum*, a plant containing glycosides of $1,25(OH)_2D_3$ in its leaves, as an inexpensive source of $1,25(OH)_2D_3$ to improve P utilization in birds and dairy cattle, and, thus decrease P excretion in animal manure.

Lack of an appropriate treatment for hypophosphatemia in cattle

Most cows experience hypocalcemia and hypophosphatemia at parturition. Milk fever develops when cows are not able to remove bone calcium sufficiently to compensate for calcium lost to milk production. From 3 to 28% of milk fever cows do not respond to calcium therapy and remain recumbent; some because of failure to correct low plasma P. In the United States, the treatment used in milk fever cows is also used to "treat" hypophosphatemia. The commercial product contains calcium gluconate, magnesium and phosphites (PO_3^{3-}) and phosphinates (PO_2^{3-}). Unfortunately, the body only uses phosphate (PO_4^{3-}) rather than phosphite. The commercial products are not useful as a phosphate supply for hypophosphatemic cows. If low plasma P is not corrected and the cow stays in recumbency for more than 48 hours, permanent nerve and muscle damage result. Hence, an appropriate treatment should be developed for hypophosphatemia to prevent further damage from recumbence in dairy cattle.

We conducted an experiment to demonstrate that phosphate solutions, especially sodium phosphate based solutions, could be successfully used as a treatment for hypophosphatemia.

Dissertation Organization

This dissertation is composed of a literature review and three manuscripts. The first chapter includes a general review relating the similarities and differences of P homeostasis between

mammals and ruminants, followed by an introduction of the calcinogenic plant *Solanum glaucophyllum*. The second chapter presents a manuscript that offers an appropriate treatment for hypophosphatemic cattle. It has been published in the *Journal of Veterinary Medicine*. The manuscripts in chapters 3 and 4 are studies of the application of a calcinogenic plant, *Solanm glaucophyllum*, as a cheap source of $1,25(OH)_2D_3$ to improve P utilization in chickens and dairy cattle. They have been prepared for submission for the Journal of Poultry Science (chapter 3) and the Journal of Dairy Science (chapter 4). A general conclusion will be made in chapter 5 including the recommendation for future research. References cited in literature review and manuscripts are included at the end of each chapter.

Literature Review

Phosphorus (P) utilization in domestic animals is dependent on the dietary P bioavailability and the P homeostasis mechanisms active in the individual animal. Therefore the literature review includes the following parts: the bioavailibility of dietary P and the homeostasis of P in animals.

The bioavailability of dietary P

Animal feeds contain organicly bound and free P. The organic compound binding P in grain legumes, oil-bearing plants and seeds (corns and soybean) is phytate, a six-carbon ring capable of binding six phosphate groups. At physiological pH (7.4) it has either one or two negatively charged oxygen atoms (Figure 1). The concentration of phytate P in feedstuffs depends on the part of the plant from which it is derived. In seeds and grains, phytate P is as high as 70% of total P whereas in non-storage organs of plant such as leaves it is almost zero (Sebastian et al. 1998). Phytate P can be dephosphorylated to less phosphorylated forms of inositol and finally to inositol by meso-inositol hexaphosphate phosphorydrolase (phytase),

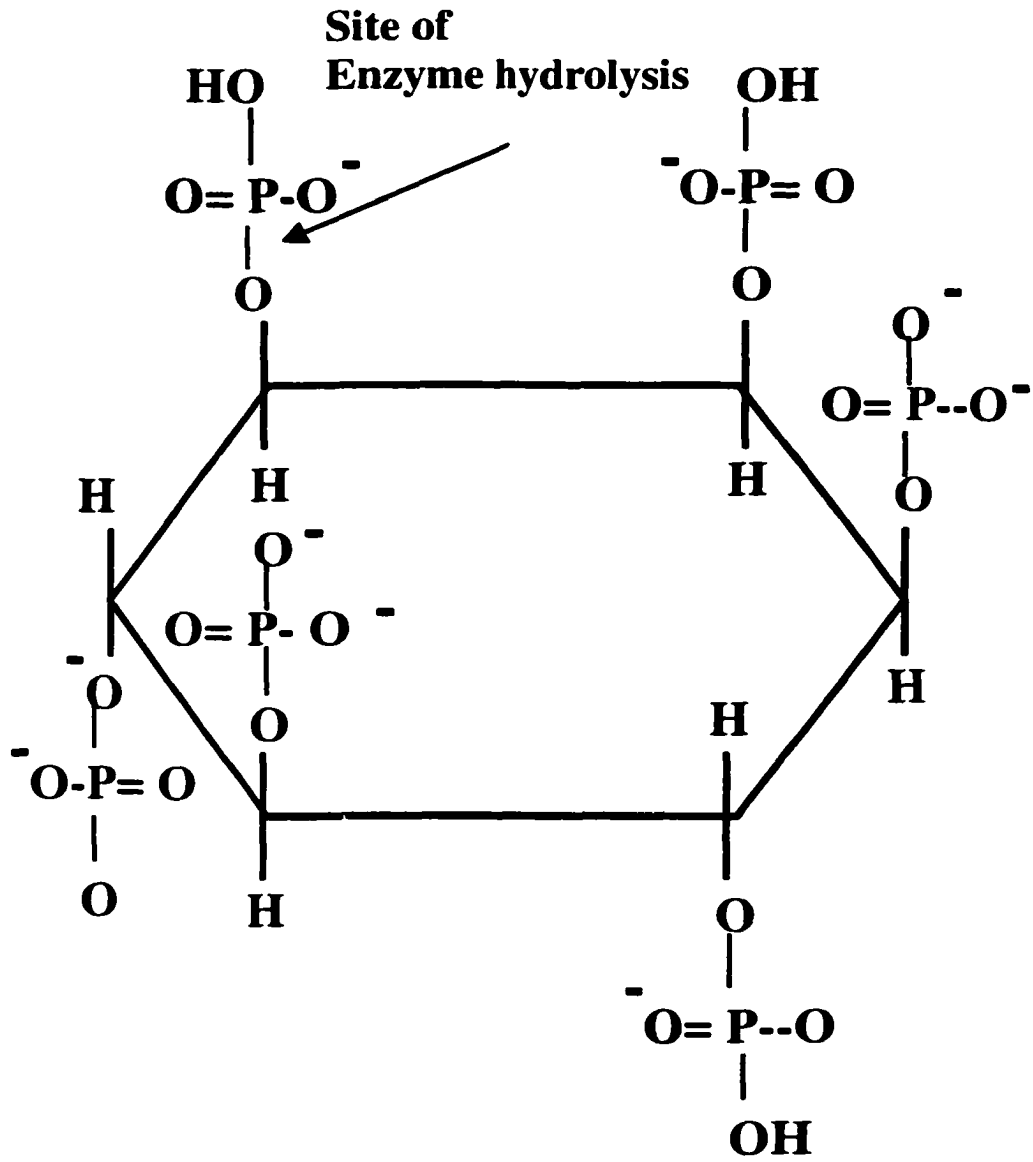

Figure 1. Myo-inositol hexaphosphoric acid

intestinal alkaline phosphate (ALP), and acid phosphatase (Reddy et al. 1982; Sandberg and Andersson 1988).

A. Phytate-P digestion in animals

In vivo tests showed the intestinal brush border contains phytase and in chicks the enzyme activity is highest in the duodenum and decreases progressively down the length of the gut (Maenz and Classen 1998). Though they are present, the small intestine mucosal phytase and ALP do not play important roles in phytate hydrolysis (Davies et al. 1970; Bitar and Reinhold 1972; Davies and Motzok 1972; Davies and Motzok 1972). The ability to digest and utilize phytate-P in poultry ranges from zero to 50% (Nelson 1976; Edwards 1983; Mohammed and Gibney 1991). It is estimated that 0 to 10% of phytate P can be used by broilers and turkeys, and up to 50% by laying hens. In swine, phytate P utilization is 10-40%. Therefore, from a practical point of view, increasing phytate dephosphorylation in monogastric animals will rely on addition of exogenous phytase derived from either plants or fungi to the diet. In ruminants, the rumen microbes produce enough phytase to break down the phytate bonds in the feedstuff which results in 90 to 98% phytate P digestion (Morse and Head 1991).

B. Factors influence intestinal phytate-P digestion

High dietary Ca or Ca:P ratio (>2) results in the formation of insoluble calcium-phytate P precipitates in the intestine (Nelson 1967). Data from chick experiments demonstrated that widening the Ca:P ratio from 1:1 to 2:1 decreased the availability of P from phytate (Qian et al. 1997). Supplemental vitamin D_3 and its metabolites increase phytate-P utilization, though the mechanism was not clear (Steenbock et al. 1953; Mohammed and Gibney 1991; Edwards 1993; Biehl et al. 1995; Edwards 1995; Mitchell and Edwards 1996; Biehl and Baker 1997; Biehl et al. 1998). It is possible that the vitamin D metabolites improve Ca absorption, which allows the phytate bound P complex to be more soluble, and therefore more available for phytase hydrolysis (Wasserman and Taylor 1973; Tanaka and Deluca 1974; Mohammed and

Gibney 1991). Some research suggested that vitamin D might directly increase synthesis or activity of intestinal phytase in chicks and rats (Fontaine et al. 1985; Shafey et al. 1991). In birds and swine, adults produce more intestinal phytase than young animals (Edwards, 1989) (Nelson 1976). The retention time of phytate-P in the gastrointestinal tract also influences phytate-P utilization. Edwards (1983) reported that the passage of digesta was slower and phytate P digestion more completely in the small intestine of leghorn chickens than in meat-type broilers (Edwards 1983).

C. Inorganic P in feeds

The major sources of free inorganic phosphorus in animal diets are supplemental minerals and animal byproducts. One group is the mono and di-calcium phosphates produced by reacting phosphoric acid with limestone. The concentration of limestone determines the amount of phosphate in the mixture. The other group is defluorinated phosphates produced by reacting phosphate rock with phosphoric acid and sodium carbonate, which then undergoes calcining at 1250°C. The biological value of the commercial defluorinated phosphates is variable due to difficulties in controlling the synthetic process (Waldroup 1999).

Bioassays for the dietary inorganic P availability usually relate the availability of a P source by comparing growth or bone parameters to those observed using reagent grade dicalcium phosphate as a standard, which is, assigned an availability of 100. The range of P bioavailability is large even when tested in the same species. In poultry, the order and ranges of bioavailability for the commercial phosphates are monocalcium phosphates (range 88.6-110), dicalcium phosphates (76.3-104) and defluorinated phosphates (68.6-89.6) (Waibel et al. 1984; Potchanakorn and Potter 1987; Potter 1988; Nelson et al. 1990). These large ranges for the availability of P sources, and the variation inherent during feed mixing may often lead to excessive P excretion or deficiency of P in rapidly growing birds (Waibel et al. 1984; Waldroup 1999). In ruminants and swine, the order of supplemental inorganic phosphates bioavailability is similar to that in poultry (Traylor and Cromwell 1998; NRC, 1998). Mono/dicalcium phosphates are more available than defluorinated phosphates and animal

byproducts. However in calves, Miller et al.(1987) reported no significant differences in the availability of defluorinated phosphates and dicalcium phosphates (Miller et al. 1987). Animal derived supplements such as meat and bone meal contain highly available P. In poultry, the availability from bone meal was similar to that of supplemental inorganic dicalcium phosphate (Sullivan et al. 1994; Sell and Jeffrey 1996). However, the P content in the animal byproducts can range from 1.8 to 7.0%, which also contributes to the possibility of overestimation or underestimation of P supply (Shutze and Benoff 1981; Waldroup 1999).

P homeostasis in domestic animals

Phosphorus homeostasis differs among monogastric animals and ruminants. In poultry and swine, P homeostasis is controlled by intestinal absorption and the kidney excretes excess P under the regulation of parathyroid hormone (PTH) and calcitriol. In ruminants, the excessive P in plasma is excreted by the salivary glands and the role of kidney in P excretion becomes minor. The major route of P excretion is through the gastrointestinal tract.

A. The physiological roles of Phosphorus (P)

Phosphorus is an essential mineral for animal growth and reproduction. Most P (85%) is stored in the skeleton in the form of hydroxyapatite with Ca. The rest (15%) is present in cell cytosol, cell membrane, and body fluid. The organic form of P is primarily present as phospholipid in cell membranes and as an energy-carrying molecule, adenosine triphosphate (ATP), in cells. Less than 1% of total body P is in blood. A large amount of blood P is within the RBC as 2-3-diphosphoglyceric acid. This compound involves the binding of oxygen to hemoglobin. The total P in blood is about 14mg/dl. However only 4-6mg/dl is in the form of inorganic phosphates. The inorganic phosphates in serum exist as phosphate salts with Ca, Mg and Na or as dibasic (HPO_4^{2-}) and monobasic ($H_2PO_4^-$) ions. Serum phosphates serves as a buffer system for the maintenance of acid/base balance. In clinical medicine, serum inorganic phosphate is also measured for diagnostic and therapeutic

reference. Young animals have higher P concentrations due to the effect of growth hormone which increases renal P reabsorption.

In addition to plasma P, serum alkaline phosphatase (ALP), renal clearance of Pi and radiographic analysis of skeleton mineralization are usually used as indices of P status (Avioli and Krane 1998).

B. Gastrointestinal phosphorus absorption

General mechanisms of Pi absorption

Intestinal inorganic phosphate (Pi) absorption occurs in 3 steps; a) Pi crosses the lumen brush-border membrane and enters the enterocyte; b) intracellular Pi is transported from the lumen side to the basolateral side of the cytosol; and c) Pi is transported across the basolateral membrane into the blood (Murer and Hildmann 1981).

Both active and passive transport mechanisms allow Pi to cross the brush border membrane. The active transport system, mainly found in the jejunum, is a Na/P protein carrier. The active mechanism is vitamin D-dependent. The driving force depends on the Na gradient, since the lumenal concentration of Na is usually about ten times higher than the intracellular concentration. The Na gradient is maintained by the Na/K ATPase located within the basolateral membrane (Peterlik and Wasserman 1978) (Cross et al. 1990; Danisi et al. 1990) (Ghishan and Kikuchi 1987) (Schroder et al. 1995). Passive absorption across the brush border is driven by a chemical gradient against an electrical gradient. The intracellular electric potential is negative relative to the lumen side. Therefore, the less negative monovalent anion $H_2PO_4^-$ diffuses across the brush border membrane more readily than HPO_4^{2-}. Since an acidic environment promotes $H_2PO_4^-$ formation, the duodenum is the major site of passive P absorption due to its relative acidic environment. The more negative HPO_4^{2-} is absorbed primarily via active transport whereas the $H_2PO_4^-$ mostly through paracellular absorption (Danisi et al. 1984; Cross et al. 1990).

Little is known about the intracellular migration of Pi across the cell. It is believed that microfilament system might be involved in intracellular Pi migration, because this process

can be blocked by drugs (such as cytochalasin B) that disrupt the microfilament network (Fuchs and Peterlik 1979). A specific carrier protein synthesized in response to Vitamin D receptor activation was also proposed to play a role (DeLuca 1988). However, no specific vitamin D-dependent cytosolic Pi carrier protein has yet been isolated.

The mechanism of Pi transport across the basolateral membrane is not well defined. Danisi et al. (1984) reported the presence of a Na-independent carrier driven by the electrical gradient (Danisi et al. 1984), whereas Ghishan et al. (1987) demonstrated a Na-dependent Pi transport system that is vitamin D-dependent and that operates under electroneutral condition in basolateral membrane vesicles isolated from rat jejunum (Ghishan and Kikuchi 1987; Ghishan 1992).

Paracellular diffusion is the mechanism of Pi absorption from the large intestine (mainly in the colon). The process is triggered by high P concentration in the lumen (Barlet et al. 1995). An *in vitro* study in rat colon showed passive diffusion of Pi, which was not associated with the effect of $1,25(OH)_2D_3$ (Lee et al. 1990). Some human medical therapies involve administration of Pi via the rectum as enemas. The phosphate clearly is absorbed but the rate of absorption is variable and there is a risk of developing severe hyperphosphatemia.

Pi absorption in ruminants

In ruminants, the small intestine is the major site of Pi absorption, though small amounts of Pi can pass through the wall of the rumen (Breves and Schroder 1991; Schroder et al. 1995).

In the fore-stomach, the passive diffusion of Pi was demonstrated in bovine omasum and sheep reticulo-rumen. The electrochemical gradient across the lumen and mucosal sides drives Pi across the epithelial cell (Breves et al. 1988). But the quantity of Pi absorbed from the fore-stomach and the overall importance of this portion to total Pi absorption in gastrointestinal tract is not clear.

Studies on small intestinal Pi transport have been done mostly in sheep and goats. It is known that plasma $1,25(OH)_2D_3$ is not changed in response to P depletion (Schroder et al. 1990) (Breves et al. 1985). However, a low Ca diet stimulates $1,25(OH)_2D_3$ formation and increases both Ca and P absorption (Abdel-Hafeez et al. 1982). Shirazi-Beechy et al. (1991) reported enhancement of a pH- but not Na-dependent Pi absorption in the brush-border

membrane, with no change in plasma $1,25(OH)_2D_3$ concentration (Shirazi-Beechey et al. 1991). Schroder et al. (1995), however, demonstrated that a Na-dependent Pi transport in sheep jejunum mediated 65% of active P absorption (Schroder et al. 1995). In an *in vitro* study of brush-border membrane vesicles isolated from goat jejunum, Schroder et al. further found Na-dependent Pi transport in goats fed a normal diet. The kinetic parameters of the transport system, K_m and V_{max}, were not changed by long-term P depletion, but V_{max} was increased when plasma $1,25(OH)_2D_3$ was increased by low dietary Ca. This Na-dependent Pi transport mechanism was also enhanced when the medium pH was decreased to 5.4, although no such proton-gradient effect on Pi transport was observed when Na was absent. This suggests that Pi transport in goat jejunum is Na- and calcitrol-dependent and can be stimulated by protons (Schroder and Breves 1996).

Factors affecting intestinal Pi absorption

a. Dietary Pi concentration

The effect of dietary P concentration on Pi absorption is mediated by $1,25(OH)_2D_3$. Low P diet results in low plasma Pi, which in turn stimulates the activity of 1α-hydroxylase in the kidneys and thus increases $1,25(OH)_2D_3$ production (Gray 1981). $1,25(OH)_2D_3$ increases Pi absorption by increasing intestinal Na/P cotransporter synthesis and the V_{max} of the Na/P cotransporter (Danisi et al. 1988). However, this ability to adapt to Pi deprivation is reduced in older animals (Lee et al. 1986).

The effect of low P diet in ruminants is vitamin D independent; plasma $1,25(OH)_2D_3$ was not increased even when intestinal Pi absorption was increased (Schroder et al. 1995). Nevertheless, increasing plasma $1,25(OH)_2D_3$ by dietary Ca restriction increased active P absorption (Abdel-Hafeez et al. 1982).

b. Dietary Ca:P ratio

Intestinal absorption of Pi is influenced by Pi solubility, and the dietary Ca:P ratio changes Pi solubility. This relationship between dietary Ca and P results from chemical association of Ca and Pi in the intestinal lumen rather than from the interaction of the two ions at the

absorption site (Hurwitz and Bar 1971). In monogastric animals, dietary Ca:P ratio between 1 and 2 are assumed to be ideal for growth and bone formation (Underwood 1981). Solubility of Pi decreased as the Ca:P ratio increased (Rao et al. 1995) because of the formation of calcium phosphate precipitates. Ruminants can tolerate wide range of Ca:P ratios from 1 to 7 without affecting the absorption of either Ca or Pi (Underwood 1981). One possible explanation is that Ca and P are more soluble in acidic environments. The pH in the upper small intestine is lower in ruminants than in monogastric animals, and this lower pH decreases the calcium phosphate precipitates and thus increases Ca and P availability.

c. Citric acid

Supplemental citric acid improves absorption of both Ca and P in the small intestine of rats and chicks (Boling et al. 1998). The mechanism is not clear. It may involve changes in intestinal pH, Ca solubility, or tight junction integrity. Soluble calcium citrate will be formed when citric acid is present in the distal intestine. Citrate may also decrease intestinal pH, increasing the solubility of Pi and thus making more Pi available for absorption. The citrate may disrupt the tight junction integrity of the epithelium barrier of the intestinal wall so that more cations pass through via the paracellular pathway.

d. Intestinal phytase and phytate-cation complex

Monogastric animals have little phytase activity and thus little ability to digest phytate P, although there is disagreement on whether phytase activity is due to or associated with alkaline phosphatase (ALP) in mammalian and avian intestine (Bitar and Reinhold 1972). Intestinal phytase may be an isoenzyme of ALP, as suggested by the fact that both enzyme activities are increased by phosphorus deficiency and vitamin D_3 supplements. Davies et al. (1970), however, demonstrated that the activities of ALP and of phytase in chick intestinal mucosa were not influenced to the same degree by the factors already mentioned. When dietary P was adequate, the activity of intestinal phytase, but not of ALP, was increased when levels of vitamin D_3 were increased (Davies et al. 1970). Nevertheless, it is clear that the alkaline phosphatase protein isolated from bovine intestinal mucosa has phytase activity (Williams et al. 1985).

The presence of phytate-P has negative impacts on mineral bioavailibility (Sebastian et al. 1998). The phosphate groups on the phytic acid carbon ring have a strong tendency to form chelates with di- and trivalent cations (Figure 2). The stability of phytate-cation complexes varies with the identity of the cation: Zn > Cu > Co > Mn > Ca at physiological pH. Therefore, Zn may become a limiting mineral in high phytate diets. Furthermore, the formation of these cation complexes reduces the availability of the phytate P itself. The degree of phosphorylation of inositol alters the degree of inhibition of phytate-P availability. At higher rates of phosphorylation (Inp5 or Inp6 phosphates), absorption of calcium, zinc and iron was significantly inhibited, whereas no effect was observed at lesser degrees of inositol phosphorylation (Lonnerdal et al. 1989; Skoglund et al. 1999). The higher availability of phytate P in fermented feeds is due to the breakdown of inositol phosphates to less saturation with phosphates.

C. Renal phosphate excretion and reabsorption

In monogastric animals, kidneys maintain the plasma Pi concentration in the normal range. Almost 95% plasma Pi is filtered by the glomerulus. The renal Pi excretion is determined by the difference between free glomerular filtration and tubular reabsorption. The tubular transport maximum (Tm) for active Pi reabsorption is 0.1mmole/min and that tubular load is reached at a serum Pi level of about 2.5mg/dl. Therefore, almost 80% of filtered Pi is reabsorbed. Renal tubular reabsorption occurs primarily in proximal tubules though a small portion of filtered P is reabsorbed in the distal tubule(Amiel, Kuntziger et al. 1970; Lassiter and Colindres 1982). When plasma Pi concentrations increase, the filtered Pi and the capacity to reabsorb Pi also increases until reaching the maximal rate of transport (Tm) (Knox et al. 1977). Pi excretion is increased linearly when plasma Pi concentration is above Tm.

