Effects of Feeding Phytase Enzyme and HAP Corn on Solubility of Phosphorus, Copper, and Zinc in Turkey Manure and Manure-Amended Soils

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(ABSTRACT)

Manure from turkey poults on five diets were extracted both fresh (wet) and after drying. Soils amended with wet manure were also extracted. Phosphorus, Cu, and Zn were extracted with 0.01 M $CaCl_2$ and acidic Mehlich III extractant (Zn in soil extracts was not evaluated). Dietary treatments were (1) normal phytic acid (NPA) corn and 0.135% inorganic P (NPA diet); (2) NPA corn, 600 units phytase enzyme, and 0.135% inorganic P (NPA+Phyt diet); (3) High available phosphorus (HAP) corn and 0.135% inorganic P (HAP diet); (4) HAP corn, 600 units phytase, and 0.135% inorganic P (HAP+Phyt diet); (5) NPA corn and 0.345% inorganic P (NPA+P diet). The NPA+P diet was similar to conventional diets being fed commercially. The NPA+Phyt diet was similar to alternative, phytase-amended diets being fed commercially.

Feeding the alternative NPA+Phyt, HAP, and HAP+Phyt diets instead of the NPA+P diet reduced total P in manures by 40%, but increased the percentage of total manure P extracted with 0.01 M $CaCl_2$ from fresh excreta ($P < 0.05$). Soils amended with wet NPA+Phyt, HAP, and HAP+Phyt manures released 29 to 49% more water-soluble P than soils amended with NPA+P manure on an equal-P basis ($P < 0.05$). Feeding the NPA+Phyt diet instead of the NPA+P diet did not affect the percentage of total P manure extracted by Mehlich III from wet excreta, while feeding the HAP and HAP+Phyt diets increased the percentage of total manure P soluble in Mehlich III ($P < 0.05$). Soils amended with wet NPA+Phyt manure did not release more Mehlich III-extractable P than soils amended with NPA+P manure on an equal-P basis. Soils amended with wet HAP and HAP+Phyt manures released more Mehlich III-extractable P than soils amended with NPA+P manure on an equal-P basis ($P < 0.05$). Treatment-induced differences in extractability of manure and soil P appeared to be caused by a higher proportion of P in calcium phosphate form in the NPA+P manure. Drying manures prior to extraction generally heightened differences in solubility of P between the NPA+P and other manures.

Feeding the alternative NPA+Phyt, HAP, and HAP+Phyt diets instead of the NPA+P diet did not affect total Cu levels in manure or the percentage of total Cu extracted from manure with 0.01 M $CaCl_2$. After soils were treated with manure on an equal-Cu and equal-N basis, soils amended with NPA+Phyt, HAP, and HAP+Phyt manures released more water-soluble Cu than NPA+P-amended soils ($P < 0.05$). Under P-based manure management, soils amended with the three alternative manures released 92 to 108% more water-soluble Cu than NPA+P-treated soils ($P < 0.05$). Extractable Cu increased so dramatically because conversion to the alternative diets boosted total manure loadings by 67%. Mehlich III extraction of soils amended with manure on an equal-N and -Cu basis indicated no effect of manure type on Cu availability.

Feeding the NPA+Phyt, HAP, and HAP+Phyt diets instead of the NPA+P diet did not affect total Zn levels in excreta, but caused at least a five-fold increase in the percentage of total manure Zn extracted by 0.01 M $CaCl_2$ from fresh excreta ($P < 0.05$). Feeding the NPA+Phyt, HAP, and HAP+Phyt diets did not alter the percentage of total manure Zn extracted by Mehlich III from wet excreta.

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1. INTRODUCTION

The intensification of animal agriculture has produced regional surpluses of manure and manure nutrients including P. In Virginia and other mid-Atlantic states, increasing concerns about the potential for loss of P from heavily manured land have stimulated interest in modifying animal rations to reduce overall P excretion. Feeding phytase enzyme to poultry is a proven method for reducing the amount of P excreted with little or no impact on bird performance or costs of production. Although this method for decreasing the amount of total P in poultry manure is being adopted by many commercial producers, a number of questions about phytase remain unanswered. For example, does feeding phytase affect the form and solubility of the P that persists in poultry manure? Researchers now consider dissolved P solubilized by runoff moving across manure-amended fields to be a key threat to water quality in animal-intensive regions. This suggests that the true environmental impact of using phytase depends not only on how it affects total P excretion, but also on how it affects the potential for dissolved P loss from manure and manure-amended soil. It is also unclear whether phytase feeding modifies the availability to plants of P in manured soils. Similar questions remain to be answered regarding the effect of phytase use on the availability of manure Cu and Zn to the environment and to plants. The primary goal of this research was to investigate by means of standard laboratory extraction procedures the effect of feeding phytase enzyme on the water solubility and plant availability of P, Cu, and Zn in turkey manure as well as in soils amended with that manure. High available phosphorus corn is another alternative feed ingredient with the potential to reduce total P excretion by poultry. A secondary goal of this project was to explore the effect of feeding HAP corn as well as HAP corn plus phytase enzyme in combination on the form and fate of P, Cu, and Zn in manure and manured soil.

1.2. LITERATURE REVIEW

1.2.1. INTENSIVE ANIMAL AGRICULTURE AND THE MANURE P PROBLEM

Animal agriculture in Virginia and the nation in general has intensified dramatically in recent decades. Economies of scale favor increasing the number of livestock housed within individual facilities and have led to high concentrations of animals in certain limited geographic regions, such as areas near slaughterhouses and processing facilities (Williams, 1996). The Shenandoah Valley of Virginia provides an example of the staggering degree to which livestock have become concentrated on limited areas of land. In 1997, the Shenandoah Valley counties of Rockingham, Augusta, and Page produced 170,690,000 broilers, or about 66% of Virginia's total. Those three counties also produced 21,500,000 turkeys, or 83% of Virginia's total (Pelletier, 1999).

When large numbers of livestock are concentrated in this manner, the demand for feed vastly exceeds the supply of grain that the local land base can produce. Therefore, feed must be imported into the area from distant crop-producing regions. For example, it has been estimated that broilers and turkeys in Rockingham, Augusta, and Page counties alone consumed 1,371,701 Mg (53,953,000 bushels (bu)) of grain corn (*Zea mays* L.) and 576,371 Mg (21,159,000 bu) of soybeans (*Glycine max* (L.) Merr.) in 1997 (Pelletier, 1999). During the same year, the total amount of grain corn and soybeans produced in the entire state of Virginia was only 768,440 Mg (30,225,000 bu) and 306,995 Mg (11,270,000 bu), respectively. Due to this obvious shortfall in Virginia production as well as other factors, the Shenandoah Valley's great poultry feed demand has been and continues to be met primarily by rail shipments of grain originating in Ohio, Indiana, and Illinois (Pelletier, 1999).

An inevitable consequence of such intensive animal concentration is that massive surpluses of feed-derived nutrients accumulate in the form of manure. Essential plant nutrients, most notably N and P, are shipped into animal-intensive regions as naturally occuring components of feed grains. Nutrients like P are also imported in the form of concentrates or supplements that are added to rations to maximize productivity. Only a small percentage of these feed nutrients eventually leave animal-intensive areas in the form of meat or other animal products, because all animals remain relatively inefficient users of feed nutrients. For example, a turkey on a typical conventional commercial diet will only retain and incorporate into its tissues about 25% of the P in its feed. The remaining 75% of the P ingested by the bird is excreted (Duval, 1996). It is estimated that poultry alone in Rockingham, Augusta, and Page counties produced 330,393 Mg (364,190 tons) of litter (excreta plus bedding and feathers) in 1997. This quantity of litter contained an estimated 10,267,664 kg (22,616,000 pounds (lb)) N and 10,416,576 kg (22,944,000 lb) P_2O_5 (Pelletier, 1999). Pelletier suggested that this quantity of nutrients would meet the N needs of roughly 72,900 ha (180,000 acres) of grain corn on typical Virginia soils, or the P needs of roughly 309,825 ha (765,000 acres) of the same. Note that total corn for grain in Virginia in 1997 consisted of only 131,625 ha (325,000 acres).

Part of the reason that nutrients accumulate in animal-intensive regions like the Shenandoah Valley is that little market incentive exists for shipping those feed-derived nutrients back to their places of origin in manure form. Although valued as soil amendments, manures are bulky materials that are relatively expensive to transport. Furthermore, the total concentration and plant availability of nutrients in manures can vary significantly. In contrast, commercial fertilizers have high nutrient-to-weight ratios and the amount of plant food they contain can be reliably predicted. Therefore, due to the relatively low cost of commercial fertilizer, market forces limit the size of the land base in and around an animal-intensive area that is available for spreading manure. Typically, more nutrients are available in manure form than are needed by crops grown on this land base. The net result is that manure management in animal-intensive areas often takes on characteristics of waste disposal, as there is a strong incentive for applying more manure nutrients to fields than necessary for crop production (Sharpley, 1995a).