During renal failure, Pi excretion per nephron is also increased (Slatopolsky et al. 1966; Slatopolsky et al. 1968). Therefore the plasma Pi concentration can be maintained in the normal range though the number of functional nephrons might be decreased.

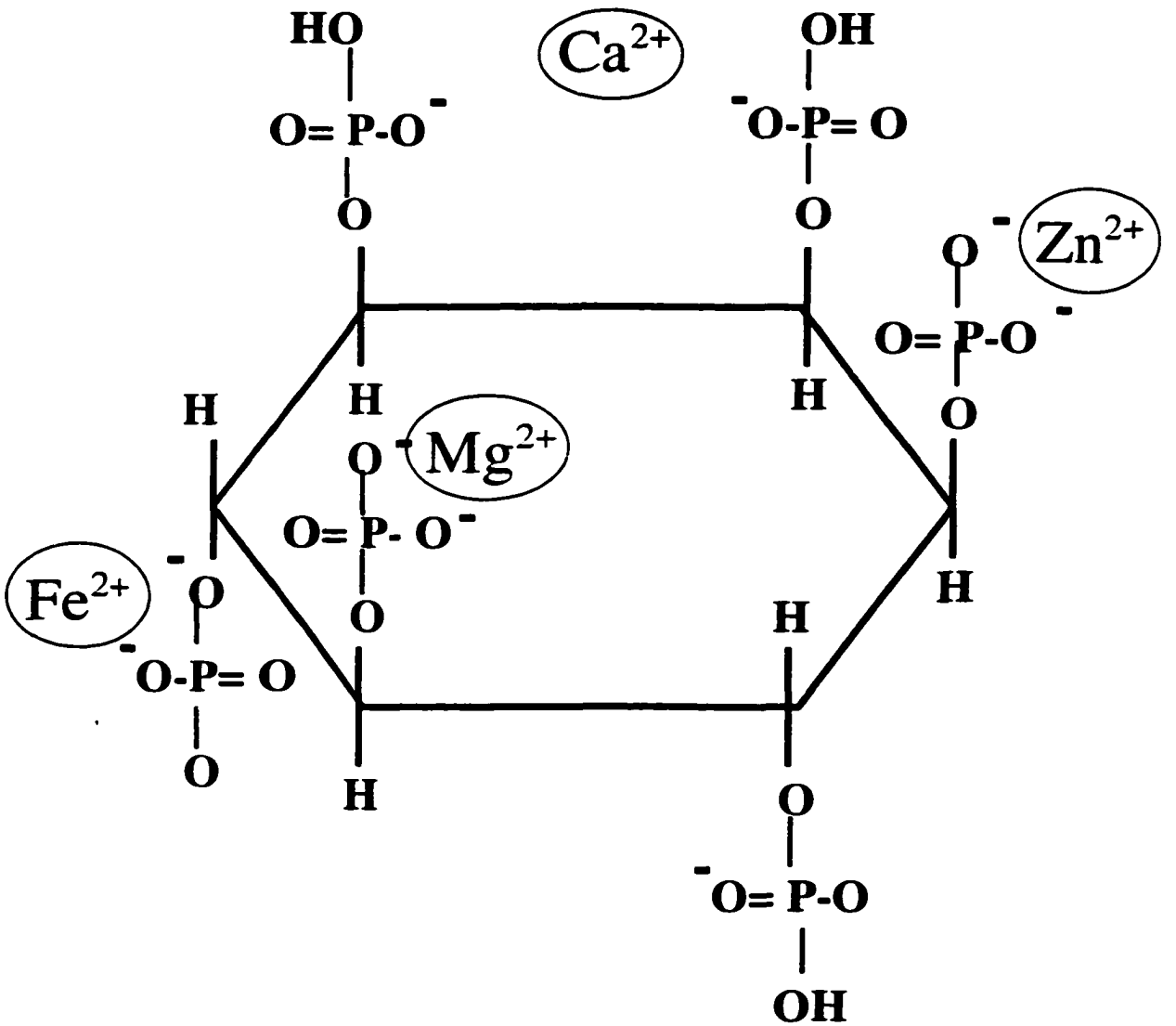

Figure 2. Phytic acid chelate at neutral pH

Hyperphosphatemia would not occur unless the glomeruler filtration rate (GFR) is decreased significantly.

Cellular mechanism of Pi reabsorption

The cellular mechanisms have been studied in many species in vivo and in vitro. A secondary active transport system known as the Na-Pi co-transporters are demonstrated to play the key role in P reabsorption. Three distinctive families of transporters are identified and named as type I, type II, and type III Na-Pi cotransporters (Murer et al. 1991; Murer and Biber 1995; Takeda et al. 1999; Murer et al. 2000). Type I Na-Pi cotransporter was first found in rabbit kidney cortex. The transporter is located in the brush-border membrane. It is not pH-dependent and it serves as an anion channel for chloride and various organic anions (Busch et al. 1996; Broer et al. 1998). Therefore, type I transporter is more like an anion channel protein rather than a P transporter. The predominant Pi uptake system in the kidneys is type II (a and b) Na/P cotransporters. Type IIa transporter is found mainly in the proximal tubular apical membrane in rat, mouse, human, rabbit and sheep. Its activity is increased with increasing membrane pH. Type IIa cotransporter is also expressed in osteoclasts, indicating its role in bone resorption (Gupta et al. 1996; Gupta et al. 1997). Type IIb transporter is the major Pi transporter in the kidney of chicks and carp. The aging process decreases the expression of this cotransporter and impairs the ability of an individual to adapt to low P diet (Sorribas et al. 1996). The mRNA expression of type III Na-Pi cotransporter has been identified in kidney, parathyroid gland and bone (Tenenhouse 1997; Tatsumi et al. 1998; Tatsumi et al. 1998). Its exact role is not clear.

In ruminants, kidneys play a minor role in P excretion because the tubular reabsorptive capacity is high during physiological conditions (Potthast et al. 1976). A single type II Na/Pi cotransporter is dominant in brush border membrane vesicles isolated from renal cortex. Compared with swine, the Na-dependent P transporter has higher V_{max} and K_m (Schroder et al. 2000). Furthermore, the plasma Pi concentration that reaches the maximal tubular reabsorptive rate (Tm) is 3 to 4 times above the normal physiological range, which explains that 99% filtered Pi is reabsorbed (Widiyono et al. 1998). Nevertheless, when the maximal

tubular reabsorptive rate is reached the renal P excretion would become significant (Liang et al. 1982; Liang et al. 1982; Sacktor et al. 1982).

Factors influencing P reabsorption

The rate-limiting step for regulation of renal Pi reabsorption is the Pi uptake from the brush-border membrane. Therefore, factors changing the activity or synthesis of type II Na-Pi cotransporter influence P reabsorption and excretion. Dietary P concentration has direct effect on the type II Na-Pi contransporter whereas PTH, $1,25(OH)_2D_3$ and calcitonin is the classical Pi regulating hormones. The role of PTH, $1,25(OH)_2D_3$ and calcitonin in renal Pi reabsorption will be included in the hormone regulation section.

a. Dietary P concentration

Low P diet directly increases renal P reabsorption rate independent of the P regulating hormones; PTH, calcitonin and $1,25(OH)_2D_3$ (Portale et al. 1987). It is believed that the adaptation to P supply is occurring at the brush border membrane Na/P cotransporter (Biber and Murer 1985). Western blots and immunohistochemistry demonstrate that an increase in brush-border membrane Na-P cotransporter activity in response to a low P diet is correlated with an increase in type IIa transporter protein (Boyer et al. 1996). However, low P diet-induced changes in the levels of specific mRNA were rather small, and transport adaptation was not prevented by actinomysin D (Biber et al. 1988). The conflict in the mRNA levels and transport protein synthesis may be explained by evidence derived from cell lines. Cells incubated in low P medium underwent a rapid increase in the V_{max} of the Na-independent P cotransporter, which was independent of protein synthesis (Biber and Murer 1985; Caverzasio et al. 1985). A slow phase of adaptation occurred several hours later and this phase could be inhibited by blocking protein synthesis (Caverzasio et al. 1985; Levi et al. 1994).

D. The recycle of salivary P in ruminants

Physiological role of salivary Pi

Saliva P is an important route of secretion of P in ruminants. It contributes 5 to 10g of P in sheep and 30 to 60g of P in cattle (Breves and Schroder 1991). Most is reabsorbed in the small intestine. However, the secretion of saliva P accounts for 80% of P in the digestive tract and therefore, is the major source of endogenous fecal P excretion (Ternouth 1989). Other sources of endogenous P are from bile, and the nuclei acids and phospholipids from the rumen microbial biomass (Tamminga 1996).

In addition to being the major source of endogenous fecal P loss, the salivary P is also important in buffering the volatile fatty acids producing in the rumen and supplying the P needed for the rumen microbes growth (Care 1994).

Composition and source of salivary Pi

The forms of P in saliva are $H_2PO_4^-$, HPO_4^{2-} or PO_4^{3-}. In sheep, salivary phosphate concentration in parotid glands varies widely, ranging between 2 and 60 mmol/l. Because salivary Pi concentration is proportional to plasma Pi and dietary P intake (Manas-Almendros et al. 1982), it is believed that excess plasma Pi is recycled by the salivary glands instead of being excreted in the urine (Kay 1960; Care 1994; Widiyono et al. 1998). Thus, the major route of P excretion in ruminants is through the digestive tract. The recycling of P through saliva may exceed fecal excretion 5 to 10 fold (Tamminga 1996). How the plasma Pi is transported to the salivary gland is not clear. Under physiological conditions, salivary Pi concentration is at least 3 times higher than plasma Pi, suggesting that Pi does not enter saliva by passive diffusion of plasma Pi. Vesicle studies showed the presence of a Na/PO_4^{3-} cotransporter in the basolateral membranes of sheep parotid cells (Vayro et al. 1991), supporting the role of an active transport system in maintaining salivary P concentration.

Factors influencing salivary Pi secretion

a. Dietary P concentration and physical nature of the diet

Experiments in sheep demonstrated that low P diets led to a decrease in plasma P concentration, which in turn, decreased the amount of P secreted in saliva. However, if roughage is fed the salivary P increased regardless of the fact that the diet is low in P (Rajaratne et al. 1996). On the other hand, pelleted or finely ground diets as well as digestible grasses decrease the rate of salivary P secretion (Scott and Buchan 1987).

b. Hormone regulation of salivary Pi secretion and concentration

The effect of PTH on salivary Pi secretion is contradictory. Some research reported that PTH increased salivary Pi secretion in non-lactating, non-pregnant sheep (Clark et al. 1975). But the increment depends on the P status of the animal and on local blood flow in the parotid gland. In P-repleted sheep, PTH increased salivary Pi concentrations and this was accompanied by increased local blood flow in the parotid glands. However, PTH given to P-depleted animals had no effect on salivary Pi (Wright et al. 1984).

Other studies have found that PTH decreased salivary P concentration and secretion in sheep and goats even the animal was in hypophosphatemia and had low salivary P (Manas-Almendros et al. 1982). In another study, $1,25(OH)_2D_3$ decreased salivary P concentration and secretion even as it induced hyperphosphatemia and hypercalcemia. When circulating $1,25(OH)_2D_3$ is inhibited, secretion and concentration of salivary Pi is increased. This suggests that plasma Pi can influence the concentration of salivary Pi but that hormones regulate the salivary Pi concentration (Manas-Almendros et al. 1982; Riad and Lefaivre 1987). Riad et al. (1994) further demonstrated that the effect of PTH on decreasing P secretion in saliva was mediated by $1,25(OH)_2D_3$ (Riad and Lefaivre 1987; Riad et al. 1994).

In cattle, $1,25(OH)_2D_3$ decreases salivary P concentration and secretion even when hyperphosphatemia is present. Salivary P secretion and concentration is increased when circulating $1,25(OH)_2D_3$ is inhibited and decreased when plasma $1,25(OH)_2D_3$ is increased. This suggests plasma $1,25(OH)_2D_3$ rather than phosphatemia regulates salivary P concentration and secretion in cattle(Riad and Lefaivre 1987).

The effect of calcitonin on salivary Pi was tested in thyroidectomized sheep. Salivary Pi was increased by administration of porcine calcitonin, and this effect was accompanied by a decrease in plasma Pi concentration (Matsui et al. 1984).

Besides the hormones, the parasympathetic nervous system, acting via muscarinic receptors, is the major stimulus for secretion by ruminant salivary glands and greatly affects the volume of saliva produced.

E. Phosphorus secretion during gestation and lactation

P transfer during gestation

In rats, Pi is transferred across the placenta by a Na-dependent mechanism. This mechanism is saturated at physiological concentrations of maternal plasma Pi. However, when the Pi on the fetal side of placenta is higher than on the maternal side, the transfer was decreased, suggesting a control by a negative feedback between fetal concentration of Pi and the Pi transfer rate (Stulc and Stulcova 1996). In humans, plasma Pi levels are normal throughout pregnancy whereas fetal Pi levels are higher than maternal (Schauberger and Pitkin 1979), suggesting Pi may be actively transported across the placenta (Weiss et al. 1998) (Stulc and Stulcova 1996).

In ruminants, transfer of Pi from high maternal plasma Pi pool to that of fetus is by passive diffusion. House and Bell (1993) reported that the conceptus in Holstein cows at 190 days and 280 days of gestation contained 1.8g and 5.4g P. Therefore, they suggested that 5.4g of P should be supplied to meet the maximal requirements for conceptus growth by the end of gestation in addition to the amount for daily maintenance. Calcitrol produced by fetal kidney increases both Ca and Pi transfer across placenta (Durand et al. 1983). Fetal PTH and PTHrP stimulate Ca transport but not P transport (Abbas et al. 1989; Care and Abbas 1990; Barlet and Davicco 1994). Even when the mother is in mineral deficiency, the fetus still has normal weight and bone mineralization at birth, indicating adequate mineral supply to the fetus is at the expense of maternal skeleton.

In reproducing sows, low Ca and low P diet results in high incidence of leg problem during gestation (Nimmo and Peo 1981). Mahan and Fetter (1982) reported the observation of trabecular bone demineralization, suggesting the mobilization of Ca and Pi from maternal bone to fetus (Mahan and Fetter 1982). However, the control on transfer of Ca and P across placenta is not clear. The average mineral deposition in a litter of pigs with a birth weight of 14kg was 2.2g/day of P and 3.7g/day of Ca (Gueguen and Perez 1981).

P secretion during lactation

The concentration of P in milk is 0.9g/kg (NRC 2000). A cow producing 9000kg milk secretes about 8.56kg P into milk in a lactation course. In order to make up the loss of P in milk she has to consume 29g/day from the diet, if the dietary P is 100% available, just for daily milk production (Horst 1986). However, only 1-2g inorganic P is present in blood. It is not likely that absorption of dietary P would make up such amount of daily P loss in a short time. Therefore, cows are in negative P balance. Bone P is released to provide the P needs when cows are in the peak of milk yield. Carstairs et al. (1981) reported 500g to 600g of Pi is mobilized from bone during early lactation (Carstairs et al. 1981).

Most studies reported no effect of dietary P on milk production unless dietary P is decreased to 0.24% (Call et al. 1987). A study showed Pi in milk was higher for cows in first lactation than multiparous cows and dietary P did not affect milk P concentration. However, milk P declined in later lactation with reduced milk yields. Interestingly, the authors also reported that month of year affects inorganic P in milk because milk P was reduced during summer months in their study (Forar et al. 1982).

F. Bone resorption in P homeostasis

Bone can serve as a mineral reservoir. About 98% of total body Ca and 80% of total body P is deposited in bone as hydroxyapatite. Since 5% of blood flow is through bones per minute, the blood rapidly equilibrates with the readily exchangeable Ca and P, as well as with the

Mg, Na and K of bone. Most phosphate is released in association with calcium as part of the mechanism for maintaining normal blood Ca concentration (Avioli and Krane 1998).

Bone is constantly remodeled. The remodeling is achieved by bone resorption coupled with bone formation. Under normal conditions, bone density and mass remain constant when bone resorption and formation are balanced. However, such equilibrium breaks when large amount of Ca and P are required for lactation or eggshell formation. Bone Ca and P are released under the regulation of hormones, which result in the decrease of bone mass and density. Nevertheless, the loss of Ca and P in bone will be replenished during the latter period of a reproductive cycle with appropriate dietary Ca and P supply (Carstairsl et al. 1981; Horst et al. 1997).

The role of bone in hens during eggshell formation

In laying hens, besides the cancellous (trabecular bone) and cortical bone formed during growth, medullary bone forms when birds reach sexual maturity at about 25-week of age. Medullary bone (MB) is composed of spicules within the marrow cavity and also a mineral layer lining the surface of the tibiae and femur. The mineral composition of MB is similar to cortical or cancellous bone except there is little orientation of collagen fibers in matrix. Therefore, it is mechanically weaker than structural bone (Whitehead and Fleming 2000).

The formation of MB is controlled by sex hormones. Administration of estradiol and testosterone to male chicks induced formation of MB in the marrow cavities of femora and tibiae accompanied with increased plasma Ca, P and ALP activity. Anti-estrogenic compounds, trioxifene and tamoxifen, inhibited MB formation and associated serum parameters (Williams et al. 1991).

MB is more important to Ca homeostasis than to P homeostasis. MB is a reservoir of readily available Ca and provides Ca for shell formation (Whitehead and Fleming 2000) (Bar and Hurwitz 1984). However, the regulation of Ca homeostasis to supply Ca for egg shell formation and MB mineralization is mediated by $1,25(OH)_2D_3$ instead of sex hormones (Bar et al. 1978). During egg-laying cycle, the activity of renal 1α-hydroxylase and plasma Ca are increased before and at the time of ovulation. Plasma $1,25(OH)_2D_3$ is increased later and

remains high until 12 hours post-ovulation (Castillo et al. 1979). Both Ca and P absorption is increased. If egg production is inhibited by drugs, the activity of 1α-hydroxylase and intestinal calcium binding protein (CaBP) are decreased but no change is observed in plasma Ca, estrogen and MB.

Under dietary Ca deficiency, MB is reabsorbed (Williams et al. 1991), whereas vitamin D deficiency results in absence of MB (Bar and Hurwitz 1984). Both induced hypertrophy of parathyroid gland. However, dietary P deficiency and hypophosphatemia had no effect on the activity of kidney 25-(OH)D_3-1-hydroxylase though the intestinal CaBP was increased. There was no change in the size of parathyroid gland either. The osteoid was increased, as an indicator of the failure of bone mineralization, suggesting the reduction of MB induced by P deficiency was due to the inhibition of bone formation rather than the stimulation of bone resorption (De Barros et al. 1981; Wilson and Duff 1991).

The presence of MB was once thought to be detrimental to bone strength, because calcium is thought to be used to form MB at the expense of the cancellous and cortical bone during the laying period (Bar and Hurwitz 1984). Recently, it was demonstrated by radiography of the humeral diaphysis that the formation of MB was not at the expense of the surrounding cortical bone. On the contrary, larger amounts of MB contributed to fracture resistance of the cortical bone (Fleming et al. 1998).

Bone in lactating cows

A cow producing 9000kg milk in a 300 days of lactation course secretes average 35g Ca and 27g P into milk ever day. The total Ca and Pi in blood pool are 2-4g and 1-2g, respectively. Even the dietary Ca and P supply meets the requirement for milk production and maintenance, bone resorption still occurs, and increasing dietary mineral concentrations does not prevent the resorption. Bone P mobilization is accompanied with Ca mobilization, whereas bone Ca mobilization is induced by the acute decrease in the plasma Ca concentration when large amount of Ca is excreted for milk production (Horst et al. 1997). PTH is released, which stimulates bone resorption and increases 1,25$(OH)_2D_3$ synthesis, as well as increases urinary Ca reabsorption. Bone loses 13% of its total Ca and provides 500 to

600g of P during early lactation (Carstairs et al. 1981; Horst et al. 1997). The loss of bone mass will be replenished in later lactation by increasing intestinal Ca and P absorption mediated by the continuously elevated plasma $1,25(OH)_2D_3$ concentration (Horst et al. 1997).

Hormonal regulation of P homeostasis

Phosphorus homeostasis is primarily regulated by 3 hormones; $1,25(OH)_2D_3$, parathyroid hormone (PTH) and calcitonin (CT). In addition, glucocorticoids and growth hormone (GH), also play roles in regulating plasma P concentrations.

Hypophosphatemia has direct and indirect effects on the small intestine to induce P absorption. Low plasma P stimulates the activity of 1-alpha hydroxylase in the kidney and therefore, the production of $1,25(OH)_2D_3$, which in turn, increases intestinal vitamin D-dependent P transport. Meanwhile, the low plasma P directly activates the Na/P symporters in the brush border membrane of small intestine to increase the V_{max} of P transport (Tenenhouse 1997; Hattenhauer et al. 1999).

PTH secretion is inhibited during hypophosphatemia. This is because the elevation of $1,25(OH)_2D_3$ induced by low plasma P increases Ca absorption, which reduces the secretion of PTH. On the other hand, $1,25(OH)_2D_3$ itself inhibits the transcription of PTH mRNA. In addition, cell size and proliferation in parathyroid glands is directly decreased by low plasma P (Wang and Paloyan 1996; Silver and Sela 1997; Funahashi et al. 1998; Silver et al. 1999) via a post-transcriptional mechanism. The overall reactions decrease plasma PTH and therefore, reduce urinary P excretion. Low plasma P also increases the renal P reabsorption capacity via intrinsic mechanisms (Audran and Kumar 1985). The reduction in renal excretion and increase in intestinal absorption re-establish the normal serum P in rats, mice, rabbits, pigs and chicks.

Unlike monogastrics, P deficiency does not stimulate $1,25(OH)_2D_3$ synthesis in goat and sheep. Nevertheless, in lactating goats, P depletion significantly increased the binding affinity of VDR in the small intestine and response to $1,25(OH)_2D_3$ (Schroder et al. 1990; Shirazi-Beechey et al. 1991).