There are few agronomic disincentives to land-applying manure and manure nutrients in excess of crop needs. When manure N and P are spread in significantly greater quantity than a crop demands, the purchase of commercial N and P fertilizers can be eliminated without risk of deficiency. Furthermore, application of P in excess of the amount taken up by crops builds up soil P levels over time, which is generally desirable from a crop production standpoint. Finally, excessive N and P applications do not lead to toxicity or other problems with most agronomic crops. The problem posed by overapplication of manure to crop land is an environmental one. Applying N and P to agricultural fields in excess of crop needs increases the potential for their movement to surface waters and groundwater (Sharpley et al., 1994). The United States Environmental Protection Agency has identified agricultural non-point source pollution as the major source of freshwater contamination that prevents fulfillment of the goals of the Clean Water Act. The agency has indicated that N and P are the contaminants of primary concern in lakes and estuaries and the third leading cause of riverine pollution (Parry, 1998). Both nutrients have been linked to eutrophication in the Chesapeake Bay. Therefore, limiting the loss of N and P to surface waters is a top priority in Virginia, Maryland, and other states in the Chesapeake Bay watershed (Sharpley et al., 1994).

The N and P pollution risks associated with land application of manures can be minimized through the proper use of nutrient management planning. Nutrient management planning is the practice of matching the amount of a nutrient applied to the land with a crop’s anticipated need for that particular nutrient. Since manures contain varying amounts of both N and P, the nutrient manager allocating manure to cropland must choose one of these two nutrients to serve as a basis for the nutrient management plan (Sharpley et al., 1994). The strategy adopted in Virginia and other states has traditionally been N-based manure management. Under this approach, the nutrient manager’s primary goal is to match the amount of manure N applied to soil with the crop’s expected demand for that nutrient. Restricting manure applications based on the P content of manure has not been a common practice. This is because soil conservation, not nutrient balancing, has generally been regarded as the key to minimizing movement of land-applied manure P to waterways (Sharpley, 1997).

Fundamental soil chemistry principles help explain the traditional reliance on nutrient management to minimize N-related risks and soil conservation to minimize P-related risks from land application of manures. Soil nitrogen can cycle into its nitrate form throughout much of the year in the humid temperate climate of Virginia. Nitrate is not readily adsorbed to soil particles. Therefore, when manure N is applied to land in excess of short-term crop needs, it is very likely that some nitrate N will be carried out of the field in dissolved form by water leaching through the soil or moving over the ground's surface (Nagle et al., 1998). In contrast, basic soil chemistry suggests that P is not likely to be carried in dissolved form by water moving through soils. This is because many soil minerals, particularly the iron and aluminum oxides so prevalent in soils of the southeastern U.S., scavenge P dissolved in soil solution and bind or fix it in highly insoluble forms (Nagle et al., 1998). As a result, nutrient managers traditionally assumed that manure P applied to fields in excess of crop needs was rapidly and tightly bound to soil solids. Erosion and transport of those P-bearing soil particles to waterways was considered the main mechanism for movement of P from soil to water. Therefore, soil conservation and retention of particulate P was seen as the key to minimizing loss of soil P to the environment (Sharpley, 1997).

It is important to note that repeated land application of manure on a N basis invariably causes soil P levels to build up over time. This is because the ratio of total plant-available N to P in poultry and other manures is usually considerably lower than the ratio of N to P consumed by crops. Therefore, when manure is land-applied to meet a crop's N need, the amount of manure P supplied exceeds the amount taken up by the crop (Sharpley, 1997). The majority of the excess P is adsorbed to soil particles, causing soil P levels to rise. When this process is repeated over a number of years, sufficient plant-available P accumulates in the soil to feed multiple future crops without additional applications of P fertilizer (Coale and Olear, 1996). It should be emphasized, however, that this buildup of soil P levels with time was not traditionally considered a major threat to water quality, as long as soil conservation measures were in place limiting movement of particulate P (Sharpley, 1997).

In recent years, researchers have increasingly questioned the traditional concepts described above regarding P mobility in soils. Although it is still believed that most soils readily fix dissolved P, it is now generally accepted that soils can become saturated with P, meaning that reactive sites on soil solids can be completely occupied by P. When P in the form of fertilizer or manure is applied to them, such saturated soils have little or no capacity to adsorb the added P. Instead, the P remains in readily soluble form and is available for transport by water moving through or over the soil, even in the absence of erosion (Sharpley et al., 1994). This suggests that land application of manure P in excess of crop needs and subsequent buildup of soil P to excessive levels can increase the risk of dissolved P movement to waterways. Loss of dissolved P is of particular concern for water quality, because P in this form is most bioavailable and is readily utilized by aquatic algae (Sharpley, 1992; Sonzogni, 1982). As a result of these new concepts, many observers now suggest that nutrient management plans should limit P applications when a significant potential for loss of dissolved P from soils exists (Moore, 1998).

Not all factors contributing to soluble P movement out of soils are understood, but it is clear that the buildup of soil P to excessive or saturation levels is an important component. On a national or statewide basis, soils with very high P levels are rare. However, in regions of high animal density like the Shenandoah Valley, P saturation of soils is much more likely to occur (Sharpley et al., 1994). In these areas, most agricultural soils have received decades of P loadings in excess of crop needs as the result of N-based or unrestricted disposal of manure. It is also widely accepted that the risk of dissolved P loss from soils is enhanced by reduced tillage. No-till agriculture reduces erosion and improves crop production efficiency, but also reduces the volume of soil that comes into direct contact with land-applied amendments. Over time, manure spreading without incorporation saturates the P adsorption capacity of soil particles near the ground's surface. Experimental evidence suggests that these circumstances greatly enhance the potential for manure and/or soil P to be solubilized and transported in dissolved form by rainwater moving across the soil surface (Sharpley et al., 1994).

In the greater Chesapeake Bay region, increasing concerns about water quality degradation due to nutrient loss from agricultural lands have prompted the passage of groundbreaking new nutrient management laws in recent years. Perhaps the most notable of these is Maryland's Water Quality Improvement Act of 1998. This measure imposes nutrient management planning requirements on virtually all farmers and land managers applying commercial fertilizer as well as organic wastes (Simpson, 1998). In early 1999, the Virginia General Assembly approved House Bill 1207, which extends nutrient management requirements to most commercial poultry producers. Prior to passage of this bill, Virginia mandated nutrient management planning primarily for managers of liquid animal wastes. As a result of this law, land application of most dry poultry litter in Virginia will be subject to nutrient management planning.

In addition to increasing the number of poultry producers and other farmers required to develop and implement nutrient management plans in Maryland and Virginia, these laws herald a new era of P-based restrictions on land application of manure. Both measures specifically mandate P-based limitations on land application of manure. It should be noted that agencies in both states have developed or are developing regulatory programs which implement the mandates of these new laws. A key question facing such regulators has been how to determine which soils present the most significant risks of dissolved and/or particulate P loss and therefore the greatest need for P-based limitations on manure spreading. The approach being taken by Maryland, Virginia, and other states is development and use of a "P Index" field assessment tool to rank sites in terms of their potential for P loss (Sharpley, 1995b). This means that "P-based" manure management in most states will actually involve a combination of N- and P-based manure allocation. On soils with low P levels and/or presenting a low overall risk of P loss, the less restrictive, traditional N-based limitations on manure loading will likely be considered appropriate. On soils with high P levels and/or presenting a relatively significant risk of P loss, more restrictive P-based limitations on manure management will most likely be required (Sharpley et al., 1994). The reader should keep this explanation in mind whenever the term "P-based" manure or nutrient management is used in the remainder of this document.

In general, the trend towards P-based restrictions on land application of manures is a major challenge for many commercial livestock producers, including the poultry farmers of Virginia's Shenandoah Valley. Most agricultural soils in the Shenandoah Valley already contain high levels of P due to years of manuring. Located on rolling topography, many fields are managed as pastures or under reduced tillage (Bosch et al, 1996). Although the regulatory program implementing House Bill 1207 and identifying priority soils for P-based manure management is still under development, it is likely that the risk of P loss from many Shenandoah Valley soils will be considered significant. Therefore, the rate at which poultry litter and other manures have been traditionally applied to Valley fields will probably be significantly reduced in order to limit P applications to crop uptake levels. Poultry litter transportation costs will increase while the economic value of litter as a fertilizer substitute will remain constant (Bosch et al., 1996). The obvious result will be a major increase in waste disposal and poultry production costs in the Valley. Another consequence of shifting to P-based manure management is that the amount of manure spread is usually reduced to levels that do not meet crop N needs. Therefore, P-based manure management in the Shenandoah Valley and other animal-intensive regions will also result in new purchases of commercial N fertilizer and increasing crop production costs (Bosch et al., 1996).