During hyperphosphatemia, renal excretion becomes the major mechanism to decrease excessive P in both monogastrics and ruminants. High plasma P inhibits the activity of renal 1-alpha-hydroxylase and reduces $1,25(OH)_2D_3$ production. The declining plasma $1,25(OH)_2D_3$ decreases both P and Ca absorption from small intestine. The secretion of PTH is induced by low plasma Ca, which enhances renal excretion of P. However, when calcium concentration is above 17mg/dl or the calcium*phosphate product in blood exceeds the solubility product (about 4.85×10^{-6} molar units), calcium-phosphate precipitates form throughout the soft tissues.

A. $1,25(OH)_2D_3$

$1,25(OH)_2D_3$ is the biologically active form of vitamin D. The two most important forms of vitamin D are vitamin D_3 and vitamin D_2. The difference in structure between these two compounds is the methyl group on carbon-24 and the presence of a 22,23-double bond in vitamin D_2 as compared with vitamin D_3. In the rachitic chick bioassay, $1,25(OH)_2D_2$ is 10 times less active than $1,25(OH)_2D_3$ though the duodenal vitamin D receptor binding capacity to both compounds is the same. Vitamin D_3 is 80 times more active than D_2, suggesting the discrimination against vitamin D_2 in chickens occurs before the formation of $1,25(OH)_2D_2$. D_2 and D_3 compounds activities are equivalent in rats (Rambeck, Weiser et al. 1984).

Dietary vitamin D_3 absorption and metabolism

Because vitamin D is lipid soluble, dietary vitamin D is transported to the liver via lymphatic circulation after being absorbed by small intestine. The circulating vitamin D_3 is converted to $25(OH)D_3$ by enzymes in the mitochondria of the liver cells. $25(OH)D_3$ is the most abundant vitamin D metabolite in plasma. It is bound to α 2-globulin and further converted to $1,25(OH)_2D_3$ in the kidneys (Reinhardt et al. 1988). When plasma $1,25(OH)_2D_3$ and/or plasma phosphate is high, the 24-hydroxylase is activated and $25(OH)D_3$ is converted to $24R\text{-}25(OH)_2D_3$ (Tanaka and Deluca 1973). Other tissues process $1,25(OH)_2D_3$ receptor also have 25(OH)D-24-hydroxylase. The physiological role of 24-hydroxylation is unclear.

In poultry, $24,25(OH)_2D_3$ promotes hatchability of eggs (Henry and Norman 1978). In humans, it increases bone mineralization with the presence of $1,25(OH)_2D_3$ (Bordier et al. 1978). But $1,24,25(OH)_3D_3$ has no function in enhancing bone mineralization (Holick et al. 1973). The presence of 24-hydroxylase in the kidney could be for side-chain catabolism of $25(OH)D_3$ and $1,25(OH)_2D_3$ (Castillo et al. 1979). In ruminants, vitamin D metabolism begins in the rumen. Both vitamin D_2 and D_3 can be converted by microorganisms to trans-10-keto-19-nor-vitamin D.

$25(OH)D_3$ and $1,25(OH)_2D_3$ are metabolized on the carbon-26 or carbon-23 position under physiological conditions (Reinhardt et al. 1981) (DeLuca et al. 1970). For review of vitamin D metabolism refer to Horst et al. (1994) (Horst et al. 1994).

Regulation of $1,25(OH)_2D_3$ secretion

Synthesis of $1,25(OH)_2D_3$ is controlled by 25(OH)-1α-hydroxylase in the kidney. 25(OH)-1α-hydroxylase is stimulated by an increase in the serum PTH, a decrease in the serum Ca and P, and a decrease in the activity of 24-hydroxylase (Gray 1981). Hypocalcemia, initially thought to act via stimulation of PTH release, has been shown to directly stimulate 1-hydroxylation in mammals. In ruminants, PTH is the most important factor that stimulates the formation of $1,25(OH)_2D_3$ (Hove 1984; Horst 1986).

a. Plasma Ca concentrations and PTH

Hypocalcemia stimulates PTH secretion, which induces the activity of 1α-hydroxylase in the renal proximal tubular cells (Omdahl et al. 1972; Henry and Norman 1984). The effect of PTH is mediated by cAMP (Rost et al. 1981). PTH also decreases the activity of 24-hydroxylase and further decreases degradation of $1,25(OH)_2D_3$ (Henry 1981). Brebza et al. (1998) reported that the effect of PTH on 1α-hydroxylase was through activation of gene promoter (Brenza et al. 1998).

b. Dietary P concentrations

The effect of low dietary P on the activation of 1α-hydroxylase is independent of PTH and plasma ionic Ca concentration (Hughes et al. 1975; Bushinsky et al. 1989). It is controlled at the mRNA level (Shinki et al. 1997). Besides, the expression of 24-hydroxylase mRNA was decreased during hypophosphatemia (Wu et al. 1996), which inhibits 1,25(OH)$_2$D$_3$ degradation. However, the activation in 1α-hydroxylase by dietary P restriction was not observed in hypophysectomized rats and diabetes rats (Gray 1987). When growth hormone and insulin were given to the animals the activation on 1α-hydroxylase was restored, suggesting IGF-I and insulin are involved in the Pi-regulated 1,25(OH)$_2$D$_3$ synthesis (Gray 1987) (Matsumoto et al. 1986).

Inhibitors of the renal 25(OH)D-1 alpha-hydroxylase include 1,25(OH)$_2$D$_3$ itself, hypercalcemia, and high blood phosphate (Breslau 1988). 1,25(OH)$_2$D$_3$ inhibits the activity of 1α-hydroxylase and stimulates 24-hydroxylase at mRNA level. Hence it inhibits its own synthesis and increases degradation (Monkawa et al. 1997). When 24-hydroxylase is activated by high plasma 1,25(OH)$_2$D$_3$ and/or plasma phosphate (Tanaka and Deluca 1973) 25(OH)D is directly converted to 24,25(OH)$_2$D$_3$. Tissues with a receptor for 1,25(OH)$_2$D$_3$ also have been shown to possess an inducible 24 hydroxylase. The degradation of 24S,25(OH)$_2$D$_3$ and 1,25(OH)$_2$D$_3$ can be found in all tissues(Horst, Goff et al. 1997).

The mechanisms of 1,25(OH)$_2$D$_3$ actions

The action of 1,25(OH)$_2$D$_3$ is mediated by a) a nuclear receptor that is involved in regulation of gene transcription in over 30 cell types possessing this receptor (genomic pathway) and b) the plasma membrane receptor that is involved in initiation of signal transduction pathways which generate rapid biological responses (non-genomic pathway). The latter occurs in 4-6 minutes whereas the former takes more than 10 hours.

a. Genomic pathway

Only 5% of circulating 1,25(OH)$_2$D$_3$ is in the free state (Bikle et al. 1984; Bikle et al. 1985) and can diffuse into target cells. The free form of 1,25(OH)$_2$D$_3$ binds with the intracellular

$1,25(OH)_2D_3$ receptor (VDR) to form heterodimer with the retinoic acid X receptor (VDR/RXR). The VDR/RXR heterodimer has high affinity for the vitamin D response elements (VDREs) in the promoter region of $1,25(OH)_2D_3$-dependent genes (Horst et al. 1997). The genes that are negatively regulated by vitamin D include avian and human PTH, protein kinase A inhibitor and rat PTHrP, whereas genes positively regulated include intestinal CaBP and Na/P cotransporter (Brown et al. 1999).

b. Non-genomic pathway

Recently, the non-genomic mechanism was demonstrated in bone cells and intestinal mucosal cells (Karsenty et al. 1985; Nemere 1996). $1,25(OH)_2D_3$ agonists open the voltage gated L type Ca channel in the cell membrane, which increases transient intracellular Ca concentration and activates both PKA and PKC signal transduction pathway (Sylvia et al. 1996). The rapid action is also mediated by altered phospholipid synthesis (Morelli et al. 1993).

Action in intestine

The vitamin D-dependent active P transport in epithelium is distinguished from that of Ca (Peterlik and Wasserman 1978). Through the genomic pathway, $1,25(OH)_2D_3$ stimulates the expression of Na-P cotransporter (Yagci et al. 1992). Nemere (1996) recently reported a non-genomic pathway of $1,25\ (OH)_2D_3$ for P absorption. Low levels of $1,25(OH)_2D_3$ stimulated rapid (4-8 minutes) phosphate transport in the perfused duodenal loop of normal chicks (Nemere 1996).

$1,25(OH)_2D_3$ stimulates active absorption of Ca by increasing synthesis of calbindin and plasma membrane Ca pump in the enterocyte. These actions are mediated by VDR (Cai et al. 1993; Wasserman and Fullmer 1995). In aged rats, the rate of Ca absorption, the synthesis of calbindin-D9k and plasma membrane Ca pump mRNA, as well as the ATP-dependent Ca uptake by basolateral membrane vesicles are decreased. Because the Ca pump mRNA was significantly increased following administration of $1,25(OH)_2D_3$ to both young and old rats, the decreased reactions in the aged animals might be due to the age-related decline in serum

$1,25(OH)_2D_3$ levels, rather than development of resistance of the Ca pump gene to $1,25(OH)_2D_3$ action (Armbrecht et al. 1988).

Action in the kidneys

The effect of $1,25(OH)_2D_3$ on renal Ca reabsorption was demonstrated in distal convoluted tubule cells (Friedman and Gesek 1993). $1,25(OH)_2D_3$ accelerated PTH-dependent calcium transport and calbindin expression (Bindels et al. 1991). Hence, the renal Ca reabsorption is increased.

The primary action of 1,25(OH)2D3 is to increase tubular P reabsorption by increasing the V_{max} of the P transport system and the synthesis of the specific Na/P cotransporter (Liang et al. 1982; Liang et al. 1982). Administration of vitamin D to normal, vitamin D-deficient, and thyroparathyroidectomized animals decreases P excretion, suggesting $1,25(OH)_2D_3$ directly induces phosphate reabsorption in the proximal tubule without the influence of PTH and plasma Ca (Gekle et al. 1969).

Action on bone

Bone mineralization induced by $1,25(OH)_2D_3$ is through increasing intestinal Ca and P absorption to supply the minerals for hydroxyapatite formation (Slovic et al. 1981). Recently, a membrane receptor for $1,25(OH)_2D_3$ was identified and confirmed a non-genomic role for $1,25(OH)_2D_3$ in the increased specific activity of alkaline phosphatase, phospholipase A2, and matrix metalloproteinases in chondrocytes. The rapid action occurred within 3 minutes and reached maximal activation by 9 minutes (Boyan, Dean et al. 1994). The non-genomic action of $1,25(OH)_2D_3$ was also shown in rat osteoblast cell-line ROS 17/2.8, which stimulated calcium influx via L-type Ca channels and therefore, elevated intracellular Ca.

The effect of $1,25(OH)_2D_3$ on bone resorption, however, is indirect and only demonstrated *in vitro* because VDR is found in mature osteoblasts rather than osteoclasts (Suda et al. 1990; Suda et al. 1992). *In vitro* test demonstrated that $1,25(OH)_2D_3$ stimulated osteoblasts to

release ostoclast-activating activity that acts through paracellular way on bone resorbing cells to mobilize Ca from bone (Ohyama et al. 1991).

B. Parathyroid hormone (PTH)

Parathyroid hormone is the most potent factor regulating Ca and P homeostasis. Its effect on stimulation of $1,25(OH)_2D_3$ synthesis contributes to its importance in the regulatory role. However, the negative feedback loop exists between PTH and plasma Ca and Pi concentrations. In addition, $1,25(OH)_2D_3$ may inhibit PTH synthesis at the mRNA levels.

Regulation of PTH synthesis and secretion

a. Plasma Ca concentration

Plasma Ca concentration regulates the synthesis of PTH at the PTH gene transcription levels (Russell et al. 1983). PTH mRNA levels was promptly increased during acute hypocalcemia and moderately decreased during hypercalcemia (Yamamoto et al. 1989). This suggests parathyroid gland is more prepared for PTH synthesis and secretion through transcription mechanism in response to the low plasma Ca. Extracellular Ca also influences the degradation of newly synthesized PTH. When bovine parathyroid gland was incubated in hypocalcemic medium, intact PTH was secreted. The secretion of degraded PTH was observed when the medium contained high Ca concentration. This suggests that parathyroid glands regulate the amount of bioactive hormone for secretion in response to Ca concentrations (Chu et al. 1973).

In addition to PTH synthesis, plasma Ca concentration regulates the secretion of PTH. When plasma Ca is low, the secretion of PTH is increased. Lowered extracellular Ca is detected by the Ca-sensing receptor, which activates the protein kinase C in parathyroid cells and triggers the secretory process (Butters et al. 1997). In calves, PTH secretion reaches maximum when plasma calcium is decreased to 7.5mg/dl (Mayer and Hurst 1978). On the other hand, when plasma Ca concentration is above normal, it does not inhibit PTH secretion completely.

b. Plasma Pi concentration

Hyperphosphatemia decreases the levels of ionized Ca, and thus stimulates PTH secretion (Sherwood et al. 1966).

c. $1,25(OH)_2D_3$

Pharmaceutical dose of $1,25(OH)_2D_3$ decreases the secretion of PTH by suppressing the PTH gene transcription (Cantley et al. 1985).

The primary function of PTH is to maintain a constant concentration of Ca in the extracellular fluid. PTH also plays a role in P homeostasis by stimulating bone resorption and increasing renal P excretion. Thus, excessive P is removed by phosphaturia.

PTH on kidney

By stimulating Na/Ca exchanger and Ca/Mg-ATPase, PTH increases Ca reabsorption in the distal nephron segments (Greger et al. 1978; Bouhtiauy et al. 1991). PTH also has effect on translocation of preformed Ca channel and functioning Na-P cotransporter. PTH triggers the translocation of intracellular Ca channels to the luminal membrane to increase Ca uptake. Meanwhile, PTH stimulates the moving of functioning Na-P cotransporter in the luminal surface into the intracellular space to decrease P uptake. Such effect explains the bi-directional action of PTH on Ca and P reabsorption (Bacskai and Friedman 1990).

PTH regulates P excretion through changing the ratio of maximum transport rate to glomeruler filtration rate (TmP/GFR). When PTH levels are low, the TmP/GFR is increased, and hence urinary P excretion is decreased. When PTH is elevated, the TmP/GFR is reduced and more P is excreted in the urine. The cellular mechanisms of PTH inhibition on renal P reabsorption involve endocytosis and lysosomal degradation of Na/P cotransporter. This action could be cAMP dependent or independent. Injection of PTH in rats or mice leads within minutes to a reduction in brush border membrane transporter. A prolonged increase in PTH leads to decrease the mRNA levels of type II Na-P cotransporter (Gmaj and Murer 1986), and thus inhibits P reabsorption.

However, the elevation of PTH induced by chronic high P diet intake had little effect in renal P excretion because urinary cAMP was decreased. The high urinary P excretion might result from decreased PTH/PTHrP receptor during high P diet feeding (Masuyama et al. 2000).

PTH on bone

Like $1,25(OH)_2D_3$, the effect of PTH on bone resorption is indirect. Mature osteoclasts do not have PTH/PTHrP receptors (Rouleau et al. 1988), whereas osteoblasts have PTH/PTHrP receptors. Therefore, it is believed that osteoblasts mediate the effect of PTH on osteoclasts (Hakeda et al. 1989; Teti et al. 1991). PTH stimulates osteoblasts proliferation and differentiation (Rouleau et al. 1988). The mature osteoblasts then activate osteoclasts, through paracrine stimulation, to undergo bone resorption process and release Ca and P into blood (Yamashita et al. 1990).

PTH on intestine

The synergetic effect of PTH and $1,25(OH)_2D_3$ on increasing intestinal calcium uptake has been reported in normal rats and normal chicks (Nemere and Szego 1981; Nemere and Norman 1986). Recently, the rapid effect (within 4 minutes) of PTH on stimulating intestinal P transport was also demonstrated in perfused duodenal loops of chicks (Nemere 1996). This suggests that PTH plays a role in intestinal Ca and P absorption. This effect might be significant to rachitic animals because these animals usually develop hyperparathyroidism. Though high plasma PTH alone has little effect in Ca and P transport, it enhances ion transport by non-nuclear pathways when vitamin D is repleted (Nemere et al. 1987; Nemere et al. 1991; Nemere 1996).

C. Calcitonin(CT)

Like PTH, the secretion and biosynthesis of CT from C cells are regulated by plasma Ca concentrations. The exact mechanism of Ca stimulation on CT exocytosis has not been fully understood. Nevertheless, the Ca-sensing receptor was demonstrated in parathyroid cells and in C cells. Its presence suggests that activation of the same receptor can either stimulate or inhibit hormone secretion in different cell types (Brown et al. 1993; Garrett et al. 1995). The half-life of calcitonin is about 10 minutes. Therefore, the hormone is most likely secreted at a continuous rate when plasma concentration Ca is normal. CT is degraded by liver and kidney to inactive fragments.

Action of CT on bone, kidney and intestine

CT directly inhibits bone resorption via osteoclastic and osteocytic cells, which contributes to hypocalcemia and hypophosphatemia (Talmage et al. 1972; MacIntyre et al. 1987). The ruffled border of the osteoclasts is decreased by CT stimulation, indicating their diminished activity in bone resorption. The depression of bone resorption decreases urinary excretion of Ca, Mg and hydroxyproline. The hypophosphatemia induced by CT results from decreased bone resorption and increased urinary P excretion.

In young pigs (<40 day old), CT inhibits net absorption of Ca flux rates in the proximal jejunum and distal ileum but not in the duodenum under physiological concentration (3.8ng/ml), but no effect on P flux rates. This suggests that the role of CT is more important to Ca than to P homeostasis (McKercher and Radde 1981).

D. Glucocorticoids

Glucocorticoids decrease the absorption of Pi in small intestine and reabsorption of Pi in renal tubules. In small intestine, glucocorticoids inhibit Pi absorption by decreasing the maximal transport capacity of the Na/Pi cotransporter. No change was observed in the affinity for phosphate or sodium. Interestingly, the inhibition of P transport by

glucocorticoid was only observed when dietary Ca and P are normal. Low P or low Ca diets that stimulates P absorption by the production of $1,25(OH)_2D_3$ was unaffected by the administration of glucocorticoid (Fox et al. 1981).

In the kidneys, the type II Na/Pi cotransport activity in brush border membrane was decreased when rats were given glucocorticoid. This effect could explain the development of osteoporosis in animals treated with long term glucocorticoid (Noronha-Blob and Sacktor 1986; Borowitz and Granrud 1992).

E. Growth hormone (GH) and insulin-like growth factor-I (IGF-I)

The effect of GH and IGF-I relates to the synthesis of $1,25(OH)_2D_3$. In hypophysectomized animals, hypophosphatemia has no effect on the activity of renal 1α-hydroxylase and the inhibition on renal P excretion. Exogenous GH restores the production of $1,25(OH)_2D_3$ (Gray 1981; Gray and Garthwaite 1985), suggesting that GH plays an direct or indirect role in regulating 1-hydroxylase activity (Caverzasio et al. 1990). Aged rats do not respond to low dietary Ca and P to stimulate $1,25(OH)_2D_3$ synthesis as young rats do. By administration of IGH-I the response is restored in old rats. Since IGF-I concentration is decreased by age, it indicates the age-related loss of 1-α hydroxylase activity is reversible and IGF-I is involved in the impairment of $1,25(OH)_2D_3$ synthesis (Wong et al. 1997; Wong et al. 2000).

<u>*Solanum glaucophyllum* research in animals</u>

Family: SOLANACEAE

Species: *Solanum glaucophyllum Desf.*

Synomyns:

Solanum amygdalifolium Stend., ex Sendth, *Solanum angustifolium* Lam.,
Solanum glaucum Dunal, *Solanum handelianum* Morong,
Solanum malacoxylon Sendth., *Solanum malacoxylon* Sendtn.var, albomarginatum Chodat,
Solanum malacoxylon Sendtn. forma vulgare Hassl.,

Solanum malacoxylon Sendtn, var. genuinum Hassl.

Common name: Waxyleaf Nightshade

Distribution: Eastern and central part of Buenos Aires Province in Argentina and Matto Grosso in Brazil (Okada et al. 1977; Boland 1986; Skliar et al. 2000).

Solanum glaucophyllum (Sg) was first described in 1846 by Sendtner. It is one of the most important calcinogenic plants in Argentina. The plants have long stems, about 1 to 3m in height with few branches. Their leaves are 9-13 cm in length and 1-2 cm in width, which continuously shed to the ground as they grow old. The flower of *Solanum* is light purple and the fruit is purple with many seeds. The root system is deep and extensive and the stems are perennial. In the winter the leaves die but new leaves grow in spring from buds on existing stems (Okada et al. 1977). The fallen leaves and small immature stems mix with pasture and may be accidentally ingested by the grazing animal. The intake of large amount of *Solanum* leaves induces toxicity characterized by loss of appetite and weight, joint stiffness and a painful gait. Animals develop hypercalcemia, hyperphosphatemia and soft tissue calcification (O'Donnell and Smith 1973). Most animals die from acute cardiac and pulmonary insufficiencies, which cause great economic loss in Argentina (Capen et al. 1966; Worker and Carrillo 1967; Mautalen 1972).

A. The structure and components of $1,25(OH)_2D_3$ glycosides in *Sg* leaves

The vitamin D-like action in *Sg* leaves was first demonstrated by O'Donnell and Smith in 1973(O'Donnell and Smith 1973). Shortly afterward, a water soluble, $1,25(OH)_2D_3$ glycoside was identified by HPLC and mass spectrometry (Wasserman et al. 1976). The A ring of $1,25(OH)_2D_3$ molecule links at least 1 glucose or fructose molecule at 1 and 3 carbon positions by β-glycosidic bonds (Vidal et al. 1985). The glycoside conjugates of vitamin D_3 and $25(OH)D_3$ were also detected in the leaf extracts and leaf cell culture (Esparza et al. 1982). Recently, 7-dehydrocholecalcesterol was found in the *Sg* cell culture grown in the dark. Vitamin D_3 and $25(OH)D_3$ appeared when the same culture system was moved under

the light, suggesting photosynthesis occurs in the leaves (Aburjai et al. 1996). Little is known about the mechanisms of the subsequent hydroxylation of vitamin D_3 in the plants though research showed low activity of the 25(OH)-1α-hydroxylase in the mitochondrial fraction (Esparza et al. 1982). It is believed that the physiological role of these vitamin D_3 glycosides affect calcium uptake and calmodulin synthesis in the root, influencing root growth and differentiation (Buchala and Schmid 1979; Vega et al. 1988; Talmon et al. 1989).

B. Equivalent of vitamin D bioactivity in *Solanum* leaves

The vitamin D bioactivity in *Sg* leaves has been evaluated on rachitic chicks by *in vitro* intestinal receptor assay and *in vivo* bioassay. Some studies reported 10 to 60 ug of $1,25(OH)_2D_3$ activity (or 82,800 IU to 300,000 IU of vitamin D3 equivalent) per kilogram of dry leaves. Others reported 120ug /g dry leaves of vitamin D3 activity. The wide ranges of vitamin D bioactivity in the *Sg* leaves result from factors such as geographic location, climate condition, and stage of development of the plant (Carrillo and Worker 1967; Procsal et al. 1976; Puche et al. 1980; Mello and Habermehl 1998).

The toxicity induced by *Sg* has been studied in poultry, rodents, ruminants and humans. It pathogenesis is mostly in bone and soft tissue calcification. However, some also reported the possibility of using *Sg* as treatment for cage-layer fatigue in hens and as a cheap source of $1,25(OH)_2D_3$ for human medicine.

C. *Solanum glaucophyllum(Sg)*-induced toxicity in animals

General mechanisms

Sg enhances Ca and P absorption by increasing Ca binding protein synthesis in duodenum and vitamin D dependent P transport in jejunum (Wasserman et al. 1976; Schneider and Schedl 1977; Peterlik and Wasserman 1978). In ruminants, the effect of *Sg* in Ca and P absorption was potentiated by the microbial activity in the rumen(de Boland et al. 1978).

Rumen microbes not only hydrolyze the glycosidic bonds but also convert the vitamin D metabolites to $1,24,25(OH)_3D_3$ (Peterlik et al. 1976; Boland et al. 1987).

Poultry

When administered to laying hens, *Sg* increased Ca binding protein synthesis and hence, increased Ca absorption, which reduced the incidence of "caged-layer fatigue". By adding 3.5g of dry *Sg* leaves powder/kg of 3.5% Ca basal diet, Morris et al. (1977) reported that the egg shell thickness was increased on the second and subsequent days of the experimental period (Morris and Jenkins 1977).

The toxicity of *Sg* on chicks was reported by Ross et al. (1971). Hypercalcemia and increased bone ash was observed when chicks were fed normal Ca and P diet for 12 weeks with 10g/kg *Sg* leaf powder. When *Sg* was given at 1g/kg diet, the breaking strength of the tibiae and weight gain was decreased. However, no soft tissue calcification was observed in any dosage of *Sg*, suggesting the level of *Sg*-induced toxicity is species dependent (Ross et al. 1971).

Rabbits

Retarded osteocytic osteolysis and osteopetrosis was observed in growing rabbits fed a normal Ca (0.6%) diet with *Sg* water extract (20g dry leaves/200ml DDW). The effect on bone induced by *Sg* could be eliminated when the dietary Ca was decreased to 0.24% (Santos et al. 1976).

Mice and rat

Solanum induced bone resorption in cultured mice calvariae (Simonite and Morris 1976; Liskova-Kiar and Proschek 1978). However, in growing rats fed *Sg* leaves for 7 days, the trabecular bone volume and bone apposition was increased (Norrdin et al. 1979). An *in vitro* test done by Stern et al. (1978) clarified this discrepancy of *Sg* on bone. Low *Sg* concentration (0.3mg/ml) induced bone resorption whereas high *Sg* concentration (1mg or above/ml) stimulated bone formation (Stern and Ness 1978).

Pig

Unlike other monogastric animals, *Sg* increased not only Ca and P but also H_2O, K and Na absorption in vitamin D depleted pigs (Fox and Care 1976; Fox and Care 1979) (Done et al. 1976). Hypercalcemia, hypophosphatemia and lung calcification were shown in weanling pigs. However, hypophosphatemia, rather than hyperphosphatemia, was enhanced when pigs were fed *Sg* incubated with rumen fluid. The authors suggested that pigs are less sensitive to the calcinogenic effect induced by *Sg* (Rucksan et al. 1978).

Human

Mautalen et al. (1977) reported that short-term treatment of renal failure patients with *Sg* leaf powder increased Ca absorption without changes in plasma P and PTH. It was proposed that *Sg* could be a good and inexpensive source of $1,25(OH)_2D_3$ for uremic patients (Mautalen et al. 1977).

Cattle

The clinical sign of the disease "enteque seco" in Argentina area is usually observed in cattle over 2 years old. The toxic effects of *Solanum* include weight loss, hypercalcemia, and hyperphosphatemia, as well as soft tissue calcification (Done and Dobereiner 1976). In response to the hypercalcemia, thyroid C cells hypertrophy and hyperplasia was observed. Under electron microscopy, parathyroid chief cells were inactivated or became atrophied (Collins et al. 1977). Experimental administration of a high dose (1g/kg/week) *Sg* for a short period of time, or a low dose (0.16g/kg/week) for a long period of time produced similar lesions to those found in the naturally occurring cases.

Calves are less susceptible to the toxicity of *Sg* than the older animal (Dobereiner and Done 1975). The dosage that could be tolerated by calves, 0.3g-2.4g/kg body weight, was sufficient to cause severe calcification of older animals. However, only moderate soft tissue calcification was observed in heart, arteries and kidney after intermittently dosing *Sg* leaves to Jersey bull calves for 87 day.

Sheep

In adult sheep fed normal Ca and P diets, *Solanum* induced defective mineralization and increased bone forming surface and trabecular bone volume. The major components in the unmineralized bone were associated with the deposition of acid mucopolysaccharide (Woodard et al. 1993).

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CHAPTER 2. RESTORING NORMAL BLOOD PHOSPHORUS CONCENTRATIONS IN HYPOPHOSPHATEMIC CATTLE WITH SODIUM PHOSPHATE

A paper published in "Veterinary Medicine"[1]

Ya-Hsin Cheng, BVSc, MS [2]; Jesse P. Goff DVM, PhD [3,4]; and Ronald L. Horst, PhD[3]

INTRODUCTION

Downer cow syndrome is an occasional sequelae to milk fever in dairy cows. Cows with milk fever are severely hypocalcemic (plasma calcium < 5 mg/dl) and usually hypophosphatemic (plasma inorganic phosphorus < 2 mg / dl) at the time of initial treatment for milk fever. In most cases intravenous administration of calcium salts raises blood calcium concentrations immediately, followed within a few hours by a rise in blood phosphorus concentration[1]. In some animals, however, plasma phosphorus concentrations fail to increase after therapy for milk fever, and it is thought that this results in a cow that fails to rise after treatment, or a "downer cows" [2,3]. Methods that restore plasma phosphorus concentrations to normal would thus help treat and prevent downer cow syndrome.

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[2] Department of Veterinary Physiology and Pharmacology, Iowa State University, Ames, IA 50010

[3] Metabolic Diseases and Immunology Unit, National Animal Disease Center, USDA-Agricultural Research Service, 2700 Dayton Ave., Ames, IA 50010

[4] To whom correspondence and reprint requests should be addressed. Phone 515-239-8547, fax 515-239-8458; e-mail jgoff@nadc.ars.usda.gov

Most products available to veterinarians in the U.S. for intravenous treatment of hypophosphatemic cattle utilize hypophosphite (PO_2^{-3}) or phosphite (PO_3^{-3}) salts as the phosphorus source. Phosphite salts are used because they are very soluble in water and remain soluble even in the presence of calcium and magnesium, allowing preparation of a single product that can be used to treat low blood calcium, magnesium, and phosphorus conditions. However, phosphorus found in blood and body tissues is almost exclusively in the form of the phosphate anion (PO_4^{3-}). To our knowledge, no pathway exists for the conversion of phosphite to phosphate salts in body tissues. In this article, we reported the results of a study initiated to determine whether intravenously injecting phosphite salts results in a rise in plasma inorganic phosphorus concentration. We also describe an intravenous and oral treatment, utilizing sodium phosphate,that will effectively raise inorganic phosphorus concentrations in the blood of cattle.

METHODS AND RESULTS

Experiment 1

In experiment 1, we evaluated the effectiveness of intravenous sodium hypophosphite and sodium phosphate as treatment for hypophosphatemia. The six animals used were 4 to 9 year old, nonpregnant, non-lactating Jersey cows. Moderate hypophosphatemia was induced in the cows by feeding them a 0.08% phosphorus diet for six days before the experiments (Adequate dietary phosphorus concentration is 0.36%). The diet was based on beet pulp and corn silage. Before feeding the low phosphorus diet, the cows' average plasma phosphorus concentration was 4.67 mg/dl. After feeding the low phosphorus diet for six days, plasma phosphorus concentrations decreased to 2.97± 0.24 mg/dl.

The six cows were treated with intravenous phosphorus solutions, which provided 7g phosphorus as the phosphite or phosphate anion in a switchback design experiment. That is, three cows received phosphite and three cows received phosphate on the first treatment day, followed by the alternate phosphorus source three days later. Treatments consisted of 30g sodium phosphate, monobasic, monohydrate or 23g sodium hypophosphite dissolved in

300ml distilled water. The pH of both solutions was brought to 7.0 with sodium hydroxide.

Autoclaved solutions were administered intravenously into the jugular vein over a period of 10 minutes. Heparinized blood samples were collected from the contralateral jugular vein immediately before treatment and at one minute, 30 minutes, and one, two, three, four, five, and six hours after each treatment. Blood samples were centrifuged at 4C and 23000 rpm for 15 minutes, and the inorganic phosphorus concentration was determined [6]. The assay used combines serum inorganic phosphate anion with molybdate to form phospho-molybdate, which reacts with paraphenylenediamine to form a stable molybdenum-blue complex obeying Beer's Law and absorbing light with a wavelength of 680nm. The assay will not detect inorganic phosphite.

A factorial analysis of variance was used to examine the effects of treatment and time on plasma phosphorus concentration. Difference were considered significant when the probability of a difference's existing exceeded 95% ($p<0.05$).

In those cows treated with intravenous sodium phosphite solution, no change was observed in plasma inorganic phosphorus concentrations. This suggested that the phosphite anion was not being converted to phosphate within the six hour time frame after treatment. In those cows treated with intravenous sodium phosphate, the plasma inorganic phosphorus concentration was significantly ($p<0.01$) increased to 13.62 ± 3.46mg/dl (mean $\pm$ SEM) one minute after phosphate administration and rapidly decreased to 3.47 ± 1.44 mg/dl within two hours after treatment (Figure 1). Six hours after treatment, the plasma inorganic phosphorus concentration was 3.08 ± 0.78 mg/dl in the phosphate-treated group and 2.25 ± 0.5 mg/dl in the phosphite-treated group.

Experiment 2

A second experiment was performed to determine if oral supplementation with 50g phosphorus as either sodium phosphate or dicalcium phosphate could quickly raise plasma inorganic phosphorus concentration. Eight cows were fed the same low-phosphorus diet as in Experiment 1 for six days to induce a mild hypophosphatemia. Four cows were drenched with a solution containing 219g sodium phosphate, monobasic, monohydrate in 0.5 L distilled water, and four cows were drenched with a slurry containing 229 g calcium

phosphate, dibasic in 0.5 L distilled water. Heparinized blood samples were collected before treatment and at 30 minutes and one, two, three, four, five, six and 12 hours after treatment to determine inorganic phosphorus concentrations as described for Experiment 1. The change in plasma phosphorus concentration observed in cows treated orally with sodium phosphate was significantly ($p<0.01$) greater than that in cows treated with calcium phosphate. Plasma phosphorus concentration was increased from 3.33 ± 0.4 mg/dl before treatment to 5.9 ± 2.88 mg/dl within one hour in cows receiving the oral sodium phosphate treatment (Figure 2). This concentration remained elevated through the 12 hours after treatment. Plasma phosphorus concentration in cows receiving oral calcium phosphate was 3.75 ± 0.75 mg/dl before treatment and was not significantly increased during the first six hours after treatment. However, 12 hours after calcium phosphate treatment, the plasma phosphorus concentration of cows treated with calcium phosphate had risen to 4.52 ± 1.13 mg/dl.

DISCUSSION

Based on findings from these experiments, hypophosphatemia in cattle can be treated effectively by intravenously injecting sodium phosphate solutions but not phosphite solutions. Sodium phosphate solutions can be made relatively inexpensively. In this experiment we raised the pH of the intravenous sodium phosphate solution to 7.0 by using sodium hydroxide before treatment. We noticed that crystallization had been beginning to occur at cooler outdoor temperatures (<41°F [5°C]). We resolved this problem by raising the pH of the sodium monophosphate solution to only 5.8, increasing the sodium phosphate's solubility. We have not noticed any problems using these slightly acidic solutions intravenously. A product using 30 g sodium phosphate, monobasic in 300ml water with no pH adjustment (pH< 3.0) is available in Australia and is thought to be effective[5]. There are sodium phosphate enemas formulated for use in people that contain 5 to 6 g phosphorus per treatment, with the pH adjusted to about 5.8(very similar to our formula described above). These enema preparations have been used for intravenous treatment of cattle [7] after they were

diluted to 1 L with water to reduce the tonicity. Sodium phosphate solutions can not be mixed with Calcium- or magnesium-containing solutions because a precipitate will rapidly form.

Oral sodium phosphate treatment (50 g phosphorus) caused a more prolonged increase in the plasma phosphorus concentration than did the intravenous treatment (7g phosphorus). More phosphate could be given intravenously, but this might result in excessive formation of calcium or magnesium phosphate precipitates in the blood since plasma phosphorus concentration are well above physiologic concentrations immediately after treatment. Intravenous phosphate solutions should not be given immediately before or after intravenous calcium treatment for this same reason. The two types of treatment should be spaced at least two hours apart. No such restrictions would be necessary for the oral sodium phosphate treatment. Sodium phosphate is more soluble than calcium phosphate, which probably explains why it is better able to raise plasma phosphorus concentrations. The speed with which the oral sodium phosphate treatment raised plasma phosphorus concentrations suggests that phosphate is being absorbed directly from the rumen or that a large amount has bypassed the rumen[8].

The etiology of the acute hypophosphatemic crisis seen with downer cow syndrome is unknown. Certainly phosphate removed from the blood during milk production contributes to hypophosphatemia development in the same manner that milk production contributes to hypocalcemia development. Hypocalcemia exacerbates the loss of phosphate from the blood as it causes parathyroid hormone secretion, which in turn rapidly stimulates the salivary glands to secrete more phosphate[9]. If gastrointestinal motility is impaired, as it is during hypocalcemia, reabsorption of the salivary phosphate will be impaired[4]. This, together with the phosphorus demands of colostrum production, can cause the acute declines in plasma phosphorus often observed in cows with milk fever. Under most circumstances, correction of hypocalcemia re-establishes gastrointestinal motility, so salivary phosphate can be reabsorbed, returning plasma phosphorus to normal concentrations within a few hours of hypocalcemia treatment.

In at least some cows classified as downer cows, the correction of hypocalcemia is not followed by correction of hypophosphatemia[5]. Why this occurs is unknown. Perhaps

insoluble phosphate complexes are formed during the period of gut stasis, or there may not be enough 1,25-dihydroxyvitamin D produced to allow efficient phosphorus absorption[10]. Perhaps gastrointestinal motility is not returning in these cows. If the latter is true, then treatment with the sodium monophosphate drench may not be effective in all downer cows. Though this generally has not been our experience, it may be necessary to intravenously inject sodium monophosphate to correct the plasma phosphorus concentration and follow the injection with an oral sodium phosphate treatment to maintain normal plasma phosphorus concentrations.

The clinical response to intravenous or oral sodium phosphate can be disappointing if downer cows are not treated early. This is because muscle and nerve damage secondary to prolonged recumbency and the resultant crush syndrome may keep the cow down despite corrected plasma phosphorus concentration[11]. Routine use of phosphate solutions to treat milk fever is unwarranted. However, on farms with a history of hypophosphatemia and downer cows, practitioners could routinely administer intravenous calcium treatment, wait two hours, and then administer intravenous sodium phosphate. Or they could give oral sodium phosphate immediately after the intravenous calcium once the swallowing reflexes are intact.

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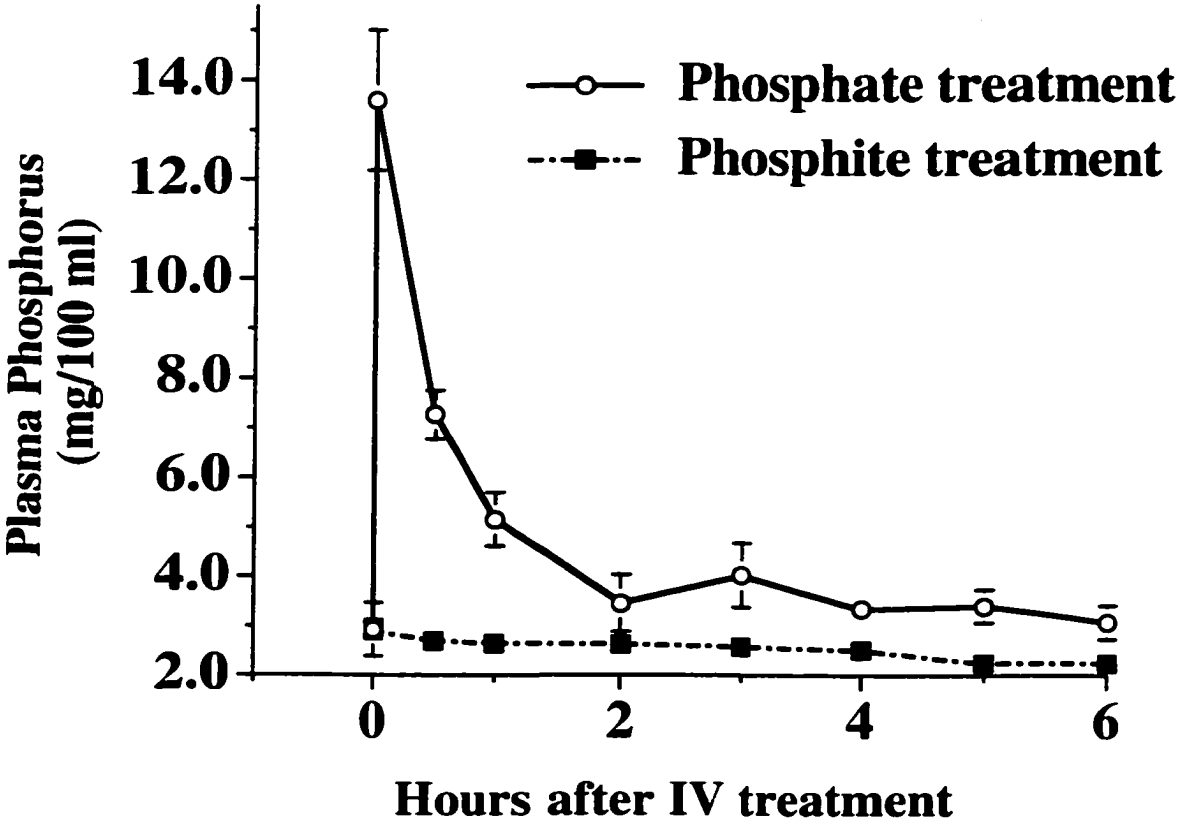

Figure 1. Mean+SEM plasma phosphorus concentrations(mg/dl) in cows receiving 7g phosphorus intravenously supplies by sodium phosphate or sodium hypophosphite. Six cows received both phosphorus cources on two separate days as treatment for hypophospatemia

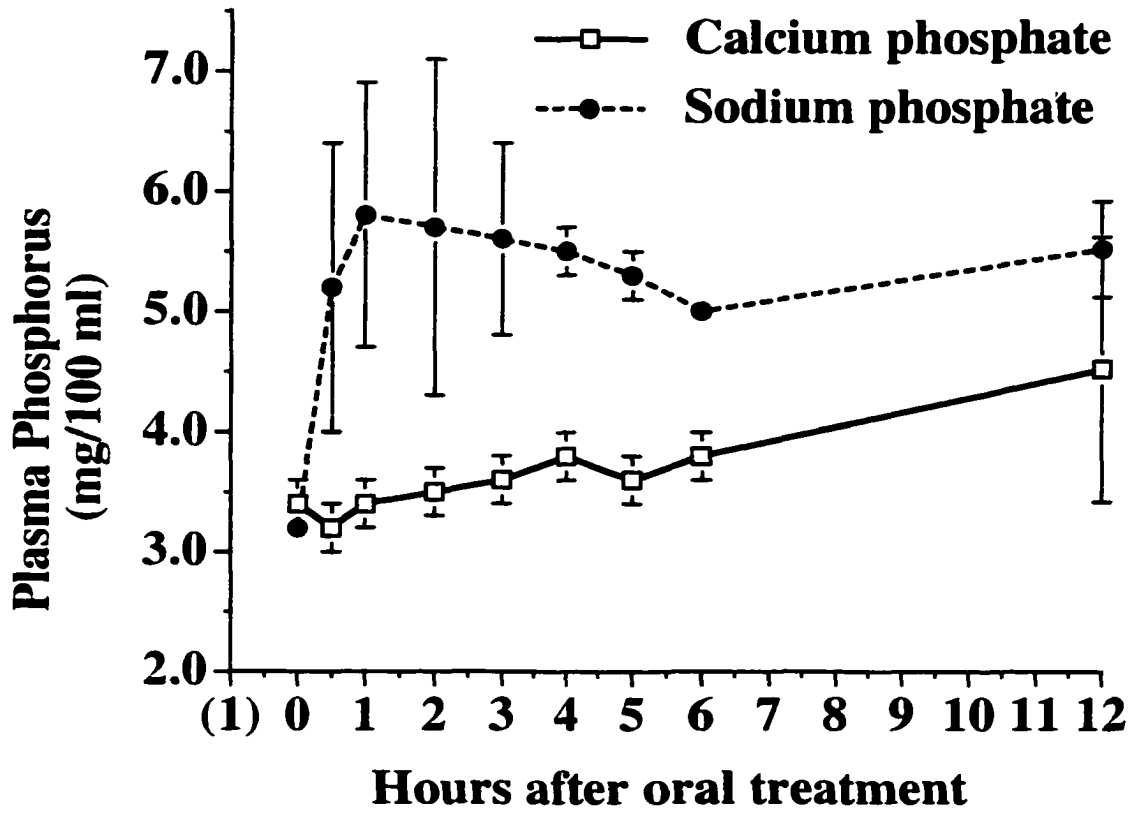

Figure 2. Mean+SEM plasma phosphorus concentrations (mg/dl) in cows receiving an oral drench containing 50g phosphorus supplied by sodium phosphate or calcium phosphate. Four cows received oral sodium phosphate and four received oral calcium phosphate

CHAPTER 3. THE USE OF *SOLANUM MALACOXYLON* ON PHOSPHORUS (P) UTILIZATION AND ITS ADDITIVE EFFECT WITH PHYTASE IN CHICKS FED LOW CALCIUM, LOW P, AND VITAMIN D_3 ADEQUATE CORN-SOYBEAN DIET

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Yahsin J. Cheng, Jesse P. Goff, Jerry L. Sell, S. Gill, E. Pawlak, M. Elena, and Ronald L. Horst

ABSTRACT Two experiments were conducted to demonstrate the effectiveness of *Solanum malacoxylon (Sg)* as an inexpensive source of $1,25(OH)_2D_3$ on P utilization in broilers. In Experiment 1, three levels of *Sg* leaf powder : 1g, 2.5g, 5g and 15 ug $1,25(OH)_2D_3$ were added per kilogram to a basal diet. The basal diet contained 0.6%Ca, 0.5% P, and adequate vitamin D. Treatments were fed to broilers from 7-day-old to 28-day-old. Growth performance and bone ash were used to compare the effect of *Sg* to synthetic $1,25(OH)_2D_3$ on P utilization. Addition of 5g *Sg* and 15ug $1,25(OH)_2D_3$ increased weight gain, plasma Ca and P concentrations, bone ash and bone density. No hypercalcemia was observed in birds in the *Sg* treatments. Bone density and mineral content were normalized by 15ug $1,25(OH)_2D_3$. In experiment 2, the additive effect of *Sg* with phytase was tested by chicks fed 0.6% Ca, 0.45% P basal diet. Two levels of *Sg* (7.5g and 10g), phytase (1200FTU) and the combination of *Sg* (7.5g) and phytase (1200FTU) were added per kilogram basal diet. *Sg* and phytase respectively increased weight gain, plasma Ca and P concentrations, bone ash and density as well as bone Ca and P content. The greatest weight gain was shown in the phytase treatment. There was no significant difference between the effect of two levels of *Sg*. No additive effect was observed by the *Sg*/phytase combination when compared with the phytase treatment alone. We concluded that *Solanum glaucophyllum* could be used as an

inexpensive source of $1,25(OH)_2D_3$ to improve P utilization in poultry industry. The hypercalcemic effect induced by low dietary P and *Sg* was prevented by lowering dietary Ca.