As the preceding paragraphs indicate, P in poultry litter has quickly become an environmental and financial liability for the poultry industry. The prospect of mandatory P-based nutrient management has added urgency to the search for technologies that can reduce P pollution risks associated with poultry manure. One approach is to avoid direct land application of manure P altogether. Options for achieving this include feeding poultry litter to cattle or burning it. Another approach is to process manure to create higher-value fertilizer materials that will justify the costs of transport out of animal-intensive regions (Day and Funk, 1998). A third approach is to continue land-applying manure P to fields in animal-intensive locations, but only after chemical amendment to reduce solubility and pollution potential of that P. Researchers have shown that mixing poultry litter with materials ranging from Ca-bearing liming products to alum to industrial wastes can significantly reduce the solubility of P in the manure (Moore and Miller, 1994; Peters and Basta, 1996). Most of these materials contain large quantities of Al, Fe, or Ca, which readily bind with P to form insoluble compounds under appropriate pH conditions. For example, Moore and Miller (1994) found that treatment with calcium oxide decreased water-soluble P levels in poultry litter from over 2000 mg kg^{-1} to less than 1 mg kg^{-1}. The fact that chemical immobilization is regarded as a potential solution to the poultry manure P problem highlights a key point: many prominent researchers currently believe that the pollution potential of manure depends on the form and solubility of P in the manure as much, if not more, than the total amount of P in the material (Robinson and Sharpley, 1996).

1.2.2. POTENTIAL SOLUTIONS: PHYTASE ENZYME AND HAP GRAINS

Another approach to the problem of excessive manure P in intensive poultry-producing regions is to simply reduce the amount of P excreted per bird. This can be accomplished by improving the efficiency with which poultry use the P that they ingest. Turkeys and other non-ruminants are particularly inefficient users of the P found in feed grains, because their digestive systems are not able to break down the phytic acid or phytate molecule (Moore, 1998). The terms phytic acid and phytate (anionic form of the acid) are used interchangeably in this document, as in most publications on this topic (Harland and Oberleas, 1996). The phytic acid or myo-inositol hexaphosphate molecule consists of a six-carbon ring to which six phosphate groups are bound. Most of the P in plant seeds, including two-thirds of the P in corn and soybeans, exists in this phytate form (Ravindran, 1996). Phytate forms of P also represent a major proportion of the organic P in soils (Anderson, 1980). The poultry digestive system does not produce phytase, the acid phosphatase enzyme that catalyzes the stepwise removal of phosphate groups from the phytate molecule. Therefore, phytate P is nutritionally unavailable to poultry. This means that roughly 65% of the naturally occuring P in a conventional corn and soybean meal ration cannot be used by turkeys and passes directly through the birds. The remaining one-third of the P in the corn and soybean meal, referred to herein as plant-derived nonphytate P, is nutritionally available to the bird (Ravindran, 1996).

Given the low availability of naturally occurring P in grain, supplemental P from inorganic sources is typically added to conventional corn and soybean meal diets in order to meet the nutrients requirements of poultry. These inorganic P sources usually consist of one of a number of forms of calcium phosphate, including mono-dicalcium phosphate (21.0% P), dicalcium phosphate (18.5% P), and thermochemically produced defluorinated phosphate P (18.0% P) (Sullivan, 1996). Inorganic as well as plant-derived forms of nonphytate P are therefore considered the most digestible forms of P in conventional poultry rations. This is why P requirements for poultry are expressed in terms of nonphytate P levels (NRC, 1994). Inorganic forms of P are much more digestible than phytate P, but they are not 100% available. Kornegay (1998) reviewed 20 experiments to estimate the percentage of P in commercial inorganic P sources actually digested or retained by poultry. He found that 0.462 g of P was retained per g of inorganic P added to rations, above an average dietary basal level of 0.52% P. This helps explain the overall inefficiency of P use by poultry on conventional diets. All of the phytate P and a portion of the supplemental inorganic P ingested pass directly through the birds, ending up in the excreta.

The efficiency with which poultry utilize feed P can be improved by enhancing the digestibility of phytate P. Once phytate P naturally present in feed grains is made nutritionally available, the amount of supplemental inorganic P added to diets can be reduced without sacrificing animal performance. The net result is a significant decrease in the amount of phytate P excreted (Coelho, 1996a). At present, the principal method available for improving the digestibility of phytate P is the addition of phytase enzyme to rations. This enzyme cleaves phosphate groups from the phytic acid molecule, thereby making P available. Feeding phytase means that more of the plant-derived P is available, less inorganic P is needed in the diet, and overall P excretion is reduced (Coelho, 1996a). Under commercial conditions, feeding phytase enzyme and reducing inorganic P supplementation can reduce the total amount of P excreted by poultry by 25 to 35% (Duval, 1996). Phytase-amended feeds do not typically supply sufficient P in plant-derived nonphytate form to completely meet the needs of young poultry. Therefore, alternative, phytase-amended rations still require supplementation with a baseline amount of inorganic P.

A secondary impact of replacing phytate with nonphytate forms of dietary P relates to the availability of cations such as Ca, Cu, and Zn. The negatively charged phytate molecule readily chelates such cations, producing salts of extremely low solubility. Therefore, the phytate molecule reduces the digestibility of essential elements like Ca and Zn. This explains why phytate is often referred to as an “antinutritional factor” (Coelho, 1996a). Addition of phytase enzyme to diets and cleavage of phosphate groups from the phytate molecule increases the availability of complexed cations for absorption. Researchers have shown that feeding phytase improves the availability of Ca, Zn, and other essential cations to poultry (Gordon and Roland, 1996).

At this juncture, it is important to point out the critical relationship between Ca and P in poultry diets. Conventional feeds are supplemented with significant quantities of inorganic Ca as well as inorganic P. Common dietary inorganic P sources (i.e., calcium phosphates) are also sources of Ca. An excess of dietary Ca is known to interfere with P utilization in poultry. Therefore, an important goal in formulating conventional poultry rations is to achieve a desirable ratio of Ca to P in the feed. A Ca:P ratio of 1.2:1 is considered ideal, although ratios of 1:1 to 1.5:1 are well tolerated (Ensminger et al., 1990). Kornegay et al. (1996) and other researchers have shown that excess Ca also interferes with utilization of P in phytase-amended rations. Therefore, maintaining the traditional Ca:P ratio is recommended when formulating both conventional and phytase diets.

As of early 2000, the use of phytase to reduce poultry manure P content is a proven practice being rapidly adopted by the industry. Advances in fermentation technology and other innovations have made the price of phytase enzyme attractive. In most cases, the cost of using phytase can now be offset by the savings associated with reduced supplemental inorganic P purchases. Pelletier's (1999) research suggests that, as of early 2000, most of the poultry produced in the Shenandoah Valley of Virginia are already on phytase-amended feeds. The shift towards P-based nutrient management standards in Virginia and other states will likely provide a strong economic incentive for further adoption of phytase technology. This is because phytase use can greatly reduce poultry manure disposal costs under P-based manure management, as shown by Bosch et al. (1996). In Maryland, poultry integrators will have no choice but to convert to feeding phytase. Maryland's Water Quality Improvement Act requires that, by December 31, 2000, all contract feed for chickens must include phytase or another enzyme or additive that reduces manure P content (Simpson, 1998).

A second method for enhancing the digestibility of phytate P in poultry rations involves feeding grains that contain greater-than-normal amounts of digestible P. One such grain is low phytic acid (LPA) corn, also known as high available phosphorus corn. This mutant hybrid with the *lpa 1-1* allele produces grain that is similar in total P content to normal corn, but which contains about 60% less phytate P (Raboy et al., 1994). A number of researchers have shown that more P is available to poultry from rations formulated with HAP corn than rations containing normal corn (Kersey et al., 1998; Li et al., 1998; Yan et al., 1998). Therefore, the net result of HAP corn use is similar to the effect achieved with phytase: since a greater proportion of P ingested is in an available form, the total amount of P in both diet and excreta can be reduced without sacrificing animal performance.

There is significant potential for feeding both phytase enzyme and HAP corn in the same poultry ration. For example, if HAP corn is used in place of normal corn in a traditional corn and soybean ration, the feed still contains a significant quantity of soybean-derived P that is in unavailable phytate form. By adding phytase enzyme to the ration, a portion of this phytate P can be made available and P retention can be further improved. A similar benefit is predicted when substituting HAP grains to a diet already containing phytase enzyme. As indicated previously, not all plant-derived P in phytase-amended feeds is digestible. Therefore, phytase-amended rations are still supplemented with some inorganic P. Substitution of HAP corn for normal corn in these phytase-amended rations have the potential to further boost plant-derived nonphytate P levels, allowing for greater reductions in inorganic feed P. The result is again improved P efficiency and further reduction in P excretion. Despite their promise, HAP grains are not currently available on a commercial scale. Therefore, while it is safe to say that phytase enzyme will be fed to commercial poultry on a massive scale in the near future, it is less clear to what extent HAP corn will be used.