(*key words*: *Solanum glaucophyllum*, $1,25(OH)_2D_3$, P utilization, broilers)

INTRODUCTION

Solanum malacoxylon (*Sg*)is a calcinogenic plant that induces the disease "enteque seco" in cattle and other grazing animals (Worker and Carrillo 1967; Gill, Singh et al. 1976; Puche, Faienza et al. 1978). Hypercalcemia, hyperphosphatemia and soft tissue calcification were observed in the sick animals, which resembles the lesions of hypervitaminosis D (Capen et al. 1966; Done and Dobereiner 1976). The vitamin D-like factor in *Solanum* leaves was identified as a water-soluble $1,25(OH)_2D_3$ glycoside (Wasserman et al. 1976; Napoli et al. 1977; Boland et al. 1987; Curino et al. 1998).

Recently, $1,25(OH)_2D_3$ and other vitamin D metabolites were demonstrated to improve P utilization in poultry (Edwards 1993; Biehl and Baker 1997; Biehl, Baker et al. 1998). Mohammed et al. (1991) reported that increasing cholecalciferol in low Ca and low P diet restored growth performance and bone development in broilers (Mohammed and Gibney 1991). The vitamin D metabolites, such as $25(OH)D_3$, $1\alpha(OH)D_3$ and $1,25(OH)_2D_3$, also increase P retention and decrease P excretion in birds (Edwards 1993; Biehl, Baker et al. 1995; Biehl and Baker 1997). Among the vitamin D metabolites, $1\alpha(OH)D_3$ and $1,25(OH)_2D_3$ increased bone ash and plasma P in growing chicks better than $25(OH)D_3$ and cholecalciferol (Edwards 1995). In addition, the incidence and severity of Tibial Dyschondroplasia (TD) was decreased by supplemental 6ug $1,25(OH)_2D_3$ per kilogram diet in growing birds (Roberson and Edwards 1996). However, the high cost of synthetic 1α-hydroxylated vitamin D compounds limits their application in the poultry industry. Finding a cheap source of $1,25(OH)_2D_3$ would be of benefit to farmers and animal welfare.

Microbial phytase has been used as a feed additive to improve phytate-P utilization. Simons et al. (1990) reported that adding phytase to low P diet increased dietary P availability 60% and decreased excreted fecal P 50% in 3-week-old growing broilers (Simons, Versteegh et al. 1990). Mitchell and Edwards (1996) suggested that $1,25(OH)_2D_3$ and phytase increased phytate P utilization through different mechanisms. Therefore, when both phytase and $1,25(OH)_2D_3$ were used, the additive effect allowed a reduction of 0.2% of diet inorganic P (Mitchell and Edwards 1996). The synergetic effect of phytase and $1,25(OH)_2D_3$ not only increased P utilization but also decreased TD incidence in growing chicks.

In the present study, we conducted two experiments to determine (1) the effect of *Sg* leaf powder, as an inexpensive source of $1,25(OH)_2D_3$, on P utilization, and (2) the additive effect of *Sg* and phytase in broiler fed low Ca, low P and vitamin D-adequate corn-soybean meal.

MATERIALS AND METHODS

General procedure

One-day-old broiler chicks were purchased from a commercial hatchery and housed in the poultry science unit in Iowa State University. The temperature was maintained at 34 to 35°C during the experimental period. All chicks were given normal diet based on the NRC (1994) requirement for 7 days. On the 8th day, chicks were weighed and selected to standardize weights, followed by random distribution into 36 floor pens. Each treatment was given to 6 pens of 8 chicks for the following 21 days. Water and treatment diets were provided *ad libitum*. Feed consumption and weight gain were recorded weekly to determine growth performance. At 28 d of age, all chicks were anesthetized with CO_2:O_2(50:50). Heparinized blood samples were obtained by cardiac puncture and centrifuged at 1735g for 20 minutes. The plasma was collected and stored at –20°C until analysis. While under anesthesia, the chicks were killed by cervical dislocation. These procedures were approved by the Iowa State University Animal Care and Use Committee.

The right tibiae from all chicks were dissected and the attached soft tissue was removed carefully. Bone density was determined by water displacement as described (Zang and Coon 1992). Briefly, each bone was placed in deionized, distilled water DDW) and placed into a vacuum chamber overnight to displace air trapped within the bone medullary cavity. The hydrated tibiae were then put on the cup of the balance and weighed in air (A). While the tibiae was still on the cup the balance was re-zeroed. The tibiae was then moved to the water and the immersed bone weight was recorded, which was the buoyancy density (P) of the tibiae. The gravity of the DDW during the weighing condition was ρ_0. Therefore, the density of the tibiae (ρ_2) could be calculated using the following formula

$\rho_2 = A^* \rho_0/P$

It was assumed that water specific gravity is 1.0g/cm^3 at room temperature 22°C.

Following the measurement of bone density, pooled bones from each pen were dried at 80°C overnight to obtain the dry bone weight. The dried bones were ashed at 600°C for 8 hours and the ash weight percentage are presented as ash weight per tibiae volume and ash weight per dry tibia. Plasma and bone Ca concentrations were determined by atomic absorption spectrophotometer (Perkin-Elmer 1965) whereas phosphorus concentrations were determined colorimetrically. Plasma 1,25(OH)$_2$D$_3$ was measured by RIA after the extraction of 1,25(OH)$_2$D$_3$ by Sep-Pak chromatography.

Experiment 1

The purpose of experiment 1 was to test the effect of *Solanum (Sg)* leaf powder as the substitute of 1,25(OH)$_2$D$_3$ in improving phosphorus utilization in broiler chicks. Six diets were designed. The basal diet contained 0.5% total P (0.21% available P) and 0.6% Ca (Table 1). Disodium phosphate and calcium phosphate were used to adjust the desired level of dietary Ca and P. One gram, 2.5g and 5g of ground dry *Sg* leaves were added to each kg of the basal diet, respectively. One treatment consisted of 15ug 1,25(OH)$_2$D$_3$/kg in the basal diet and was used to compare the *Sg*-induced activity to pure 1,25(OH)$_2$D$_3$ activity. The normal diet contained 1% Ca and 0.7% total P (NRC, 1994) and was used as the positive control.

Experiment 2

Experiment 2 was designed to determine if *Sg* had an additive effect with phytase to improve P utilization in the Ca and P deficient diet. The source of *Sg* leaves in this experiment was different from the one used in experiment 1. Rat bioassay showed that the $1,25(OH)_2D_3$ equivalent in 1g of *Sg* used in experiment 1 was equal to the activity in 1.5g of *Sg* used in experiment 2 (data did not show). Therefore, in Experiment 2, we used 7.5g of *Sg*/kg (equal to the 5g effect in Experiment 1) as one treatment and increased the Sg dose to 10g/kg for another treatment.

Total P level in the basal diet was decreased to 0.45% (0.16% non-phytate P; Table 1) in consideration of the expected phytase effect. The dietary Ca level was maintained at 0.6%. In order to compare the maximum effect of phytase[1] to *Sg* effect, the levels of phytase used in this experiment was 1200 FTU/kg of basal diet. Therefore, the five treatments in Experiment 2 were basal diet, basal diet with 7.5g or 10g of *Sg* leaves/kg, basal diet with 1200 FTU of phytase/kg and basal diet with the combination of 1200FTU phytase and 7.5g *Sg* leaves, respectively. A diet containing 1% Ca and 0.7% total P was used as the positive control.

Statistical analysis

All statistics were conducted on the basis of pen means where pen is the experimental unit. Treatment means were analyzed by ANOVA and significant differences among treatment means were assessed using the Fisher's LSD multiple pair-wise comparison procedure with a 5% level of probability.

[1] A generous gift from BASF

RESULTS

Experiment 1

Experiment 1 was designed to see if *Sg* would improve P utilization as well as synthetic $1,25(OH)^2D_3$ when 0.6% Ca and 0.5% P corn soybean diet was fed to broilers from 1-4 weeks of age. There were no significant differences in feed efficiency and plasma Ca concentrations among dietary treatments (Table 2). Weight gain and plasma P concentrations were increased and normalized by 15ug $1,25(OH)_2D_3$ and 5g *Sg* ($P<0.01$). The highest plasma $1,25(OH)_2D_3$ concentrations were shown by the treatment of 5g *Sg*. Nevertheless, the elevation of $1,25(OH)_2D_3$ by 5g *Sg* was not significantly different from that observed in chicks fed basal and 15ug $1,25(OH)_2D_3$ treatments (109.11 vs. 89 and 76.22pg/ml).

When compared to basal treatment, *Sg* treatments significantly increased bone density ($P<0.01$) (Table 3). However, tibiae ash and bone calcium concentrations were only significantly increased by the 5g dose of *Sg* when compared to the basal treatment. None of the *Sg* treatments increased bone P concentrations. Addition of 15ug $1,25(OH)_2D_3$ increased tibiae ash percentage and normalized bone density and bone Ca and P concentrations.

Experiment 2

Based on the results from experiment 1, it was expected that dietary concentration of *Sg* greater than 5g/kg would normalize both plasma and bone parameters as compared with 15g of synthetic $1,25(OH)_2D_3$/kg. On the other hand, we questioned whether the effect of *Sg* on bone ash could be improved by additional phytase. Therefore, the purpose of the experiment was to test the effect of higher doses of *Sg* and its additive effect with phytase on P utilization. In order to avoid hyperphosphatemia induced by the *Sg*/phytase combination, dietary P was reduced from the P used in Experiment 1, 0.5%, to 0.45% whereas the Ca levels remained at 0.6%.

The ANOVA table showed that the treatment effects of phytase were significant in weight gain, bone density, tibiae ash and ash percentage ($P<0.01$, Table 4). The effect of *Sg* was significant in tibiae ash ($P<0.01$) whereas a significant interaction of *Sg* with phytase was also observed in tibiae ash ($P<0.05$).

There were no significant differences in feed efficiency among treatments (Table 5). Compared to the basal treatment and normal treatment, weight gain was significantly increased by *Sg*, phytase and the combination of *Sg*/phytase ($P<0.01$). The greatest weight gain was observed with phytase treatment alone. Plasma Ca concentration was decreased significantly by phytase treatment alone compared to other groups. No hypercalcemia was observed by *Sg* treatments. Low dietary P resulted in low plasma P in the basal treatment (4.56mg/dl), which was significantly increased ($P<0.01$) and normalized by *Sg*, phytase and the combination of *Sg*/phytase. Compared to the normal treatment, plasma $1,25(OH)_2D_3$ was significantly increased by basal diet and *Sg* treatments. Nevertheless, there was no statistical difference in plasma $1,25(OH)_2D_3$ concentrations between basal and *Sg* treatments. Compared to basal and *Sg* treatments, phytase and the combination of *Sg*/phytase significantly decreased plasma $1,25(OH)_2D_3$ ($P<0.05$) though the value was still higher than in the normal treatment birds.

Compared to the basal treatment, *Sg*, phytase and the combination of *Sg*/phytase significantly increased tibiae ash, ash percentage and bone density ($P<0.05$, Table 6). The effect of phytase and the combination of *Sg*/phytase on tibiae ash and ash percentage was significantly greater than that of *Sg* treatments ($P<0.05$). There were no differences between the effect of phytase alone and the combination of *Sg*/phytase on tibiae ash, ash percentage. The concentrations of bone Ca and P were significantly increased ($P<0.05$), but not normalized by *Sg*, phytase and the combination of *Sg*/phytase treatments. No differences were observed in tibia ash, bone density and bone mineral concentrations between the two levels of *Sg* treatments. Compared to *Sg* treatment alone, the combination of *Sg*/phytase significantly increased bone Ca concentrations but not bone P concentrations. The difference in bone Ca and P concentrations between phytase alone and the combination of *Sg*/phytase was not significant.

DISCUSSION

Our experiments focused on the effect of *Sg* as a substitute of $1,25(OH)_2D_3$ to improve phosphorus utilization in broilers fed low Ca, low P corn soybean diet. We demonstrated that 5g *Sg* dry leaves per kilogram in Ca and P deficient diet normalized weight gain and plasma P concentrations in growing chicks.

The South America calcinogenic plant, *Solanum malacoxylon*, is also known as *Solanum glaucophyllum Dest* (Okada et al. 1977). The mechanism of *Sg*–induced hypercalcemia and hyperphosphatemia in animals is due to its vitamin D activity in Ca and P absorption. The $1,25(OH)_2D_3$ glycosides have been extracted and identified from *Sg* leaves (Wasserman et al. 1976; Haussler et al. 1977; Boland et al. 1987; Curino et al. 1998). In rachitic chicks and rats, *Sg* leaves extracts restored intestinal Ca-binding protein synthesis (Wasserman et al. 1976; Schneider and Schedl 1977), hence increased Ca absorption. P uptake is mediated by increasing transcription of DNA to RNA and protein synthesis on the lumenal side (Basudde and Humphreys 1975; Haussler et al. 1977; Peterlik and Wasserman 1978). In our studies, *Sg* increased plasma $1,25(OH)_2D_3$ (Table 2 and 5), and therefore improved Ca and P absorption in broilers. Plasma Ca and P concentrations were restored by *Sg* treatments when chicks were fed low Ca and low P diet (Table 5).

Ross et al. (1971) reported hypercalcemia in chicks fed 5g *Sg* for 3 weeks. The hypercalcemia they observed should be due to the relatively high dietary Ca used in their experiment (Ross et al. 1971). Wasserman (1975) found that the *Sg*-induced toxicity in animals only occurs when the plant is ingested and an adequate dietary Ca and P is intake (Wasserman 1975). By decreasing dietary Ca from 1% to 0.6%, the *Sg*-induced hypercalcemia was not observed in our study, suggesting *Sg* toxicity in elevating plasma calcium could be prevented by decreasing dietary Ca. Therefore, *Sg* could be used as a cheap source of $1.25(OH)_2D_3$ to improve both Ca and P utilization in broilers. However, Canas et al. (1977) observed the increment of Ca absorption but no effect on P absorption in chicks fed *Sg* methanol extract. This result might be because little $1,25(OH)_2D_3$ activity is present in methanol extraction of *Sg* leaves because the $1,25(OH)_2D_3$ glycosides are more soluble in water than in methanol (Canas et al. 1977).

The effect of *Sg* on bone is controversial. Norrdin et al. (1979) reported the acute effect of *Sg* in growing rats was a stimulation of trabecular bone formation associated with an increase in the bone apposition rate on trabecular surfaces (Norrdin et al. 1979). Others observed release of hydroxyproline and calcium, and increasing of glucose utilization in the medium of mice calvariae cultured with *Sg* extracts (Lloyd et al. 1975; Simonite and Morris 1976). Liskova-Kiar et al. (1978) further supported the effect of *Sg*-induced bone resorption by *in vivo* test. In their study, mobilization of Ca from mouse fetal fibulae cells resulted from increasing the population of osteoclasts (Liskova-Kiar and Proschek 1978). The discrepancy of *Sg* on bone formation and resorption was explained by Stern and Ness (1978). These authors demonstrated *in vitro* that *Sg* had biphasic effects on bone. At low concentration of *Sg* extract (0.3mg/ml) bone resorption was stimulated whereas at high concentrations of *Sg* extract (more than 1mg/ml) bone resorption was inhibited. Santos et.al. (1976) reported that the *Sg*-induced osteocytic osteolysis and osteopetrosis disappeared when dietary Ca was decreased (Santos et al. 1976), indicating that *Sg* dosage and dietary Ca are critical in determining the response of bone resorption. It is now known that $1,25(OH)_2D_3$ increased bone mineralization by increasing intestinal absorption of Ca and P, and thus increases plasma Ca and P concentrations, which lead to hydroxyapatite formation. Its effect on bone resorption, on the other hand, is mediated by stimulation of osteoblast differentiation (Slovic et al. 1981; Suda et al. 1990; Suda et al. 1992). In our experiments, tibiae ash and bone density were increased by *Sg* in the Ca and P deficient diet. Compared to the basal treatment, *Sg* leaves significantly increased bone Ca and bone P concentrations (Table 3 and 6), indicating the improvement of bone mineralization.

In our preliminary experiment, 20 ug of synthetic $1,25(OH)_2D_3$ /kg increased bone ash and bone density to the levels higher than the birds received normal treatment (data did not show). Therefore, we suspected lower dose of synthetic $1,25(OH)_2D_3$/kg at 15ug would be appropriate to normalized growth rate and bone development in the same animal model. However, in Experiment 1, 15ug of synthetic $1,25(OH)_2D_3$/kg in the 0.5% P and 0.6% Ca diet restored growth performance and bone density. Tibiae ash and ash percentage were increased but still lower than the normal treatment (1.895 *vs.* 1.961 and 39.7% *vs.* 41.9%, Table 3). Edward (1993) reported that 5ug $1,25(OH)_2D_3$ in low P diet greatly increased bone

ash, bone Ca and P, and decreased P rickets retention in a 9-day experimental period (Edwards 1993). They also reported the decrease of the incidence and severity of TD by additional $1,25(OH)_2D_3$ in the same diet (Mitchell and Edwards 1996; Mitchell and Edwards 1996). Baker and Biehl (1995) demonstrated 10ug of $1,25(OH)_2D_3$ increased bone ash and body weight in 2-week-old of chicks fed 0.63% Ca, 0.43% P (0.1% nonphytate P)(Biehl et al. 1995). Our results showed that additional 20ug of synthetic $1,25(OH)_2D_3$/kg was necessary for growing birds from 2-week to 4 week-old of age to normalize growth performance and bone development when dietary Ca was 0.6% Ca and dietary P was 0.5% P. the differences in the requirement of 1,25(OH)2D3 to normalize bone Ca and P might be due to the differences in the duration of experimental period.

The effect of different levels of dietary $1,25(OH)_2D_3$ on bone development in broilers at 3 week of age was tested by Roberson and Edwards. There were no significant differences in plasma 1,25(OH)2D3 concentrations (267 to 288 pg/ml) when chicks were fed 0, 3, 6, 9ug of 1,25(OH)2D3, respectively, /kg diet containing 0.8% Ca and 0.77% P (Roberson and Edwards 1996). Though the plasma $1,25(OH)_2D_3$ concentrations were not significant differences, bone ash was significantly increased and the incidence and severity of TD was decreased by the treatment of 6ug of $1,25(OH)_2D_3$/kg. This suggests supplementation of $1,25(OH)_2D_3$ improves bone mineralization in fast growing birds. Physiologically, the absorbed P was first shown in plasma and used for growth performance. Bone calcification is the last criteria to indicate the P status of the individual. In our experiment, plasma $1,25(OH)_2D_3$ concentrations in 15ug of $1,25(OH)_2D_3$/kg and 5g of *Sg*/kg treatments were 89 and 109 pg/ml (Table 2) when chicks fed 0.6% Ca and 0.5% P diet, which was less than half of the value from Roberson and Edwards'. Such plasma $1,25(OH)_2D_3$ concentrations in our experiment significantly increased tibiae ash and Ca and P concentrations in bone when compared to the basal treatment, but not normalized the bone criteria when compared to the positive control(Table 3). This indicates *Sg* and $1,25(OH)_2D_3$ improve Ca and P utilization and therefore, improve bone mineralization when chicks fed low Ca and low P diet. It also suggests that the bone parameters could be restored when higher dose of *Sg* is given by increasing higher plasma $1,25(OH)_2D_3$, which in turn, increases more P absorption and offer P for bone development. However, in Experiment 2 bone and blood parameters were not

significantly different from chicks fed 7.5g and 10g of *Sg* (Table 6). One possibility was the available P in the basal diet, 0.16%, was too low and therefore restricted the effect of *Sg*. It indicates the elevation of *Sg* dosage is not the major determining factor to normalize bone development in chick fed 0.45% P and 0.6% Ca diet.