1.2.3. UNRESOLVED QUESTIONS

1.2.3.a. Phosphorus

Although phytase and HAP grains have the potential to help solve the poultry industry's manure P problem, a number of questions remain to be answered. It is known that these alternative feed components reduce the total amount of P excreted by chickens and turkeys. But, how do dietary phytase and HAP grains affect the forms of P that persist in poultry manure? In particular, is the P in manure derived from alternative feeds in a more soluble and environmentally available form than P in conventional manure? Both phytase and HAP grains make feed P more digestible by converting highly insoluble phytate P into bioavailable, nonphytate forms. It is therefore logical to ask whether a similar increase in solubility and bioavailability is observed after the P leaves the bird's digestive system in manure. The answers to these questions are critical, because without them, the true impact of feeding phytase and HAP grains on P pollution potential cannot be assessed. This is especially true when P is the nutrient limiting manure applications. In these cases, conversion to alternative feeds increases the amount of manure that can be disposed per unit of land, but does not change the total amount of P applied per unit of land. If feeding phytase or HAP corn significantly increases the solubility of manure P, their use under these circumstances could conceivably increase the potential for soluble P loss from manured fields. It is intriguing to note that the greatest incentive and likelihood for phytase use exists when manure applications are limited on a P basis. These factors suggest that investigating how phytase enzyme and HAP grains affect the solubility of P in poultry manure is a very meaningful undertaking.

Another question that has not been addressed is whether land application of manures derived from feeding phytase and/or HAP corn changes the availability of soil P for plant uptake. A number of researchers have suggested that the availability of soil P to plants as well as to the environment may be affected by differences in the original source and form of P (Robinson and Sharpley, 1996). It seems unlikely that feeding phytase and/or HAP corn to poultry could change the forms of P in manure in such a way as to ultimately produce significant impacts on plant availability of P in manured soils. The question nevertheless deserves attention, if only because the crop acreage that will soon be fertilized with manure from phytase-fed birds is so extensive.

1.2.3.b. Copper and Zinc

Feeding phytase enzyme to poultry increases the solubility and nutritional availability of feed metals such as Cu and Zn (Kornegay et al, 1996). Therefore, it is also logical to ask whether alternative feeds increase the solubility of Cu and Zn in poultry manure. As explained below, metals in land-applied poultry manures have been shown to pose potential agronomic and environmental risks. Those risks would likely be heightened if feeding phytase or HAP corn increased the solubility of Cu or Zn found in land-applied poultry litter.

Commercial poultry rations are commonly supplemented with heavy metals including Cu and Zn. These trace metal supplements help prevent diseases and improve weight gain and feed efficiency. The great majority of these metals pass directly through the birds and are concentrated in the excreta (Tufft and Nockels, 1991). Parker and Zublena (1996) indicated that commercial poultry litter typically contains between 230 to 360 mg kg^{-1} Zn and 110 to 300 mg Cu kg^{-1}. However, Stephenson et al. (1990) found a wider range of 25 to 1000 mg Cu kg^{-1} in litter. This variability in manure metal content can be influenced by the quantity of metal supplements fed, the type of bedding material used, and the manner in which the litter is managed in the house and after cleanout.

Long-term land application of poultry litter can cause high levels of metals to accumulate in soils (Kingery et al., 1994). Although elevated levels of Cu and Zn in soils can cause toxicity to crops, instances of phytotoxicity due to land application of poultry manure are rare. This is because, under the relatively high pH conditions maintained in most agricultural soils, metals like Cu and Zn are readily bound to soil particles and are not available for plant uptake (Moore, 1998; Anderson et al., 1991; Payne et al., 1988b). However, cases of Cu and Zn toxicity to crops due to long-term applications of poultry litter have been reported (Zublena, 1994). Factors increasing the likelihood of Cu and Zn phytotoxicity include production of particularly sensitive crops on sandy soils with low potential for metal adsorption. A dramatic increase in the solubility or plant availability of manure Cu or Zn caused by feeding phytase or HAP grains could increase the risk to crops grown on land receiving litter. Greater crop uptake of Cu and Zn could also conceivably enhance accumulation of these metals in plant tissues, increasing risks for human and animal consumers (Moore, 1998).

Traditional soil chemistry principles suggest that Cu and Zn are strongly adsorbed to soil particles and that, when applied to soils in manure form, these metals are unlikely to be solubilized by runoff water moving over the soil surface. However, recent research indicates that the potential for dissolved transport of poultry-derived Cu and Zn in runoff exists. For example, Moore et al. (1998b) applied poultry litter to grassed plots and collected runoff produced by simulated rainfall. Dissolved Cu concentrations as high as 1.0 mg L^{-1} were observed in the runoff water, suggesting a potential threat to algae in aquatic environments. This research as well as that of del Castilho et al. (1993) supports the theory that chelation of Cu and Zn by soluble organic compounds may greatly enhance the solubility and mobility of manure-derived metals in the soil environment. It is intriguing to consider that feeding phytase or HAP grains might further enhance the solubility and potential for movement of Cu and Zn from manure compounds.

It is undeniable that environmental concerns about the fate of Cu and Zn in land-applied poultry litter will continue to grow. Informed observers like Williams (1996) suggest that restrictions on land application of poultry waste will eventually take into consideration metals as well as N and P. The potential for movement of dissolved Cu and Zn out of manured soils will certainly be a critical aspect of this issue, as suggested by the fact that researchers like Moore et al. (1998a) are currently evaluating ways of inhibiting the solubility of Cu and Zn in poultry litter. Investigation of the influence of feeding phytase and HAP grains on the solubility of Cu and Zn in poultry manure is therefore a highly relevant undertaking.

1.2.4. SUMMARY OF RELATED RESEARCH

1.2.4.a. Effect of alternative diets on solubility and availability of manure P

Other researchers have investigated how feeding phytase and/or HAP corn changes the forms and solubility of P in poultry manure and manure-amended soil. However, detailed reports on the results of most of these projects have not been published in the literature at this time. Studies have included both laboratory and field research.

Sims et al. (1999) compared the water-soluble P content of broiler litter from chickens on six different dietary treatments. These researchers used three methods to determine water-soluble P content of litter. The six diets fed included (1) a diet formulated with normal corn, no phytase enzyme, and control levels of inorganic P; (2) a diet formulated with HAP corn, no phytase, and control inorganic P; (3 & 4) diets formulated with phytase, 0.10% inorganic P, and normal or HAP corn; and (5 & 6) diets formulated with phytase, 0.20% inorganic P, and normal or HAP corn. Phytase was included at 800 units per kg of feed in starter diets and at 600 units per kg of feed in all other diets. In this study, modifying poultry diets by use of phytase and HAP corn decreased total P as well as soluble P in broiler litter. The litter for diets 1 through 6, respectively, contained the following concentrations of total P: 1.43, 1.38, 1.21, 1.11, 0.97, and 0.84%. The litter for diets 1 through 6, respectively, contained the following concentrations of soluble P: 0.24, 0.23, 0.22, 0.12, 0.09, and 0.04%. The method used to determine water solubility of P affected the magnitude, but not the trend in the results. The authors stated that they identified no other changes in broiler litter composition that would impact the use of litter as an agricultural soil amendment. Given the many similarities between their work and our own, it is expected that eventual comparison of the two studies' results will be extremely useful.

Moore et al. (1998b) applied litter from broilers on conventional and alternative diets to replicated grassed field plots (3.05 x 1.52 m), used rainfall simulators to produce two runoff events, and collected runoff water for comparison of P levels. The dietary treatments included a conventional diet, a diet formulated with 500 units of phytase enzyme per kg of feed, a diet prepared with HAP corn, and a diet formulated with both HAP corn and phytase enzyme. Litter was applied to all plots at a rate of 8.98 Mg ha^{-1} (8000 lb $acre^{-1}$). Manure application significantly increased the total amount of P in runoff compared with control (unfertilized) plots. However, the runoff from plots fertilized with different manures did not differ significantly in either total or soluble reactive (below for an explanation of this term) P levels. Mean total P levels in runoff water were 18.0, 17.9, 13.9, and 13.2 mg L^{-1} for phytase, conventional, HAP, and HAP plus phytase diets, respectively, for the first runoff event. It is important to note that litter was applied to these fields on an equal-weight basis. Since the use of phytase and HAP corn typically reduces total P levels in manure by 25% or more, one can assume that the plots in this experiment fertilized with phytase- and HAP-derived manures were loaded with at least 25% less total P than plots fertilized with conventional manure. The fact that the phytase and conventional plots yielded statistically and numerically identical amounts of P to runoff may indicate that P in the alternative manure was more environmentally available on a relative basis than P in the conventional manure. However, overall environmental loadings from the two treatments did not change. Note that the HAP corn and HAP plus phytase diets lowered P runoff by 22% and 26%, respectively, over the conventional diets.

Since swine and poultry are both non-ruminants, feeding phytase and/or HAP corn can improve the availability of phytate P and reduce P excretion for both types of animals. Given this similarity, research on the form and fate of P in swine manures may be relevant to similar questions for poultry. Baxter et al. (1998) compared the water solubility of P and amounts of phytate P in manures from swine fed a conventional control diet and three alternative diets formulated with phytase, HAP corn, and HAP corn plus phytase. Feeding the phytase, HAP corn, and HAP corn plus phytase diets decreased total P excretion by 21%, 23%, and 41%, respectively, relative to the conventional control diet. However, feeding the alternative diets increased the percentage of total manure P extracted with distilled deionized water from 56% for the conventional control to 64%, 67%, and 73% for the phytase, HAP corn, and HAP corn plus phytase diets, respectively (only the conventional and HAP plus phytase manures differed significantly in P solubility percentage at the $p = 0.01$ level). Baxter et al. (1998) also found that feeding the alternative diets caused statistically significant reductions in the percentage of total manure P in phytate form. This percentage dropped from 15% for the conventional manure, to 10% for the phytase and HAP corn manures, to 6% for the HAP plus phytase manure. The researchers did not explore whether the treatment-induced decreases in phytate P content among the manures caused the observed increases in water solubility of manure P.

Joern et al. (1996) compared the extractability of P in manures of swine fed conventional and phytase-amended diets. Manures were extracted both fresh and after 110 d of storage with water as well as with 0.5 M NaOH. The percentage of total fresh manure P in water-soluble form was 1% and 8% for the conventional and phytase treatments, respectively. Storage for 110 d led to P water solubility percentages of 5% and 10% for the conventional and phytase treatments, respectively. Fewer differences in NaOH extractability of P was observed between the conventional and alternative manures. The 0.5 M NaOH solution extracted 39% and 41% of total P in fresh manures for the conventional and alternative treatments, respectively. After storage, 47% and 59% of total P was extracted from the conventional and alternative manures, respectively.

1.2.4.b. Effects of alternative diets on solubility and availability of manure Cu and Zn

Thorough review of the literature suggests that little research has been conducted about how dietary phytase and HAP grains affect the solubility of Cu and Zn in poultry manure. The available information about the research described above does not suggest that manure extracts or runoff water from manured plots were analyzed for metals.

1.2.4.c. Other research relevant to choice of materials and methods

As indicated above, relatively few researchers have assessed how feeding phytase or HAP grains to poultry changes the solubility and availability of P, Cu, and Zn in manures and manure-amended soils. However, a greater number of workers have used laboratory extraction procedures in order to estimate the environmental and plant availability of P and metals from poultry manures or manured soils. Although their ultimate goals differed from those of this research project, the strategies and methods used by these researchers are relevant.

A number of laboratory extraction procedures have been developed and standardized for estimating the availability of P, Cu, and Zn from soils. As Sims et al. (1999) observed, however, similar standardized procedures are lacking for poultry manure. Researchers seeking to compare the solubility and environmental availability of P in different types of manures have employed a variety of methods. For example, Moore and Miller (1994) sought to assess whether Al, Ca, and/or Fe amendments reduced the solubility of P in poultry litter. Fresh (i.e., field moisture) poultry litter including rice hull bedding was mixed with amendments, incubated for 1 wk, and extracted with deionized water. The extraction procedure involved shaking 20 g (dry weight) of litter in 200 mL water for 2 h in polycarbonate centrifuge tubes, followed by centrigation at 4066 rpm for 20 min and subsequent filtration of decanted supernatant through 0.45-μm filters. Baxter et al. (1998) evaluated the solubility of P in lyophilized swine manure samples by shaking for 24 h using a 1:100 ratio of sample to water extractant. As indicated previously, Sims et al. (1999) used three different extraction procedures to compare how dietary treatments affected the solubility of P in manures.

Researchers also have a variety of extraction methods to select from when estimating the environmental and plant availability of P, Cu, and Zn in soils. Water-extractable forms of P in soils typically represent a very small fraction of total available soil P. These water-soluble forms of P are those most likely to be dissolved and transported in soil solution. They are also the forms of P most readily available for uptake by plant roots (Kuo, 1996). The same concepts hold true for water-soluble metals (Li and Shuman, 1997a). For obvious reasons, many researchers have used water as an extracting solution when assessing under laboratory conditions the potential for desorption and transport of soil P by rainwater and runoff. However, an extracting solution of 0.01 M $CaCl_2$ can be used in place of water to estimate readily available soil P. This dilute $CaCl_2$ solution has comparable ionic strength to that of the natural soil solution on typical agricultural lands (Li and Shuman, 1997a). Use of the $CaCl_2$ extractant instead of water also encourages flocculation of particulates when separating solids from solution after extraction is complete (Kuo, 1996). A number of researchers have used 0.01 M $CaCl_2$ solutions for estimating the amount of readily soluble P in soils treated with poultry manure. For example, Mozaffari and Sims (1996) periodically leached litter-amended soils with 0.01 M $CaCl_2$ to determine the rate and extent of soluble P release from those soils. Peters and Basta (1996) evaluated the potential for using municipal and industrial wastes to reduce P solubility in soils with a history of poultry litter applications. These researchers estimated the bioavailability of P in these soils by extraction with 0.01 M $CaCl_2$. Li and Shuman (1997a) leached soils with 0.01 M $CaCl_2$ in order to estimate Zn solubilization by the natural soil solution.

While water or dilute salt solutions provide a measure of readily soluble P and metals in soils, they do not provide a full assessment of the quantity of those elements available to plant roots.

Stronger extractants like the acidic, chelate-containing Mehlich III solution are commonly used by soil testing labs to estimate plant-available P in soils. The Mehlich III extraction procedure has been used extensively to estimate the plant availability of P in soils amended with poultry manures. For example, Peters and Basta (1996) sought to chemically immobilize the P in soils with a history of poultry litter applications. These researchers used the Melich III extraction procedure to estimate how the treatments affected plant-availability of soil P.

The preceding paragraphs have repeatedly differentiated between dissolved and other forms of P. In actuality, distinguishing particulate P bound to microsopic clay particles suspended in solution from P completely solubilized in water is no simple matter. Many researchers have adopted the operational definition of dissolved or soluble P as any P that remains in solution after filtration through a membrane with a pore size of 0.45 µm. Any P removed from solution by the 0.45 µm filter is considered particulate P (Sharpley et al., 1994). The total quantity of dissolved P in a filtered extract or solution can be quantified by inductively coupled plasma (ICP) spectroscopy. Total dissolved or soluble P can be further divided into inorganic and organic fractions. Dissolved inorganic P is generally regarded as soluble P in molybdate-reactive orthophosphate form. This fraction of dissolved P can be quantified colorimetrically using the method of Murphy and Reilly (Anderson et al., 1996). Since this dissolved inorganic or reactive P is readily utilized by aquatic algae, it is often referred to as bioavailable P and is considered an especially potent pollutant (Sonzogni et al., 1982). The portion of dissolved P which is not molybdate reactive is sometimes called organic P. It can be quantified by subtracting the amount of molybdate-reactive soluble P from total P in the filtered solution or extract.

CHAPTER 2: MANURE EXTRACTION STUDY

2.1. OBJECTIVES

2.1.1. PRIMARY OBJECTIVES

a) Investigate how converting from a conventional diet to an alternative diet formulated with phytase enzyme changes the water solubility of P in turkey manure at the point of excretion and after drying.
b) Relate any observed changes in solubility of manure P to diet formulation and/or manure composition.

2.1.2. SECONDARY OBJECTIVES

a) Investigate how converting from a conventional diet to an alternative diet formulated with phytase changes the water solubility of Cu and Zn in turkey manure at the point of excretion and after drying.
b) Relate any observed changes in solubility of manure Cu and Zn to diet formulation and/or manure composition.
c) Investigate how converting from a conventional diet to alternative diets formulated either with HAP corn or HAP corn plus phytase affects the water solubility of P, Cu, and Zn in turkey manures at the point of excretion and after drying.
d) Relate any observed changes in solubility of manure P, Cu, and Zn to diet formulation and/or manure composition.

2.2. MATERIALS AND METHODS

2.2.1. MANURE PRODUCTION

All turkey manure used in this extraction study was produced during a feeding experiment conducted by the Department of Animal and Poultry Sciences at Virginia Tech. The objective of the feeding experiment was to compare the bioavailability to young turkeys of P in HAP corn and normal phytic acid (NPA) corn and to determine the influence of microbial phytase supplementation to each corn type. The feeding experiment involved feeding 12 dietary treatments to turkeys from 1 to 5 wk of age.

On the day of hatch, nine turkey poults were randomly assigned to 96 Petersime pens. All birds were fed a commercial starter diet for 1 wk. At the end of this 1 wk adjustment, turkey numbers were equalized to eight birds per pen. Individual birds were wing banded, weighed, and started on test diets. The 12 test diets were randomly assigned to pens within replicate blocks of 12 pens. Therefore, each dietary treatment was fed to eight replicate pens of birds. When the poults reached 3 wk of age (2 wk on the test diets), the number of poults per pen was reduced to seven and birds were transferred to grower pens for an additional 2 wk.

The pens containing the birds were housed in environmentally controlled rooms. Small feeder troughs located along the front edge of each pen provided birds with unlimited access to feed and water. No bedding material was used and the birds stood directly on the steel mesh bottoms of the pens. Manure (excreta plus feathers) as well as spilled feed and water dropped through the steel mesh bottoms of the pens onto metal manure trays. Each pen was underlain by its own manure tray, which prevented waste from dropping to the pen below. During the feeding trial, these manure trays were pulled from the batteries daily, scraped clean of manure, and replaced. Manure removed from the trays in this manner was discarded, except during the three manure collection days described below.