The phytase effect in Experiment 2 was consistent with other reports that phytase increases bone ash and growth performance in chicks fed P deficient diets (Nelson et al. 1971; Qian et al. 1997). In table 5 and 6, 1200FTU phytase decreased plasma $1,25(OH)_2D_3$ and increased weight gain, plasma P, tibiae ash and bone mineral in a greater manner than *Sg* treatments, suggesting phytase is superior to *Sg* on P utilization especially in high phytate-P diet. Sebastian (1996) reported the phytase concentrations to optimal growth performance and mineral utilization in 3 week old chicks fed 0.5% P and 0.6% Ca was at 600 FTU (Sebastian et al. 1996). When total P was decreased to 0.45% the maximum growth performance was observed at 1000 to 1500FTU/kg. Our data showed that 1200U phytase induced the greatest growth performance in broiler chicks (Table 5), which would have overwhelmed the possible additive effect with *Sg*. Hence, the combination of phytase and *Sg* treatment did not result in significant differences on growth performance and bone mineralization when it was compared to phytase treatment alone. Nevertheless, the synergistic effect of vitamin D metabolites with phytase was reported (Edwards 1993; Roberson and Edwards 1994; Biehl et al. 1995; Mitchell and Edwards 1996; Mitchell and Edwards 1996; Qian et al. 1997). Baker and Biehl (1995) observed an additive effect of 1200 FTU phytase and 10ug $1,25(OH)_2D_3$ in increasing bone ash when chicks were fed 0.63% Ca and 0.43% P (Biehl et al. 1995). Mitchell and Edwards (1996a and 1996b) reported 5ug $1,25(OH)_2D_3$ with 600FTU phytase in increasing body weight, bone ash and decreasing rickets and TD incidence, as well as decreasing fecal P excretion when chicks were fed 0.45% P and 0.83% Ca (Mitchell and Edwards 1996; Mitchell and Edwards 1996). In our experiment, the only significant interaction of *Sg* and phytase was in increasing tibiae ash (Table 4). Paired comparison of our data also indicated that the combination of *Sg* and phytase had significantly higher weight gain, tibiae ash and bone Ca concentrations than those by *Sg* treatment alone (Table 5 and 6). Bone P concentrations were not different between *Sg* and the combination of *Sg* and phytase. It indicates that when *Sg* was used to improve dietary P utilization in broilers, the

presence of phytase would further increase growth performance. As far as the interaction of phytase and *Sg* on bone, it is mainly in increasing bone Ca rather than bone P concentrations.

CONCLUSION

The effect of phytase and $1,25(OH)_2D_3$ in growing broilers promises a good future for improving P utilization and bone development, which in turn, will decrease P pollution and TD incidence.

In our studies we demonstrated *Solanum* could be used as a less expensive source of $1,25(OH)_2D_3$ in 0.6% Ca and 0.5% (or 0.45%) P to improve P utilization. Though tibiae ash was slightly lower than the normal treatment, adequate phytase should be able to compensate the disadvantage.

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Table 1. Composition (as-fed basis) of basal and normal corn-soybean meal diet in Experiment 1 and 2

Ingredients	Basal for Experiment 1 (%)	Basal for Experiment 2 (%)	Normal
Corn	52.63	51.22	52.63
Soybean meal	39.56	41.53	38.7
Soy oil	5.15	5.15	5.15
Limestone	1.22	0.99	1.22
Mineral premix1	0.3	0.3	0.3
Vitamin premix2	0.3	0.3	0.3
Salt	0.2	0.2	0.2
DL-Methionine(98%)	0.14	0.14	0.14
Disodium phosphate	0.5	0.19	-
Calcium phosphate	-	-	1.36
Calculated composition			
ME. Kcal/kg	3200	3200	3200
Crude Protein	23	23	23
Calcium	0.6	0.6	1
Total phosphorus	0.5	0.45	0.7
Non-phytate phosphorus	0.23	0.18	0.43

1 Mineral premix provided the following(per kilogram of diet): Mn, 70mg(MnSO4 H2O); Zn, 40mg(ZnSO4 H2O); Fe, 37mg(FeSO4 7H2O); Cu, 6mg(CuSO4 5H2O); Se, 0.15mg(Na2SeO3); NaCl, 2.6g (Iodized).
2 Vitamin premix provided the following(per kilogram of diet): vitamin A(retinyl acetate), 8065 IU; cholecalciferol, 1580 IU; menadione sodium bissulfite, 4mg; vitamin E 15 IU; vitamin B12 16ug; ribolfavin, 7.8mg; pantothenic acid 12.8mg; folic acid, 1.62mg; niacin, 75mg; biotin, 270ug; choline chloride, 509mg.

Table 2. Effect of *Solanum(Sg)* and 1,25-dihydroxycholecalciferol on growth performance and plasma parameters on broilers fed 0.6% Ca, 0.5% P and vitamin D3 adequate corn soybean diets in Experiment 1 [1]

Diet	Weight	Gain:Feed	Plasma		
	Gain (g)	g/kg	Ca(mg/dl)	P(mg/dl)	$1,25(OH)_2D_3$ (pg/ml)
Basal	1213±23[a]	663±83	10.23±0.22	7.11±0.06[a]	89.0±19.6[a]
Basal+*Sg* 1g/kg	1260±18[a]	677±33	10.35±0.15	7.26±0.15[a]	-
Basal+*Sg* 2.5g/kg	1213±18[a]	696±19	10.44±0.19	7.72±0.24[b]	-
Basal+*Sg* 5g/kg	1286±16[b]	679±27	10.26±0.21	8.08±0.24[bc]	109.1±8.6[a]
Basal +15 ug $1,25(OH)_2D_3$/kg	1298±16[b]	714±25	10.27±0.16	8.28±0.2[c]	76.2±5.6[ab]
Normal	1278±14[b]	668±100	10.27±0.09	8.01±0.09[c]	52.1±3.7[b]

Means in columns with different superscript letters are different (P< 0.05)

[1] data are means±SEM of 6 pens of 8 chicks during the period 8 to 28 day post-hatching; average initial weight was 156g/chick

[2] data were means of 4 pens/treatment

Table 3. Effect of *Solanum(Sg)* and 1,25-dihydroxycholecalciferol on bone mineralization in broilers fed 0.6% Ca, 0.5% total P and vitamin D3 adequate corn soybean diets in Experiment 1 [1]

Diet	Tibial ash		Bone	Mineral content	
	g	%	Density	Ca	P
			-g/cm^3-	- mmole/cm^3-	
Basal	1.61±0.03^a	38.5±0.3^{ab}	1.139±0.003^a	1.35±0.17^a	0.84±0.15^{ab}
Basal+*Sg* 1g/kg	1.69±0.02^a	37.7±0.2^a	1.148±0.001^b	1.34±0.20^a	0.82±0.18^a
Basal+*Sg* 2.5g/kg	1.66±0.03^a	38.9±0.6^{bc}	1.148±0.002^b	1.37±0.21^{ab}	0.83±0.13^{ab}
Basal+*Sg* 5g/kg	1.75±0.03^b	38.4±0.5^{ab}	1.149±0.002^b	1.41±0.13^b	0.86±0.12^b
Basal+ 15ug $1,25(OH)_2D_3$/kg	1.86±0.03^c	39.7±0.5^c	1.163±0.002^c	1.53±0.22^c	0.93±0.11^c
Normal	1.96±0.04^d	41.9±0.2^d	1.162±.003^c	1.55±0.12^c	0.94±0.16^c

Means in columns with different superscript letters are different ($P< 0.05$)

[1] data are means±SEM of 6 pens of 8 chicks during the period 8 to 28 day posthatching; average initial weight was 156g/chick

Table 4. Analysis of variance summary of the effect of *Solanum* and Phytase in broilers fed 0.6% Ca, 0.45% P and vitamin D3 adequate corn soybean diet in Experiment 2[1]

		Weight gain	Bone density	Tibiae ash	
		-g-	-g/cm^3-	g	%
	df		----------Probability----------		
Treatment	3	<0.01	<0.01	<0.01	<0.01
Solanum	1	0.24	0.06	<0.01	0.66
Phytase	1	<0.01	<0.01	<0.01	<0.01
*Solanum** Phytase	1	0.09	0.09	0.02	0.11

Table 5. Effect of *Solanum(Sg)* and phytase on growth performance and plasma parameters in broilers fed 0.6% Ca, 0.45% total P and vitamin D3 adequate corn soybean diet in Experiment 2 [1]

Diet	Weight	Gain:Feed	Plasma		
	Gain (g)	g/kg	Ca (mg/dl)	P (mg/dl)	$1,25(OH)_2D_3$ (pg/ml)
Basal	984±16[a]	688±13	9.23±0.23[ab]	4.56±0.26[a]	157.3±7.1[a]
Basal+*Sg* 7.5g/kg	1045±31[b]	697±16	9.51±0.21[a]	6.89±0.26[b]	176.1±14.0[a]
Basal+*Sg* 10g/kg	1046±23[b]	702±15	9.20±0.14[a]	7.02±0.29[b]	169.7±8.2[a]
Basal+phytase 1200 FTU/kg	1136±11[c]	727±7	8.85±0.13[b]	7.06±0.15[b]	127.7±6.5[c]
Basal+*Sg* 7.5g +phytase 1200 FTU	1124±16[c]	727±6	9.35±0.15[a]	7.46±0.22[b]	143.0±10.7[ac]
Normal	1033±20[a]	685±24	9.04±0.12[a]	7.54±0.30[b]	80.7±4.4[b]

Means in columns with different superscript letters are different ($P < 0.05$)

[1] data are means±SEM of 6 pens of 8 chicks during the period 8 to 28 day post-hatching; the average initial weight was 133 g/chick

Table 6. Effect of *Solanum(Sg)* and phytase on bone mineralization performance in broilers fed 0.6% Ca, 0.45% total P and vitamin D3 adequate corn soybean diets in Experiment 2 [1]

Diet	Tibial ash		Bone	Mineral concentrations	
	g	%	Density	Ca	P
			-gw/cm^3-	-mmole/cm^3-	
Basal	1.02±0.03^a	36.8±0.4^a	1.117±0.007^a	1.01±0.02^a	0.68±0.02^a
Basal+*Sg* 7.5g/kg	1.19±0.03^b	37.9±0.2^{ab}	1.132±0.003^b	1.18±0.02^b	0.78±0.01^b
Basal+*Sg* 10g/kg	1.25±0.05^b	38.7±0.5^b	1.128±0.002^{ab}	1.19±0.02^b	0.80±0.01^b
Basal+phytase 1200FTU/kg	1.35±0.02^c	40.8±0.8^c	1.136±0.002^b	1.24±0.01^{bc}	0.81±0.02^b
Basal+*Sg*7.5g +phytase 1200FTU	1.38±0.02^c	40.4±0.3^c	1.137±0.001^b	1.29±0..02^c	0.80±0.03^b
Normal	1.49±0.03^d	42.8±0.4^d	1.158±0.006^c	1.39±0.04^d	0.92±0.02^c

Means in columns with different superscript letters are different (P< 0.01)

[1] data are means±SEM of 6 pens of 8 chicks during the period 8 to 28 day post-hatching; the average initial weight was 133 g/chick

CHAPTER 4. THE EFFECT OF *SOLANUM GLAUCOPHYLLUM* ON PHOSPHORUS UTILIZATION IN LACTATING COWS

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Yahsin J. Cheng, Jesse P. Goff, Ronald L. Horst

ABSTRACT

Ten primiparous Holstein cows were used to demonstrate the effect of *Solanum glaucophyllun(Sg)* on phorphorus(P) utilization. Four cows received positive control diet. The positive control diet contained 0.7% Ca and 0.37% P. The remaining 6 cows were fed basal diet containing 0.6% Ca and 0.27% P. After two weeks of feed adjustment, 2g *Sg* per cow per day was bolus administered to 3 cows fed the basal diet. *Solanum* administration continued for 7 days and its effect on fecal P excretion was compared to that of cows fed basal diet or positive control diet . Body weight and milk yield were not significantly different among treatments. Fecal P concentration was significantly decreased by reducing dietary P. Compared to the positive control, total fecal P was decreased 49% in cows fed basal diet and 60% in cows with Sg bolus. Apparent P digestibility was increased 60% by decreasing dietary P to 0.27% and increased 90% by *Sg* administration when compared to apparent P digestibility of cows on the positive control diet. Plasma $1,25(OH)_2D_3$, Ca and P concentrations were significantly increased by *Sg* bolus, suggesting the stimulation of intestinal P and Ca absorption. Bone resorption was inhibited by *Sg* in cows fed basal diet. We concluded that *Solanum* improves P utilization by increasing intestinal P absorption.

(**Key words**: dairy cows, fecal phosphorus excretion, *Solanum glaucophyllum*)

Abbreviation key: **DM**= dry matter, **DMI**= dry matter intake, **P**= phosphorus, ***Sg***= *Solanum glcaucophyllum*, **CP**= crude protein, **ADF**= acid detergent fiber, **NDF**= neutral detergent fiber

INTRODUCTION

In ruminants, dietary P absorption is directly related to P requirement. Once the P supply reaches the requirement, higher P intake would decrease absorption rate [1]. However, the P requirement for dairy cows is not well established because there are differences in dietary P availability and difficulties in estimating endogenous P losses [2]. Hence, farmers are prone to give higher P than the NRC recommendation to maintain reproductive performance and high milk yield. High P intake results in high fecal P output, which raises concerns of P pollution from dairy farms. Therefore, in order to decrease fecal P the practical way is to decrease dietary P.

Reducing dietary P too much (to 0.24%) decreased body weights, feed consumption and lowered inorganic serum P concentration in lactating cows [3]. Most reports suggest that prolonged feeding of P at 0.28% to 0.31% is sufficient to meet the P requirement for dairy cows producing 7500kg to 9000kg milk per lactation without influencing reproductive performance [4, 5]. Wu and Satter (2001) reported that feeding 0.31% P for 2 years does decrease bone P content even though the breaking strength was not changed [6]. This suggests that negative P balance results in bone resorption when dietary P is decreased to reduce fecal P excretion, even though reproductive performance is not affected.

Decreasing P intake might be sufficient to reduce fecal P. Increasing active intestinal P absorption by supplemental vitamin D metabolites could be an alternative way. The P regulating hormone, $1,25(OH)_2D_3$ stimulates active P transport, and therefore increases intestinal P absorption [7]. However, in ruminants, plasma $1,25(OH)_2D_3$ would not be stimulated by the level of dietary P recommended for decreasing fecal P output. Our hypothesis is that by giving exogenous $1,25(OH)_2D_3$ the active P absorption mechanisms can be increased, which in turn, would decrease fecal P excretion and increase apparent P

digestibility. In addition, the effect of $1,25(OH)_2D_3$ on bone mineralization may avoid possible bone P mobilization when cows are fed a low P diet. We conducted a pilot experiment to determine whether *Solanum glaucophyllum*, a plant containing $1,25(OH)_2D_3$ glycosides in its leaves [8] [9] [10], could be used as a source of $1,25(OH)_2D_3$ to improve P utilization, and thus further decrease P excretion when dairy cattle are fed 0.6% Ca and 0.27% P.

MATERIALS AND METHODS

Animal and diet

Ten primiparous Holstein cows in mid lactation (15-19kg milk/day) were used in the experiment. Animals were housed in a free-stall barn and randomly divided into 3 groups. Two groups of 3 cows each were assigned to basal diet which contained 0.6% Ca and 0.27% P on a dry matter (DM) basis. The energy and all nutrients except Ca and P met the NRC (2000) requirements for cows at 550 kg body weight producing 23kg milk per day during mid-lactating period. The remaining 4 cows were given 0.7% Ca and 0.37% P (NRC 2000 requirement) as a positive control group. Dicalcium phosphate was added to the basal diet to obtain desired Ca and P concentrations in the positive control diet. Individual cows in each group were the experimental units. All animals were allowed free access to water. Basal and positive control diets were made every two days and sampled for DM, CP, ADF, NDF, as well as Ca and P analysis. Composition of diets is presented in Table 1.

Experimental design

The experimental period consisted of 3 weeks: 2 weeks of diet adaptation followed by one week of sample collection. Body weights were recorded one day before and at the end of the 3 week trial. Before the experiment, cows were fed an alfalfa hay- corn silage based diet. In order to achieve a low P diet, the alfalfa hay was replaced by beet pulp. Corn silage

comprised a large part of the diet. Wheat straw was added to provide NDF to avoid rumen acidosis. The daily feed intake from each group decreased slightly during the first week of diet adjustment. Therefore, to ensure that most cows consumed all of the experimental diet, the dry matter intake was restricted to 13.5kg per cow per day. The positive control group received diet containing 0.7% Ca and 0.37% P through the 3 weeks. The other two groups received basal diet containing 0.6% Ca and 0.27% P during the experimental period. The experimental diet was divided into two part and given at 9:00am and 3:00pm. Ytterbium (Yb) was used as an indigestible marker to estimate daily fecal output. The Yb was first mixed with beet pulp which was added to the diets at concentration of 40mg/kg of DM (40ppm) as prepared by Wu and Satter [4]. The analysis of Yb concentration in the feed samples indicated only 30ppm was actually in the diets. During the first 2 weeks of feed adjustment, blood samples were taken at 3 day intervals. On the 3rd week, two grams of *Sg* leaf powder per cow per day was bolus administered to one group of 3 cows fed the basal diet. *Solanum* administration continued for 7 days and samples including blood, urine and feces were collected every day. Feed refusal and milk production were recorded daily.

Sample collection and analysis

Blood samples were collected at 1:00pm via jugular vein into heparinized tubes and centrifuged for 30 minutes shortly after bleeding. Urine samples were collected at the same time and mixed with 50ul of concentrated HCl before storage to prevent crystallization of calcium salt and creatinine degradation. Cows were milked at 7:00am and 2:00pm daily. Milk samples were collected after each milking. Plasma, urine and milk samples from each collection were stored at –20°C until analysis. Fecal samples were collected from the rectum at 1:00pm and 7:00pm. Thirty grams of manure from each collection were pooled per cow per day and dried at 90°C overnight. After calculating the dry matter of the feces, samples were ground into powder by electrical mill passing through 1mm screen. A 0.25g aliquot of fecal powder was wet ashed in 5ml concentrated HNO_3 and boiled at 60°C overnight. The Yb concentration in fecal sample was determined by plasma emission spectroscopy [11].

One gram of the ground fecal powder was ashed at 550°C for 4 hours for Ca and P determination.

Plasma $1,25(OH)_2D_3$, Ca and P are used as indicators for the $1,25(OH)_2D_3$ activity in *Sg*. Plasma free hydroxyproline is an indicator of bone resorption because hydroxyproline is released into plasma when type I bone collagen is degraded. The percentage of urinary Ca and P clearance was calculated and used as a reference for renal function and mineral excretion. Calcium concentration was determined by atomic absorption spectrophotometer (Perkin-Elmer Model 2380), whereas P, creatinine and hydroxyproline were determined by colorimetric methods. Plasma $1,25(OH)_2D_3$ was measured by RIA after the extraction of $1,25(OH)_2D_3$ by Sep-Pak chromatography.

Statistics

Phosphorus balance was determined by the differences between P intake and milk P, fecal P and urinary P clearance calculated from each treatment. Treatment means were compared by analysis of variance and significant differences among treatments were assessed using the Fisher's LSD multiple pair-wise comparison procedure with a 5% level of probability.

RESULTS

Diet analysis and feed intake

Originally, we planned to have the basal diet at 0.55% Ca and 0.27% P in order to prevent possible hypercalcemia that might be induced by Sg treatment. We estimated the diet mineral composition based on the NRC (2000) feed tables. However, the actual feed analysis showed 0.62% Ca and 0.27% P in the basal diet. The unexpected high Ca content in the basal diet resulted from an unexpectedly high Ca concentration in the corn silage used during the experiment. The Ca content in our corn silage was 0.35%, which was 0.07% higher than that listed on the NRC(2000) feed ingredient table (0.28%). Therefore, the Ca content in

positive control diet was also increased to 0.71% instead of 0.66% (Table 1). In addition, the acid detergent fiber (ADF) and neutral detergent fiber (NDF) in the feeds were higher than what we calculated. It could be due to the amount of straw and beet pulp in the feeds. Dry matter intake among groups was not significantly different. Most cows consumed all 13.5 kg of diet per day during the 3-week experimental period, except one cow fed the positive control diet and one fed the basal diet (assigned in the *Sg* treatment group later), resulting in a small variation in the feed intake in these 2 groups (Table 2). The treatment effect was not significant for body weight and milk yield ($P \geq 0.05$). However, cows in the positive control group had slightly lower body weight than the other two groups before the trial, which became significantly lower than those on the basal and Sg groups. Before the trial, milk yield was 18.6, 19.1 and 17.4kg per day in the positive control, basal and *Sg* treatment, respectively. One week after the beginning of the experiment, while cows were still in the feed adjusting period, one cow assigned in the *Sg* group was found to have mastitis and received antibiotics. Therefore, the mean milk yield from the *Sg* group was decreased to 15.8kg.

P balance

Daily P intake was 49.7, 36.5 and 36g in the positive control, basal and *Sg* treatments, respectively (Table 3). Mean fecal output on dry matter base was 5kg in positive control and basal groups, respectively, and 4.2kg in Sg treatment. The difference in fecal output was not significant among treatments. Decreasing dietary P from 0.37% to 0.27% significantly reduced fecal P concentration from 6.00g/kg to 3.41g/kg($P<0.01$). Fecal P concentration was 3.15g/kg in Sg treatment, which was not significantly different from basal group but also significantly lower than that in positive control. Compared to positive control, daily fecal P excretion was significantly decreased by basal diet and Sg treatment. Daily fecal P excretion was decreased 49% in cows fed basal diet and decreased 60% in cows fed *Sg* treatment when compared to positive control cow fecal P excretion ($P<0.01$). Compared to the basal diet, daily fecal P was reduced 22% by the presence of *Sg*. However, the reduction was not significant. Milk P concentration was not significantly different among treatments, being

0.78, 0.85 and 0.83g/kg of milk in positive control, basal group and *Sg* treatment, respectively. Urinary P clearance was higher in cows fed 0.37% P diet than in cows fed 0.27% P diet, but the difference was not significant. Since the glomerular filtration rate (GFR) and the daily urine volume was not measured, twenty liter per cow per day was assumed to be the minimum urine volume [12]. Hence, the urinary P excretion was calculated as 0.5g/d for estimating P balance since the highest urinary P concentration was less than 0.015g/L. Variance between the assumed and the true P excreted in urine should not greatly influence the P balance because of the low urinary P concentration. Phosphorus balance in the positive control, basal and *Sg* treatments were 2.19g, 3.14g and 8.92g, respectively. The treatment effects on P balance were not significant due to the small cow numbers in each group (n=3) and relatively big variation in individual. Nevertheless, compared to the positive control and basal group, *Sg* increased 6.73g and 5.7g of P retention. Decreasing P intake significantly increased apparent digestibility of dietary P ($P<0.01$). Compared to cows fed 0.37% P, apparent P digestibility was increased 60% by decreasing dietary P to 0.27% and 90% by *Sg* in the 0.27% P diet. The presence of *Sg* in the basal diet further increased 18.7% of P digestibility than without *Sg*.