Although 12 dietary treatments were fed and evaluated as part of the original feeding experiment, only five diets (described in detail below) were selected for use in this manure extraction study. Therefore, manure samples were collected from 40 pens (five dietary treatments x eight replicate pens). The extraction study manure samples were collected on each of three consecutive days during the fourth week of the feeding trial. Since feeder troughs were located along the front edge of each pen, spilled feed and water were concentrated near the front edge of each pen's manure tray. Therefore, manure was not sampled from the front six inches of the trays. As much manure as possible (approximately 500 g d^{-1}) from the remaining portion of the trays was collected. Excreta were sampled from manure trays using metal paint scrapers or putty knives. On each sampling day, the manure from each of the 40 selected pens was scraped into 3.8-L (1-gallon) plastic freezer bags. These bags were then packed into plastic storage bins and immediately frozen. Since a new set of 40 bags was used for manure collection on each of the three sampling days, a total of 120 manure solubility research samples were collected and frozen. At a later date, the 120 manure samples were thawed. The three samples collected from each of the 40 selected pens were combined in hard plastic freezer containers consisting of 7.6-L

(2-gallon) buckets with air-tight lids. The three samples in each bucket were thoroughly mixed using a metal paint mixer driven by an electric hand drill. The resulting 40 manure samples corresponding to the 40 selected pens (five dietary treatments x eight replicate pens) were then returned to the freezer.

2.2.2. DIET FORMULATION

The principal ingredients used in formulating the five different feeds selected for the extraction study are shown in Table 1. Note that all analyses of feeds and feed ingredients in this document are presented on an as-fed basis (i.e., not an oven-dry-weight basis). The same type of soybean meal made up 51.06% of all five diets. Ground corn made up 41.06% of all diets, but the type of corn used varied by diet. Coventional or NPA corn was used in the NPA, NPA+P, and NPA+Phyt feeds. Optimum Quality Grains supplied the HAP corn used in the HAP and HAP+Phyt feeds. All five diets were formulated to contain a baseline of 0.75% defluorinated phosphate (PCS Phosphate Company, Inc.) as an inorganic P source. The exception was the NPA+P feed, which contained 1.92% defluorinated phosphate. Note that all diets contained identical quantities of Ca-containing limestone. Natuphos microbial phytase enzyme with 600 units g^{-1} of activity was added only to the NPA+Phyt and HAP+Phyt diets and represented 0.10% of these feeds. A trace mineral premix made up 0.10% of all diets. This premix supplied 95 mg Zn and 12.5 mg Cu per kg of feed. Starch/dextrose levels in the five diets varied in order to equalize for differences in the other feed ingredients. The five feeds contained equal amounts of all other ingredients. The five diets therefore supplied equal amounts of nutrients other than P and Ca. The five diets also supplied all nutrients other than P and Ca at or above levels recommended by the National Research Council for turkeys in the 0 to 4 and 4 to 8 wk age range (NRC, 1994).

Table 1: Composition of Feeds

Ingredient	% (as fed)				
	Diet				
	NPA	HAP	NPA+Phyt	HAP+Phyt	NPA+P
NPA corn	41.06	0.00	41.06	0.00	41.06
HAP corn	0.00	41.06	0.00	41.06	0.00
Soybean meal	51.06	51.06	51.06	51.06	51.06
Defluorinated phosphate (18% P & 32% Ca)	0.75	0.75	0.75	0.75	1.92
Limestone (38% Ca)	0.72	0.72	0.72	0.72	0.72
Natuphos 600 phytase enzyme	0.00	0.00	0.10	0.10	0.00
Trace mineral premix[a]	0.10	0.10	0.10	0.10	0.10
Starch/Dextrose	2.25	2.25	2.15	2.15	1.08
Other ingredients	4.06	4.06	4.06	4.06	4.06
Total	100.00	100.00	100.00	100.00	100.00

[a] Trace mineral premix supplied 12.5 mg Cu and 95 mg Zn kg^{-1} diet (as fed)

Table 2 shows the calculated forms and amounts of P in the five diets. Phytate P and plant-derived nonphytate P originated from the soybean and corn component of the feeds. Inorganic nonphytate P originated from the defluorinated phosphate component of the feeds. Total nonphytate P is the sum of plant-derived and inorganic nonphytate P. For poultry, nonphytate P is generally considered available P and NRC-recommended dietary P levels are given in terms of nonphytate P (NRC, 1994). Total feed P is the sum of phytate P and total nonphytate P.

The NPA diet can be considered a negative control treatment. It was prepared using conventional corn, a baseline amount of supplemental inorganic P, and no microbial phytase enzyme. As a result, it was formulated to provide only 0.282% total nonphytate P on an as-fed basis. The NRC-recommended levels of nonphytate P in turkey diets is 0.6% for birds from 0 to 4 wk old and 0.5% for birds from 4 to 8 wk old (NRC, 1994). Since the NPA feed was expected to provide significantly lower-than-optimum levels of P to turkeys throughout the feeding experiment, it was not representative of a commercially acceptable diet. However, the NPA treatment provides a useful baseline against which the effects on manure properties of dietary defluorinated phosphate addition, phytase enzyme supplementation, and HAP corn substitution can be evaluated.

Table 2: Forms and calculated amounts of P in feeds (as-fed basis)

	% of Feed					% of Total Feed P			
Diet	Phytate P	Nonphytate P			Total P	Phytate P	Nonphytate P		
		Plant-derived	Inorganic	Total			Plant-derived	Inorganic	Total
	Prior to Phytase Activity								
NPA	0.269	0.147	0.135	0.282	0.551	48.8	26.7	24.5	51.2
HAP	0.236	0.185	0.135	0.320	0.556	42.5	33.2	24.3	57.5
NPA+Phyt	0.269	0.147	0.135	0.282	0.551	48.8	26.7	24.5	51.2
HAP+Phyt	0.236	0.185	0.135	0.320	0.556	42.5	33.2	24.3	57.5
NPA+P	0.269	0.147	0.345	0.492	0.761	35.3	19.3	45.3	64.7
	Predicted after Phytase Activity								
NPA	0.269	0.147	0.135	0.282	0.551	48.8	26.7	24.5	51.2
HAP	0.236	0.185	0.135	0.320	0.556	42.5	33.2	24.3	57.5
NPA+Phyt	0.169	0.247	0.135	0.382	0.551	30.6	44.9	24.5	69.4
HAP+Phyt	0.136	0.285	0.135	0.420	0.556	24.5	51.2	24.3	75.5
NPA+P	0.269	0.147	0.345	0.492	0.761	35.3	19.3	45.3	64.7

†600 units phytase activity per kg feed is expected to convert 0.100% of feed from phytate to plant-derived nonphytate form

Substitution of HAP corn for NPA corn was the only difference between the HAP diet and the baseline NPA diet. The P in HAP corn grain is considered more available to poultry because a greater proportion of it is in nonphytate forms. This is reflected in Table 2, which shows higher levels of plant-derived and total nonphytate P for the HAP diet than for the NPA diet. However, the 0.320% total nonphytate P in the HAP feed was still significantly lower than NRC-recommended levels of 0.5 to 0.6%. For this reason and because HAP corn is not commercially available, the HAP feed is not representative of a diet currently in use in the poultry industry. The HAP treatment does provide a basis for evaluating the effect of dietary HAP corn substitution on manure characteristics.

The NPA+Phyt diet was the same as the NPA diet with 600 units of phytase activity added per kg of feed. Table 2 shows that prior to phytase enzyme activation, the NPA and NPA+Phyt feeds were formulated to contain equal quantities of all forms of P. Differences between these diets in forms and amounts of P were only predicted after phytase activity. The additional phytase enzyme in the NPA+Phyt feed was expected to cause the conversion of approximately 0.100% P from non-available phytate form to digestible nonphytate forms (Duval, 1996). Therefore, the NPA+Phyt diet was formulated to supply approximately 0.382% (0.282% + 0.100%) total nonphytate P to birds in the feeding trial. The NPA+Phyt diet was the experimental diet most similar to the alternative, phytase-containing diets currently being fed by U.S. turkey producers. It should be noted that the 0.382% nonphytate P supplied by the NPA+Phyt diet was still lower than NRC-recommended levels. A true phytase-amended commercial diet would likely contain additional inorganic phosphate in order to increase nonphytate P to recommended levels. Additional phytase enzyme would not be economical in a commercial situation and would therefore not be used to boost nonphytate P levels (Duval, 1996). The NPA+Phyt diet provides a basis for assessing the effect of dietary phytase enzyme additions on manure characteristics.

The HAP+Phyt diet was the same as the HAP diet with 600 units of phytase enzyme added per kg of feed. After activation of this phytase, the HAP+Phyt diet was expected to supply approximately 0.420% (0.320% + 0.100%) nonphytate P to birds in the feeding experiment (Table 2). Although this diet provided levels of digestible P approaching those recommended by NRC (1994), it was not representative of a commercial ration currently in use, because it contained HAP corn. The HAP+Phyt diet provides a basis for assessing the combined effect of dietary HAP corn substitution and phytase enzyme addition on turkey manure properties.

The only difference between the NPA+P diet and the NPA diet was that the former contained higher levels of defluorinated phosphate than the latter. This extra inorganic P boosted calculated total nonphytate levels in the NPA+P feed to 0.492%. The NPA+P feed was thus formulated to provide close-to-recommended levels of nonphytate P to birds during the feeding experiment. Therefore, the NPA+P feed was the experimental diet most similar to conventional commercial diets currently being fed by U.S. turkey producers. The NPA+P diet also provides a basis for evaluating the effect of dietary defluorinated phosphate addition on manure properties..

Table 3 shows the total P, Ca, Cu, and Zn contents of the five feeds selected for evaluation in the manure extraction study. Calculated concentrations were estimated using knowledge of the amount and composition of ingredients used to formulate the feeds. Analyzed concentrations were determined by laboratory analysis of feed samples. In the lab, feed samples were ground to pass a 1-mm sieve. After a nitric-perchloric acid wet digestion, P concentrations were determined colorimetrically using the vanadomolybdate procedure and Ca concentrations were determined using atomic absorption spectroscopy. Note that feed samples were not analyzed for total Cu and Zn contents. Comparison of calculated and analyzed P and Ca concentrations shows only minor differences, supporting the validity of other calculated values related to feed composition presented in Tables 1, 2, and 3. Table 3 also shows that the NPA+P feed differed from the other four feeds in both total P and total Ca content. The amount of Ca added in the experimental diets was adjusted in order to maintain a total Ca:total P ratio in the recommended range of 1.2 to 1.3:1. An inevitable consequence of maintaining this ratio was that the total dietary Ca supplied by the NPA, HAP, NPA+Phyt, and HAP+Phyt feeds were significantly lower than NRC-recommended levels of 1.2% and 1.0% for turkeys 0 to 4 wk and 4 to 8 wk of age, respectively.

2.2.3. MANURE HANDLING AND CHARACTERIZATION

The turkey manure sampled during the feeding experiment was a semi-solid material containing approximately 80% moisture. It was decided that most analyses and extractions would be conducted on manure in this fresh or “wet” form. This approach was selected in order to compare P, Cu, and Zn solubility differences between the five manures as they existed at the point of excretion. It was hypothesized that drying or other pretreatment of the manure prior to analysis might transform manure compounds controlling the solubility of P, Cu, and/or Zn. In order to avoid the potentially confounding effects of such transformations, manure samples were kept frozen (-10 °C) to the maximum extent possible. However, six of the 40 buckets containing manure from the 40 individual pens sampled in the feeding experiment were not consistently handled in this manner. The manure from these six buckets was partially consumed during preliminary trials in which extraction of wet manure was attempted. Furthermore, the manure in these six buckets was not kept frozen during this preliminary work. Therefore, the remaining portions of these six manure samples were not used in any subsequent parts of this research project. The samples discarded had been collected from individual pens in the feeding experiment numbered 3, 15, and 29 (these pens received the NPA+P diet) and 8, 19, and 27 (these pens received the NPA+Phytase diet).

Table 3: Feed analysis (as-fed)

Diet	Calculated Concentration				Analyzed Concentration)		
	%		mg kg^{-1}		%		
	P	Ca	Cu	Zn	Dry Matter	P	Ca
NPA	0.551	0.645	12.5	95.0	90.3	0.550	0.654
HAP	0.556	0.645	12.5	95.0	90.8	0.560	0.667
NPA+P	0.761	1.019	12.5	95.0	91.1	0.753	1.057
NPA+Phyt	0.551	0.645	12.5	95.0	91.0	0.556	0.652
HAP+Phyt	0.556	0.645	12.5	95.0	90.6	0.563	0.633

The excreta in the remaining 36 buckets were thawed and thoroughly mixed using a long-handled plastic spoon prior to removal of subsamples for manure characterization. Dry matter was determined by weighing subsamples (≅ 1.5 g of wet material) before and after 24 h of incubation in an oven maintained at 110 °C. Manure pH was determined after mixing wet manure and distilled deionized water at a ratio of 1 g manure dry matter:10 mL water. Note that all water used in this project was distilled and deionized, unless otherwise specified. The amount of water added to the wet manure was adjusted to account for the 80% entrained water already present in the excreta. Subsamples of wet manure equivalent to 2 g of dry matter was weighed into 30-mL plastic vials. After addition of the appropriate amount of distilled deionized water, the manure was vigorously stirred for 10 s with a glass rod and the pH of the mixture was immediately measured. Total P, Ca, Cu, and Zn content of the wet manure was determined after wet digestion of excreta samples using nitric and perchloric acid. Phosphorus concentrations in the resulting digested solution were determined colorimetrically using the vanadomolybdate procedure. Concentrations of Ca, Cu, and Zn in the digests were measured by atomic absorption spectroscopy.

After the six individual pen manure samples were discarded as described previously, 36 samples remained. This number included eight samples (one from each of eight replicate pens) for each of the following three diets: NPA, HAP, and HAP+Phyt. Five samples were available from replicate pens that had been fed the NPA+P diet and five were available from pens that had received the NPA+Phyt diet. A portion of each of these 36 manure samples was used to create five composite manures corresponding to the five dietary treatments. These composites were prepared primarily in order to have one manure sample derived from each of the five dietary treatments for use in the soil mixing and extraction study. Composite manure samples were prepared as follows. The five or eight individual pen manure samples derived from the same diet were thawed. A quantity of wet manure from each individual pen sample equivalent to 150 g of manure dry matter was placed in a 19-L (5-gallon) bucket. After individual pen samples were weighed into the bucket, the composite manure was thoroughly mixed using a long-handled plastic spoon. Subsamples of the composite manure were removed for characterization and for air drying. The bucket was then sealed with an air-tight lid and placed back in the freezer.

In order to evaluate the effect of drying at moderate temperature on the properties of the five different composite manures, approximately 200 g of wet excreta from each of the five composites was spread in a thin layer on a plastic dish and dried in an oven maintained at 40 °C. After 4 d in the oven, each of the five dried manure samples was thoroughly ground by hand using a mortar and pestle. Although most of the manure was ground to a fine powder, feathers in the sample resisted grinding. These were removed by seiving through a 2-mm mesh sieve. The ground, dried composite manure samples were then sealed in air-tight plastic containers and stored at room temperature. In the remainder of this document, manure dried in this manner at low temperature (40 °C) will be referred to as “dry”, “dried”, or “air-dried” manure. The terms “oven-dried” or “dry-weight basis” will refer to samples dried at 110 °C to determine moisture content.

Subsamples of the wet and air-dried composite manure samples were subjected to the same characterization analyses conducted on the wet individual pen samples. This included dry matter determination, pH measurement, and analysis of total P, Ca, Cu, and Zn content as described above. Additional subsamples of wet composite manure were submitted to a commercial soil and manure testing lab for general analysis. The main reason for using the commercial lab was to rapidly obtain a general estimate of the total and plant-available N content of the manures.

2.2.4. MANURE AND FEED EXTRACTION

The solubility of manure P, Cu, and Zn was evaluated by extracting subsamples of excreta with three different solutions: a dilute $CaCl_2$ solution, the acidic Mehlich III extractant, and a buffered alkaline $NaHCO_3$ solution (Olsen extractant). Details about each of the three extraction procedures are presented below. All individual pen manure samples were extracted with dilute $CaCl_2$ and Mehlich III, but none were extracted with $NaHCO_3$ solution. Three subsamples from each of the 36 individual pen manure samples were subjected to each of the two extraction procedures. Therefore, approximately 216 extractions were performed on wet manures from individual pens. The five wet composite manures were extracted using all three solutions. Four subsamples of each wet composite manure were subjected to each of the three extraction procedures. Therefore, approximately 60 extractions were conducted on wet composite manures. Similarly, the five air-dried composite manures (four subsamples for each) were extracted using all three extraction procedures. Therefore, approximately 60 extractions were performed on dry composite manures.