Ca balance

Daily Ca intake was 95.4g, 83.9g, 82.4g in the control, basal and *Sg* treatment, respectively. The highest fecal Ca concentration and total fecal Ca were observed in the basal group at 19.01g/kg and 97.37g/d (Table 4). Compared to control and basal treatments, fecal Ca concentration was significantly lower in the *Sg* treatment ($P<0.05$). However, the effect of Sg on fecal Ca excretion was not statistically significant. No treatment effect was observed in the milk Ca concentration and total milk Ca. Total milk Ca was lower in the *Sg* group was resulted by the lower daily milk yield. Percentage of urinary Ca clearance was 0.33, 0.18 and 3.91 in control, basal and Sg treatments. Urinary Ca clearance was significantly higher in *Sg* treatment than the other groups ($P<0.01$). Most cows had urinary Ca concentration below 0.02g/L in the normal and basal groups. Therefore, 0.5g/d was used as the Ca lost in the urine for calculating Ca balance when 20L was used as an estimation of urine volume. The

urinary Ca excretion in the Sg group was 2.72g/day because the average urinary Ca concentration in this group was 0.136g/L. Positive Ca balance was observed in *Sg* group at 7.75g/day whereas –18.68g and –30.05g/day of Ca balance was shown in the control and basal treatment.

Plasma parameters

Plasma $1,25(OH)_2D_3$ was not increased by basal diet during the experimental period (Figure 1, a and b). Plasma $1,25(OH)_2D_3$ was significantly increased after 24 hours of *Sg* bolus, reached peak at 270.7pg/ml(mean) 4 days later, followed by a decrease on the 5th day and maintained at 180 pg/ml for the rest 3 days when the cows were continuously bolus (Figure 1, a and c).

No significant changes were observed in the plasma P concentration by cows fed basal diet during the 3-week experimental period (Figure 2, a and c). Plasma P in the *Sg* treatment was not different from the basal treatment during the first 2 weeks of diet adjustment. Significant elevation of plasma P appeared 3 day after *Sg* administration. *Solanum* treatment stimulated a gradual increment of plasma P and reached a plateau at 7mg/dl until the end of the experiment (Figure 3, a and b).

Plasma Ca concentration was not increased significantly during the first 2 weeks of experimental period until 3 days after *Sg* administration. The peak plasma Ca was at 11.8 mg/dl on the 6th day after daily *Sg* bolus. Hypercalcemia sustained the rest of the experimental period (Figure 2, a and c). On the other hand, plasma Ca concentration was not changed in the basal group during the whole 3-week experimental period (Figure 2, a and b).

Plasma free hydroxyproline was 4 and 3.61ug/ml in the basal and *Sg* treatments after 2 weeks of diet-adjusting period. Mean plasma free hydroxyproline was further increased during the 3rd week trial in the basal treatment (Figure 4, a) due to the reaction from one cow (Figure 4, b). The administration of *Sg* tended to decrease plasma hydroxyproline the 3rd week experimental period (Figure 4, c).

Low dietary P did not significantly increase plasma $1,25(OH)_2D_3$ in basal group (Table 5). Compared to control and basal treatments, means of plasma $1,25(OH)_2D_3$ and P

concentrations were significantly increased by *Sg* treatment ($P<0.05$). Plasma Ca was significantly increased by basal treatment and *Sg* treatment ($P<0.01$). Though the basal group had higher plasma free hydroxyproline during the 3rd week it was not significantly different from that of *Sg* treatment.

DISCUSSION

The purpose of the study was to determine whether *Solanum* could serve as a source of $1,25(OH)_2D_3$ to increase P utilization and decrease P excretion in lactating cows.

In order to ensure cows consumed all assigned diets we restricted feed intake to 13.5kg DM during the 3-week experimental period. According to NRC (2000) for lactating cows weighing 550kg and producing 25kg milk/day during their 6th month of lactation, the predicted dry matter intake is 18.9kg. Therefore, the actual daily dry matter intake in our experiment was 28% less than that of NRC predicted. Nevertheless, the net energy intake, net protein intake and mineral contents in the 13.5kg were supposed to maintain cows producing 22.8kg milk/day without negative energy or protein balance. Assuming the P requirement for lactating cows was based on the sum of inevitable loss of P at 1.2g/kg DMI [12] and the total milk P at 0.9g/kg(NRC,2000), the minimum P intake to cows in our experiment should be 33g/d regardless the urinary P excretion. The P intake in basal and *Sg* treatments was 36.5g/d and 36g/d, which was theoretically above their daily requirement. The P in positive control (0.37%) was then 51% above their daily needs. In fact, the milk yield of our cows did not exceed 22kg milk/day before the trial. Hence, feed restriction and dietary P concentration should not result in decreasing milk yield and/or body weights. At the end of the trial, body weight was increased in all groups. Compared to the milk yield before the experiment, a slightly reduction was shown in all treatments. Mean milk P concentration in our experiment was 0.82g/kg, which is a little bit lower than the NRC published value at 0.9g/kg. Florar et al. (1982) investigated the changes of inorganic P in milk of lactating Holstein cows and reported that milk P concentration was affected by month of year and month of calving but not dietary P. Milk P concentration was lower during

summer months and declined in later lactation with reduced milk yields [13]. Our experiment was conducted in August when cows were during the 6 and 7 months of lactation. Thus, the reduction in milk P concentration and milk yield could be due to the season and length of lactation rather than treatment effects.

Means of fecal output in the 3 groups were consistent at about 5kg/day, which makes the fecal P concentration a determining factor for daily P excretion and a reliable indicator for net apparent P absorption. There was significant treatment effect on fecal P concentration. Fecal P concentration was decreased 29% when dietary P was decreased from 0.37% to 0.27%. Though there was no significant difference between basal and *Sg* treatment, Solanum tended to decrease 8% more of fecal P concentration than that of in basal treatment. It indicates that net P absorption was increased when P intake was decreased and *Sg* further increased net P absorption when it is added with low P diet.

Treatment effect on fecal P excretion was significant. Cows fed 0.37% P excreted the highest fecal P at 33.14g/day among 3 treatments. Therefore, the apparent P digestibility was 33%, the lowest among all treatments. High dietary P resulted in high fecal P and low apparent P digestibility in positive control, suggesting the higher the P intake the less the % P absorbed in the small intestine, and the more the P excreted in feces. This is supported by Challa et al. (1989) that higher P intake would decrease absorption rate when the P supply reaches the requirement [1]. Wu et al. (2000) reported similar P digestibility in cows fed 0.4% P. In their study, when dietary P was increased from 0.31% to 0.4%, the apparent digestibility was decreased from 45% to 34% in high milk yield cows during the 23th week of lactation [4]. This indicates that 0.37% P in our experiment and 0.4% P in Wu's experiment are above the P requirement for lactating cows. Hence, fecal P excretion was increased by higher P intake. When compared to the basal treatment, the apparent P digestibility was further increased 19% by *Sg*. However, the reduction induced by *Sg* was not significant even there was big differences between the two value (53% vs 63%). It might be due to the small number in each group (n=3) and variance among individuals. Nevertheless, it could be concluded that *Sg* tends to decrease fecal P excretion and increase P digestibility via increasing P absorption.

The sources of fecal P could be exogenous and endogenous. The major contribution of endogenous P is from saliva. Large amount of salivary P is secreted into the rumen and mixed with digesta before it flows to small intestine [1]. Riad et al. (1987) reported that $1,25(OH)_2D_3$ decreased salivary P concentration and secretion even though it induced hyperphosphatemia and hypercalcemia [14]. Because *Sg* increased plasma $1,25(OH)_2D_3$ it is possible that salivary P secretion was reduced, and therefore, decreased endogenous P lost in feces. However, we did not measure salivary P to demonstrate the influence of *Sg* on endogenous P loss.

Spiekers et al. (1993) reported that fecal P could be divided into 3 parts: the unavailable dietary P that was undigested and unabsorbed, the inevitable P excreting during normal physiological conditions. The latter included microbial debris containing P, salivary P and P sloughed from gut tissue. The third part was the regulated P, which is the amount of P absorbed beyond the tissue requirements [12]. The regulated P depends on the physiological events and vitamin D status of the animals. The major form of unavailable P in feed is phytate-P. Unlike monogastric animals, rumen microbes hydrolyze 98% of dietary phytate-P even when large quantities of feeds and significant amounts of grain are consumed. Therefore, phytate-P should be considered as available to lactating cows when rations to meet their P requirement [15, 16]. In this case, dietary P availability should be counted close to 100%. Because salivary P is changed with the DMI, the inevitable P loss is also determined by DMI. Decreasing dietary P would decrease the excretion of inevitable P and regulated P, and thus decrease the excretion of total P in feces.

Because the DMI and dietary P concentration was the same in basal and *Sg* treatments, the inevitable P loss was supposed to be the same. We calculated the P requirement in our experimental cows was 33.39g for the basal group and 31.62g for the *Sg* group based on the sum of inevitable P and milk P excretion [12]. Assuming dietary P is almost 100% available, calculation of the regulated P by subtracting P requirement from the P intake would be 3.11g and 4.38g for the basal and *Sg* groups. Thus, the mean of the regulated P for cows fed 0.27% P is 3.74g. When *Sg* was added in the basal diet, the fecal P was decreased 3.8g/day compared to the basal group. That is, the amount of P decreased by 2g *Sg* (3.8g) is almost identical to the amount of regulated P (3.74g), suggesting *Sg*, as an exogenous $1,25(OH)_2D_3$.

further enhances the absorption of regulated P to the minimum and decreases fecal P excretion to the least. Since the calculation in P requirement is based on the assumption proposed by Spiekers' (1993) our data support his conclusion that 1.2g/kg DMI should be used for estimation of inevitable P loss for dairy cows [12].

One may argue that by decreasing P intake to the P requirement at 33.39g would have the same results as adding *Sg*. However, when P intake was decreased to 33.39g in our experiment the dietary P would be 24%. Call et al. (1987) reported the reduction of feed consumption and decreased body weight was shown when cows fed 0.24% P [3] whereas Valk et al. (1999) proposed that 0.28% P was sufficient to meet the P requirement of dairy cows with 9000kg/year milk yield [5]. The question here is if it is appropriate to give the P intake at the level only meet their P requirement in order to minimize the fecal P output. Though plasma P concentration was in the normal range, data from Spiekers (1993) experiment showed negative P balance when P supply only met the P requirement, indicating bone resorption would have to occur to maintain normal plasma P concentration [12].

We did not observed hypocalcemia and hypophosphatemia in cows fed 0.6% Ca and 0.27% P basal diet during the first two weeks of feed adjustment (Figure 2 and 3). Mean plasma P in the basal treatment was slightly decreased from 6mg/dl to 5.6mg/dl but still in the normal range for late lactation cows (4.1-8.7mg/dl)(Table 4)[17]. Wu et al. (2001) reported decreased bone P in cows fed 0.31% P for 2 years even though plasma P was not decreased significantly [6]. Plasma P concentration reflects actual P supply in ruminants. However it may not indicate the real P status in the animal because plasma P is well controlled by parathyroid hormone, $1,25(OH)_2D_3$ and calcitonin [18]. Bone resorption occurs in a few days to maintain normal plasma P concentration when dietary P is below the requirement [19]. In our experiment, cows fed 0.6% Ca and 0.27% P diet had normal plasma Ca and P concentrations but free plasma hydroxyproline was increased after 2 weeks diet adjustment period (data not shown). Though the differences in plasma hydroxyproline between the pre- and after treatment was not significant it indicates a trend toward bone resorption. The slow elevation of plasma hydroxyproline in cows fed 0.27% P diet was inhibited when *Sg* treatment was added to the basal diet (Figure 4, a and c). *Solanum* increased plasma $1,25(OH)_2D_3$ (Figure 1, a and c), which in turn, stimulated active intestinal Ca and P

absorption and increased plasma Ca and P concentrations, hence increased Ca and P deposition in bone. Meanwhile, the hypercalcemia induced by *Sg* stimulated calcitonin secretion, which blocked bone resorption [20]. Goff et al. (1986) demonstrated that administration of $1,25(OH)_2D_3$ did not increase urinary hydroxyproline excretion rate and plasma hydroxyproline concentration. However, these authors observed severe reduction in glomerular filtration rate (GFR) and urine specific gravity when 400ug of synthetic $1,25(OH)_2D_3$ was given. They concluded that $1,25(OH)_2D_3$ does not induce bone resorption in nonlactating Jersey cows but results in the impairment of renal function at high dose of this compound [21]. In our experiment we did not measure the GFR and urine specific gravity. The percentage of urinary Ca clearance was elevated from 0.33 to 3.19 by the Sg treatment when compared to the positive control (Table 5). If the renal function was impaired and the high Ca clearance was due to the decreased GFR, the urinary P clearance should be also increased. However, the urinary P clearance was not different among treatments, indicating the renal function was normal and the high Ca excretion was due to hyperclacemia in the *Sg* treatment.

Dooley et al. (2001) reported that supplemental excess vitamin D had no effect on P absorption in dairy cattle fed 0.31% P when compared to cows fed 0.31% P alone [22]. The possible reason is a large amount of the vitamin D in their experiment was degraded by the rumen microbes before it could be absorbed [23, 24]. The advantage of oral administration of $1,25(OH)_2D_3$ is the compound may have local effects on small intestine to stimulate intestinal Ca and P absorption [25] when it forms lipid-mixed micelles and is taken up by the intestinal mucosa cells. Solanum leaves contain $1,25(OH)_2D_3$ glycosides. The glycosidic bonds could be hydrolyzed by the microbial enzyme before it is absorbed [9, 26]. Although it may also be subject to degradation by rumen microbes, the elevation of plasma $1,25(OH)_2D_3$ in *Sg* treated cows suggests that some $1,25(OH)_2D_3$ was absorbed and therefore, increased P utilization in dairy cattle.

CONCLUSION

Our data support the premise that decreasing dietary P significantly increases apparent P digestibility and reduces fecal P excretion, which may greatly contribute to the prevention of P pollution. Decreasing dietary P from 0.37% to 0.27% in mid-lactating cows increased apparent P digestibility from 33% to 53%. Fecal P excreted by cows fed 0.27% P was 52% of the P excreted by cows fed 0.37% P. *Solanum* leaves enhance active intestinal P absorption and therefore, decrease fecal P excretion and increase P digestibility in lactating cows. Another benefit is decreasing the possibility of bone Ca and P mobilization as it increases Ca and P balance. With 2g of *Sg*/kg in 0.27% P diet fecal P was reduced to 40% of the P excreted by cows fed 0.37% P. The effect of *Sg* on improving P utilization would be especially appreciated by cows during early lactation when their feed intake was decreased and negative P balance was occurred. Future research should focus on the concentration of dietary Ca in the 0.27% P diet needed to prevent the hypercalcemic effect induced by *Sg* in our experiment.

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Table 1. Ingredient and nutrient composition of diets(DM basis)

Item	Basal diet %	Positive control %
Ingredient		
Corn silage	22.36	21.41
Corn grain, cracked, dry	17.19	16.9
Beet pulp, dried	21.76	21.41
Wheat straw	12.99	14.53
Soybean, meal, solv, 46% CP	14.93	14.69
Soybean, meal, expellers	4.23	4.17
Calcium soaps of fatty acids	1.44	1.43
Vegetable oil	1.44	1.42
Salts	0.47	0.44
Vitamin premix	0.47	0.44
Magnesium oxide	0.24	0.24
Magnesium sulfate($7H_2O$)	0.62	0.6
Sodium bicarbonate	1.16	1.14
Calcium phosphate	-	0.5
Calculated chemical composition		
CP	18.4	18.1
ADF	19.7	20.1
NDF	34	33.5
Ca	0.56	0.66
P	0.27	0.37
Mg	0.38	0.38
Analyzed chemical composition		
CP	16	15.23
ADF	30.23	31.29
NDF	45.20	48.39
Ca	0.62	0.71
P	0.27	0.37
Mg	0.42	0.39

ADF = acid detergent fiber
NDF = neutral detergent fiber

Table 2. Body weights, daily intake, and milk yield of cows with different treatments before and after experiment*

Treatment	Positive control	Basal	Basal+2g *Sg*	P value
Number of cows	4	3	3	
DMI, kg/d	13.4±0.1	13.5±0.0	13.3±0.2	0.58
Body weight, kg				
Before experiment	506.8±14.6[a]	556.2±17.9[b]	545.1±10.3[ab]	0.09
After experiment	515.6±12.3[a]	562.4±11.5[b]	556.6±12.7[b]	0.05
Milk yield, kg/d				
Before experiment	18.6±0.9	19.1±0.8	17.4±1.7	0.59
After experiment	17.8±0.9	18.7±0.7	15.8±1.7	0.29

*Data are expressed as mean±SEM.
Means with different superscripts indicate significant difference ($P<0.05$)

Table 3. Phosphorus balance in dairy cows fed different treatments*

Treatment	Positive control	Basal	Basal+2g *Sg*	P value
Number of cows	4	3	3	
P intake, g/d	49.7	36.5	36	
Fecal output, kg/d	5.0±0.3	5.0±0.7	4.2±0.3	0.38
P in feces, g/d	33.14±2.47[a]	17.01±1.81[b]	13.27±0.74[b]	<0.01
P in feces, g/kg DM	6.00±0.27[a]	3.41±0.09[b]	3.15±0.04[b]	<0.01
P in milk, g/d	13.87±0.59	15.87±0.99	13.30±1.98	0.36
Milk P, g/kg	0.78±0.02	0.85±0.04	0.83±0.03	0.31
% Urinary P clearance**	1.30±0.8	0.07±0.04	0.11±0.06	0.26
P balance, g/d***	2.19±2.5	3.14±1.69	8.92±2.60	0.18
P digestibility, %	33.25±4.94[a]	53.33±4.98[b]	63.33±1.76[b]	<0.01

*Data are expressed as mean±SEM.

** % of urinary P clearance = 100 {[creatinine] $_{\text{in plasma}}$×[P] $_{\text{in urine}}$/ [creatinine] $_{\text{in urine}}$×[P] $_{\text{in plasma}}$}

*** P balance = P intake – [P in feces + P in milk + P in urine]. Urinary P less than 0.015g/L was counted as 0.5g/day in the balance table; as the daily urine volume was not determined. There may be a deviation between P balance and P retention.

Means with different superscripts indicate significant difference (P<0.05)

Table 4. Calcium balance in dairy cows fed different treatments*

Treatment	Positive control	Basal	Basal+2g Sg	P value
Number of cows	4	3	3	
Ca intake, g/d	95.4	83.9	82.7	
Ca in feces, g/d	92.80±10.22	97.37±20.44	54.32±5	0.1
Ca in feces, g/kg DM	17.75±1.32[a]	19.01±1.42[a]	12.83±0.41[b]	0.02
Ca in milk, g/d	20.44±0.95	21.43±0.52	17.91±2.30	0.27
Milk Ca, g/kg	1.15±0.02	1.14±0.01	1.13±0.02	0.74
% urinary Ca clearance**	0.33±0.09[a]	0.18±0.09[a]	3.91±0.70[b]	<0.01
Ca balance, g/d	-18.34	-35.4	7.75	

*Data are expressed as mean±SEM.

**Urinary Ca less than 0.02g/day is counted as 0.5g/d in the basal and normal treatments whereas 2.72g/d was used in the *Sg* treatment for the Ca balance

Means with different superscripts indicate significant difference (P<0.05)

Table 5. Mean value of treatment effects on Plasma $1,25(OH)_2D_3$, Ca, P and hydroxyproline*

Treatment	Positive control	Basal	Basal+2g *Sg*	P value
Number of cows	4	3	3	
Plasma $1,25(OH)_2D_3$, pg/ml	43.1±3.3[a]	79.79±5.83[a]	185.67±17.88[b]	< 0.01
Plasma Ca, mg/dl	9.66±0.02[a]	9.38±0.06[b]	10.83±0.04[c]	< 0.01
Plasma P, mg/dl	5.26±0.27[a]	5.46±0.24[a]	6.58±0.30[b]	0.04
Plasma hydroxyproline, ug/ml	3.31±0.16	4.79±0.76	3.28±0.29	0.16

*Data are presented as mean±SEM.
Means with different superscripts indicate significant difference (P<0.05)

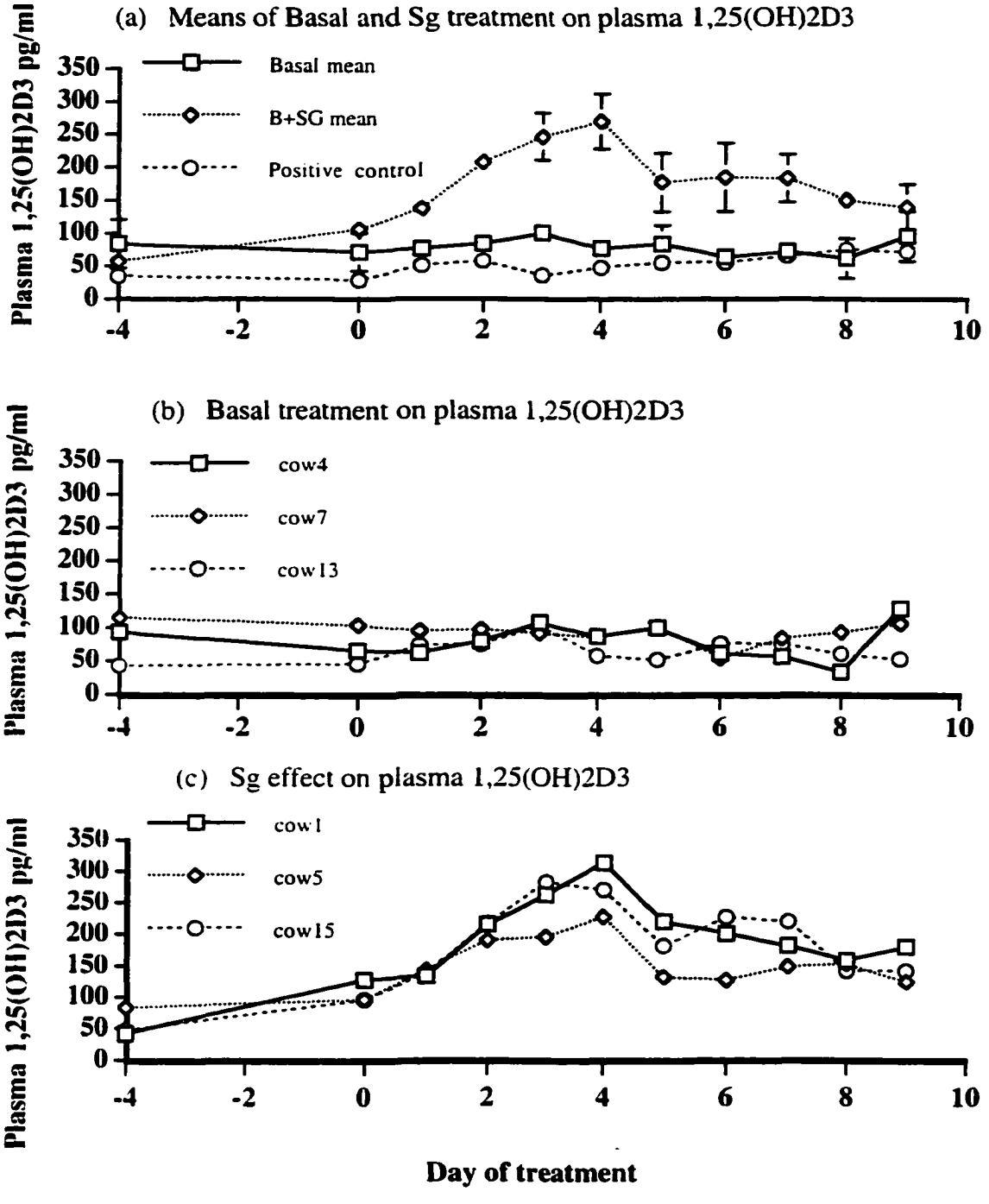

Figure 1. The effect of Solanum(Sg) on plasma 1,25(OH)2D3; (a) mean curves of basal and Sg treatment with the positive control as reference(dot line), (b) individual curves for basal treatment, and (c) indivual curves for Sg treatment. Sg was given at day zero.

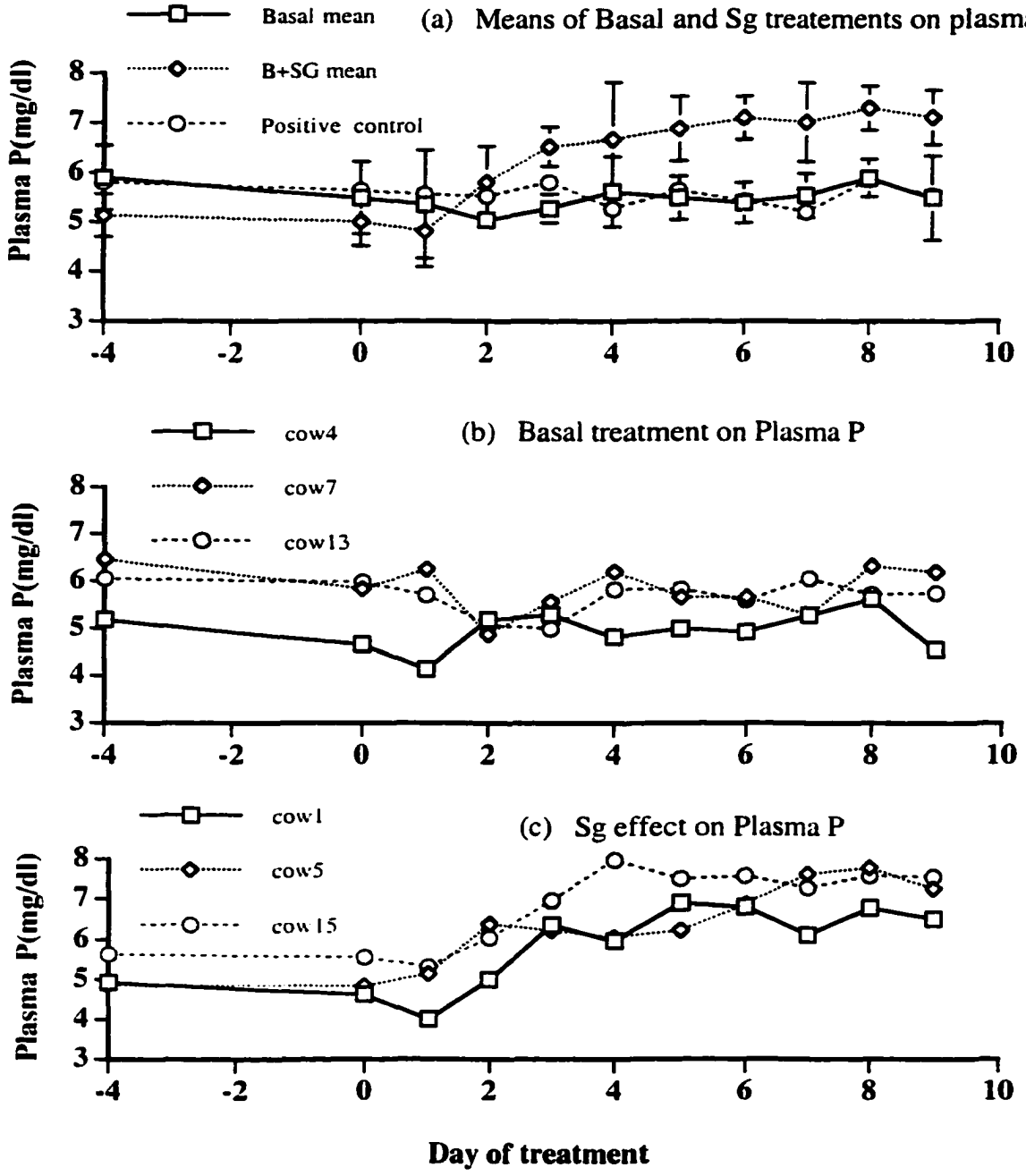

Figure 2. The effect of Solanum on plasma P; (a) mean curves of basal and Sg treatment with the positive control as reference(dot line), (b) individual curves for basal treatment, and (c) individual curves for Sg treatment. Sg was given at day zero.

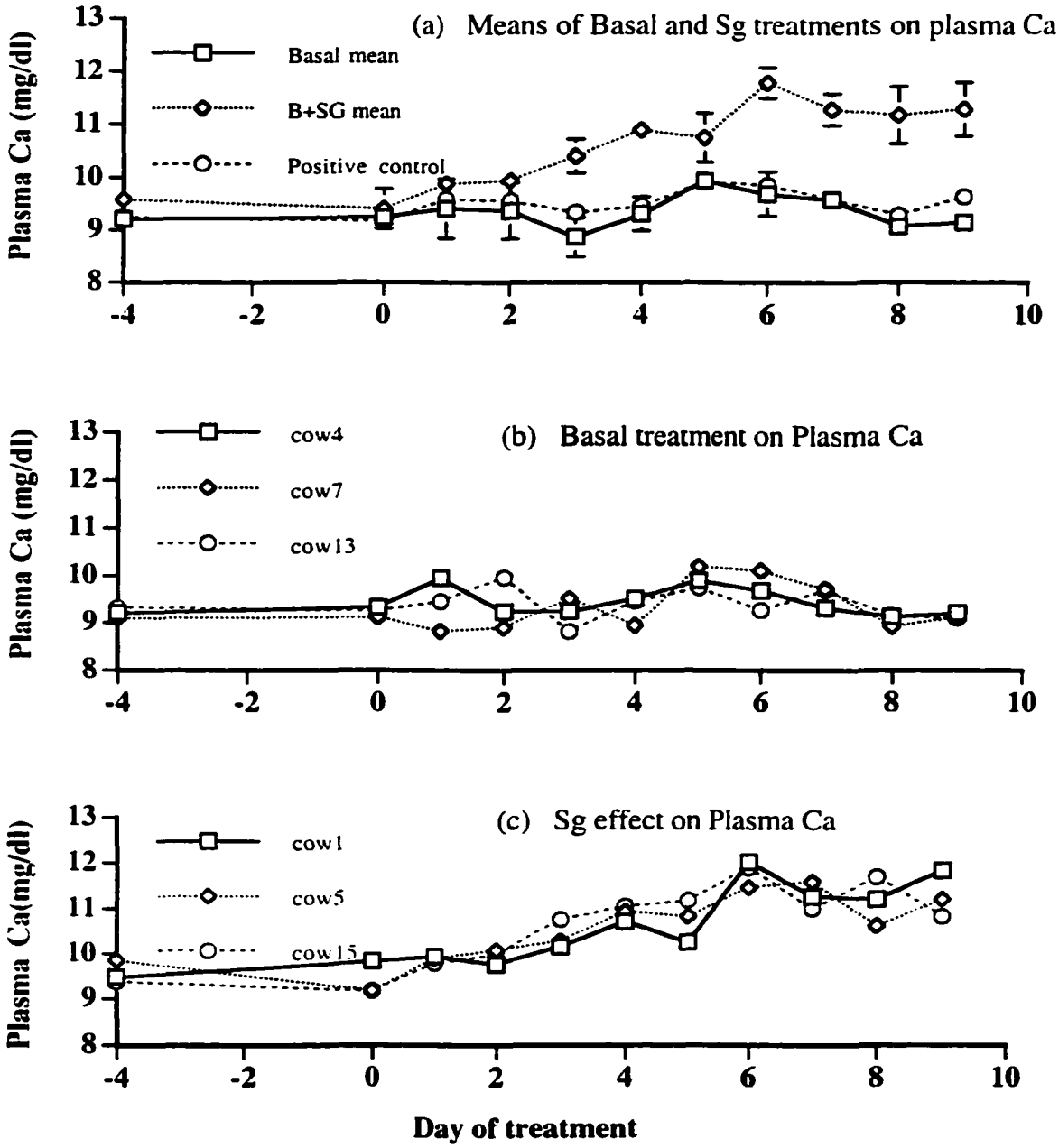

Figure 3. The effect of Solanum on plasma Ca; (a) mean curves of basal and Sg treatment with the positive control as reference(dot line), (b) individual curves for basal treatment, and (c) individual curves for Sg treatment. Sg was given at day zero.

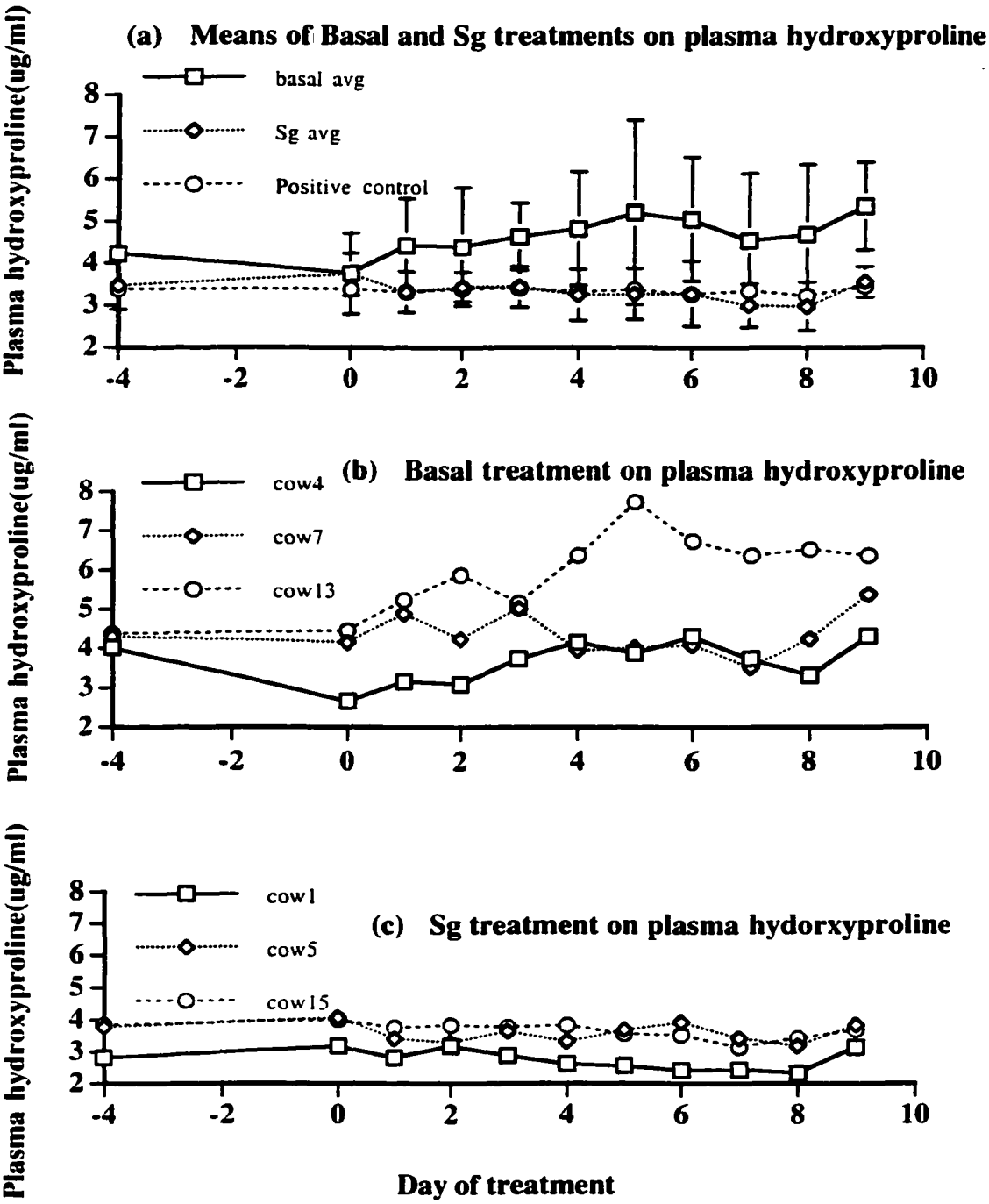

Figure 4. The effect of Solanum on plasma free hydroxyproline; (a) mean curves of basal and Sg treatment with the positive control as reference(dot line), (b) individual curves for basal treatment, and (c) individual curves for Sg treatment. Sg was given at the day zero.

CHAPTER 5. GENERAL CONCLUSIONS

Hypophosphatemic cows should be treated with phosphate solution to prevent muscle and nerve damage

Phosphorus (P) utilization in animals depends on P availability in the diet and P homeostasis of the individual. Phosphate ($H_2PO_4^{2-}$, HPO_4^{2-}) is the form of phosphorus that is absorbed by the digestive tract and regulated by the P homeostatic mechanisms. In chapter two, we point out that current treatment for hypophosphatemia in dairy cattle is not appropriate because the P is in the phosphite (PO_2^{3-}, PO_3^{3-}), rather than the phosphate form, in the commercial solution. Our data demonstrated that when P was intravenously administered, plasma inorganic P was increased by sodium phosphate solution but not by sodium phosphite solution in hypophosphatemic cows. We also demonstrated that the sodium phosphate salt was more effectively absorbed in the small intestine than the calcium phosphate salt when the two were compared as sources of oral P. It is important to note that phosphate would react with calcium ions and precipitate as calcium phosphate under physiological pH (7.4) and block the vessels when they are given intravenously at the same time. Therefore, to cows suffering from milk fever and having a history of hypophosphatemia, sodium phosphate should be administered intravenously 2 to 3 hours after the Ca is administered.

Solanum glaucophyllum could be used as an inexpensive source of $1,25(OH)_2D_3$ to improve P utilization in broilers and dairy cattle

In chapter three and four, we reported on the use of *Solanum glaucophyllum* as an inexpensive source of $1,25(OH)_2D_3$ to improve P utilization and hence decrease fecal P excretion in broilers and dairy cattle. *Solanum glaucophyllum (Sg)* is a calcinogenic plant native to South America countries (Boland 1988). Its leaves contain water-soluble $1,25(OH)_2D_3$ glycosides that can be absorbed and utilized as a source of $1,25(OH)_2D_3$ when it is taken up by animals (Wasserman et al. 1976). $1,25(OH)_2D_3$ increases vitamin D-

dependent Ca and P absorption in the small intestine, thereby increasing plasma Ca and P concentrations. Toxicity induced by *Sg*, including hypercalcemia, hyperphosphatemia, weight loss and soft tissue calcification, has been reported in cattle (Capen et al. 1966). Similar clinical signs were also observed in other animals with experimentally induced toxicity (Ross et al. 1971; Woodard et al. 1993). Santos et al. (1976) demonstrated that the *Sg*-induced toxicity could be eliminated by decreasing dietary Ca (Santos et al. 1976). Thus, in our experiments, we tried to improve P utilization in broilers and dairy cattle by using *Sg* as an inexpensive source of $1,25(OH)_2D_3$ and eliminate its hypercalcemic effect by modifying the levels of dietary Ca and P.

Different factors result in high fecal P in birds and cattle. In broilers, the low level of intestinal phytase is insufficient for digestion of high dietary phytate-P, contributing to high fecal P excretion (Nelson 1967; Nelson and Ferrara 1968; Nelson 1976). In corn and soybean meal, phytate-P accounts for 60-70% of total P, and this phytate-P easily chelates minerals such as Ca and Zn ions in the diet to form calcium phytate complexes. Because the calcium phytate complex is insoluble, Ca absorption is decreases. Meanwhile, chelation of Ca and phytate reduces the available space on phytate molecules for phytase attachment, thus decreasing phytate hydrolysis (Sebastian et al. 1998). $1,25(OH)_2D_3$ regulates Ca and P homeostasis via increasing active intestinal Ca and P absorption. Under normal physiological condition, synthesis of $1,25(OH)_2D_3$ is stimulated by parathyroid hormone or by low dietary Ca and P (Gray 1981; Gray and Garthwaite 1985) (Hove 1984). Administration of exogenous $1,25(OH)_2D_3$ to birds would increase dietary Ca and P absorption, allowing phytase of either endogenous or exogenous origin to break down phytate bonds and release phosphate for absorption, thereby decreasing fecal P. In chapter three, we demonstrated that 5g *Solanum* or 15ug $1,25(OH)_2D_3$ per kg diet increased weight gain, plasma Ca and P, bone ash and bone density in broilers fed a low Ca(0.6%) and low P(0.5%) corn-soybean diet. Bone density and mineral content were normalized by 15ug $1,25(OH)_2D_3$. The *Sg* effect on hypercalcemia was prevented by lowering dietary Ca. However, our data did not support an additive effect of *Sg* and phytase in combination on P utilization. This could be due to the high concentration of phytase used in the study. Nevertheless, the effect of $1,25(OH)_2D_3$ on bone development and its synergetic effect with phytase on P utilization in growing chicks

has been demonstrated in many studies (Mohammed and Gibney 1991; Edwards 1993; Biehl et al. 1995). The effect of *Solanum* on improving P absorption makes it a candidate for decreasing fecal P excretion in the poultry industry. Additional studies should focus on the additive effect of various levels of phytase combined with 5g *Solanum* to minimize fecal P excretion by poultry.

In dairy cattle, fecal P excretion increases when dietary P intake increases (Braithwaite 1984; Braithwaite 1985). High fecal P results from excessive dietary P given to maintain reproductive performance and milk yield (Sansinena et al. 1999). Such a diet also contains high phytate-P. Nevertheless, the enzyme activity produced by rumen microbes hydrolyzes dietary phytate, which suggests that phytate-P is 90-98% available to ruminants (Morse and Head 1992a). Therefore, decreasing dietary P to reduce fecal P excretion becomes a practical way of avoiding P pollution from dairy farms. Although research showed that decreasing dietary P from 0.41% (NRC requirement) to 0.31% in lactating cows did not decrease reproductive performance, bone P was decreased after a 2 year experimental trial (Wu et al. 2000; Wu et al. 2001). This indicates that decreasing dietary P may be at the expense of bone P mobilization.

Alternatively, increasing the P absorption rate may increase P digestibility and decrease fecal P excretion. P is absorbed through two mechanisms: vitamin D-dependent active transport and passive diffusion. In ruminants, large dry matter (DM) intake results in short retention time. Continuous digestion, which is the nature of ruminant digestion, leads to a constant high flow rate of digesta in the gastrointestinal tract (GIT) (Church 1988). It is not likely that passive P absorption could be increased when digesta flow is fast and retention time is short, along with decreased dietary P. Supplying exogenous $1,25(OH)_2D_3$ with lowered dietary P to lactating cows would enhance active P absorption, thus decreasing fecal P excretion. In chapter four, we reported on results with decreased dietary P, from 0.37% to 0.27%, and compared fecal P output with use of the basal diet and of the *Solanum*/basal diet. Without *Sg*, the 0.27% P diet was associated with a 48% decrease of fecal P, whereas with *Sg*, fecal P was decreased 60% when cows fed 0.27% P were compared to cows fed 0.37% P. Fecal P could be divided into 3 categories: the unavailable P, the inevitable P and the regulatory P. The regulatory P is controlled by the homeostatic mechanisms to the levels that

actual available P supply exceeds the P requirement (Spiekers et al. 1993). In our study, the amount by which fecal P was decreased by *Sg* treatment equaled the amount of P calculated as regulated P. In other words, *Sg* as an exogenous $1,25(OH)_2D_3$ source may enhance absorption of dietary P maximally, so that less P would be excreted in the feces. Such an effect also resulted in more positive Ca and P balance, which inhibited bone resorption in our experiment. We demonstrated that when dietary P is decreased for the purpose of decreasing fecal P output, *Sg* could be used as a source of exogenous $1,25(OH)_2D_3$ to improve intestinal P absorption and further decrease fecal P excretion in lactating cows. In our experiment, 0.6% dietary Ca along with *Sg* resulted in hypercalcemia. Future research should focus on determining the appropriate combination of dietary Ca and P with *2g Solanum/kg* diet to avoid the hypercalcemic effect.

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APPENDIX. CALCULATION OF FECAL OUTPUT BY USING YTTERBIUM AS INDIGESTABLE MARKER IN COW FEEDS

Ytterbium (Yb) intake = Yb output in feces

Therefore, Yb intake (mg/day of DM) = [Yb] in feces (ppm of DM) × Fecal output

Fecal output (kg) = Yb intake/ [Yb] in feces

Raw numbers of Yb intake, [Yb] in feces and Fecal output from each cows

Treatment/cow	Yb intake (mg/day)*	[Yb] in feces (ppm)**	Fecal output (kg)
Positive control			
Cow 2	405.9	88.4	4.59
Cow 6	394.2	75.3	5.23
Cow 8	405.9	64.9	5.85
Cow 14	405.9	90.5	4.49
Mean	403	79.8	5.04
Basal			
Cow 4	405.9	87.9	4.62
Cow 7	405.9	64.3	6.31
Cow 13	405.9	98	4.14
Mean	405.9	83.4	5.02
Basal + Sg			
Cow 1	405.9	85.1	4.77
Cow 5	388.2	95.3	4.07
Cow 15	405.9	106	3.83
Mean	400	95.5	4.22

* Yb intake = [Yb] in feeds × daily feed intake. [Yb] in feeds was 30ppm (on dry matter base) and daily feed intake was 13.5kg on dry matter base. Cows with decreased Yb intake was due to feed refusal

** [Yb] in fecal sample was based on dry matter base and determined by plasma emission spectrophotometer

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