2.2.4.a. $CaCl_2$ Extraction of Manures

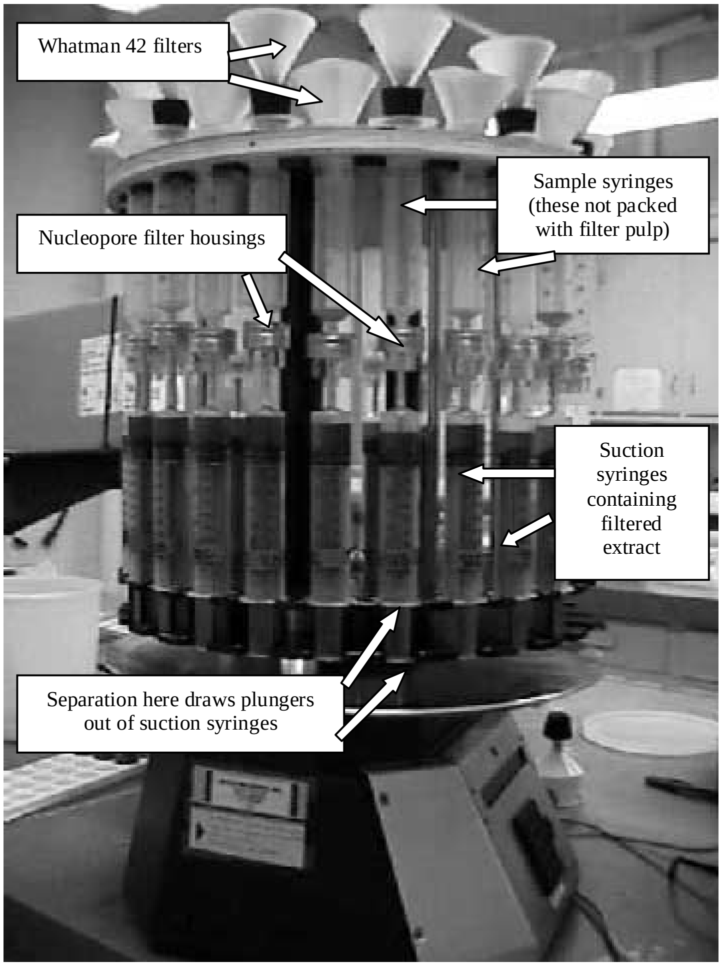
The following approach was taken in selecting all three extractants used in this study. A goal of the study was to subject both manures and soils amended with those manures to identical extraction procedures. Since standardized soil extraction procedures were available, while established manure extraction procedures were not, soil extractants were selected first. The same soil extraction procedures were then used for extracting manure samples. An extracting solution 0.01 M $CaCl_2$ was selected for evaluating the water solubility of P and Cu in manure-amended soils. As explained in the preceeding literature review, extraction with 0.01 M $CaCl_2$ is a standardized procedure for extracting water-soluble components of soils. Therefore, 0.01 M $CaCl_2$ was also used to evaluate the water solubility of manure P, Cu, and Zn. Since the $CaCl_2$ extraction was designed to evaluate water solubility of both manure and soil components, it will be referred to as water extraction from this point forward in this document.

The desired extractant:solid ratio for the $CaCl_2$ extraction was 10 mL 0.01 M $CaCl_2$:1 g of manure dry matter. Since most manure samples extracted were wet, the moisture entrained in the excreta was counted as part of the volume of extractant. Therefore, 0.02 M $CaCl_2$ was prepared and combined with manure at a ratio of 5 mL 0.02 M $CaCl_2$: 1 g manure dry matter. Distilled deionized water was then added as necessary to achieve a total water:solid ratio of 5 mL total water (added plus entrained):1 g of manure dry matter. The final result was the desired ratio of 10 mL 0.01 M $CaCl_2$:1 g of manure dry matter. This strategy of using a “double-strength” extracting solution to make up 50% of extractant volume and using water (including water entrained in manure) to make up the remaining 50% of extractant volume was adopted for all extractions in this study.

The actual water extraction procedure was based on the technique of Kuo (1996) and was conducted as follows. Manure (wet or air-dried, depending on the sample) was weighed into 100-mL plastic centrifuge tubes so as to provide 3 g of manure dry matter per tube. Sufficient distilled deionized water and 0.02 M $CaCl_2$ were added to the tubes to achieve the desired 10 mL 0.01 M $CaCl_2$:1 g manure dry matter ratio. Electronic pipettes were used to deliver the proper quantities of liquid by volume. Tubes were sealed with plastic caps and agitated on a reciprocal shaker for 1 h. Tubes were then centrifuged at 2500 rpm for 10 min. Supernatant liquid in the centrifuge tubes was decanted into syringes for filtration (one syringe per tube). The basic filtration system used for all extracts in the study consisted of 60-cm^3 syringes with Luerlok tips. Nucleopore filter housings (25-mm diameter) were fitted to the Luerlok tips of the syringes. Filter housings were loaded with 25-mm diameter Nucleopore filters with pore size of 0.45 μm. Non-reactive filter pulp (1 g) packed into each syringe provided pre-filtration prior to extract passage through the Nucleopore filter housing and the 0.45-μm membrane. Whenever possible, extracts were forced through this double filter system by simply inserting the syringe plunger and pressing down on the plunger by hand. However, extraction of raw manures generally produced extracts containing significant quantities of suspended material, even after centrifugation. Typically, filtration of such extracts was so slow that pressing them through the syringes by hand was prohibitively time-consuming. In such cases, a SampleTek vacuum extraction machine was used to draw the extracts through the syringe filters, as explained below.

The SampleTek vacuum extractor (Figure 1) creates a vacuum inside multiple syringes by means of a simple mechanical system that pulls the plungers out of the syringes at a controlled rate. These syringes in which vacuum is created are suction syringes. The suction syringes are attached with pieces of Tygon tubing a few cm in length to a second set of syringes (or other vessels) containing samples. The tubing creates an air-tight connection between the tips of the suction syringes and the tips of the sample syringes. In this project, the syringes packed with filter pulp and fitted with Nucleopore filter housings were used as sample syringes. Tygon tubing was used to connect the tips of the suction syringes to the outlets from the Nucleopore filter housings. As a result, when the device's extracting mechanism was activated, a vacuum was created in the suction tubes and liquid extract in the sample syringes was drawn through the filter pulp, through the Nuclopore filter housings, and finally into the suction syringes. For extracts filtered using the SampleTek vacuum extractor, an additional method of pre-filtration was used. Extracts were also gravity filtered through Whatman No. 42 filter paper while being poured into the sample syringes on the SampleTek extractor. All manure water extracts were filtered using the SampleTek Vacuum extractor in the manner described above. The quantity of suspended material in the extracts dictated the length of time required for filtration of samples. In most cases, the vacuum extractor was programmed to extract 50 mL over a 15-h period.

Figure 1: SampleTek Vacuum Extractor



After filtration, water extracts of individual pen manure samples were diluted either 25x or 50x with distilled deionized water. Samples of diluted extracts were refrigerated prior to analysis. Extracts were analyzed for total P, Cu, and Zn content using inductively coupled plasma (ICP) emission spectrometry. The instrument used was a SpectroFlame Modula Tabletop ICP. Individual pen water extracts were also analyzed colorimetrically for molybdate-reactive orthophosphate using the ascorbic acid method of Murphy & Riley (1962) as modified by Watanabe and Olsen (1965) and a Spectronic 21 D-V spectrophotometer with wavelength set at 880 nm. Filtered water extracts of both wet and dry composite manure samples were all diluted 25x with water. These composite manure extracts were analyzed for ICP-detectable total P, Cu, and Zn, but were not analyzed for molybdate-reactive orthophosphate.

2.2.4.b. Mehlich III Extraction of Manures

The Mehlich III extraction procedure was selected primarily for assessing plant-available P and metals in soils. Manure samples were extracted with Mehlich III in order to assess the acid-solubility of P and metals in the excreta. The traditional Mehlich III extracting solution has a pH of 2.0 and consists of 0.2 M acetic acid (CH_3COOH), 0.25 M ammonimum nitrate (NH_4NO_3), 0.015 M ammonium fluoride (NH_4F), 0.013 M nitric acid (HNO_3), and 0.001 M EDTA (ethylenediaminetetraacetic acid) (Mehlich, 1984). The desired extractant:solid ratio for the Mehlich III extraction was 10 mL Mehlich III:1 g manure dry matter. To account for the significant amount of moisture entrained in the wet manures subjected to extraction, the technique described previously of extracting with "double-strength" extractant plus water was used. Therefore, the Mehlich III solution prepared for this study contained 0.4 M CH_3COOH, 0.5 M NH_4NO_3, 0.03 M NH_4F, 0.026 M HNO_3, and 0.002 M EDTA. Manure to be extracted was mixed with this double-strength Mehlich III extractant so as to achieve a ratio of 5 mL double strength Mehlich III:1 g manure dry matter. Distilled deionized water was then added to the extraction tube to achieve a ratio of 5 mL water (added plus entrained):1 g manure dry matter. The final result was the desired Mehlich III:manure dry matter ratio.

The Mehlich III extraction procedure differed slightly from the water extraction procedure. Although the same extractant:solid ratio was used in both extractions, a quantity of manure equal to 4 g of dry matter was weighed into each Mehlich III extraction tube. After addition of extracting solution, Mehlich III extraction tubes were only shaken for 5 min prior to centrifugation and filtration of supernatant. Melich III manure extracts were filtered using the SampleTek Vacuum Extractor as described previously. Mehlich III extracts of individual pen manure samples were diluted 25x or 50x with distilled deionized water. Mehlich III extracts of both wet and dry composite manures were all diluted 25x with distilled deionized water. All Mehlich III samples were analyzed for total P, Cu, and Zn by ICP spectrometry. No Mehlich III samples were analyzed for molybdate-reactive orthophosphate.

2.2.4.c. Alkaline Olsen Extraction of Manures

The buffered alkaline solution known as the Olsen extractant was the third extracting solution used in the study. This extractant was chosen in order to assess the base solubility of the elements of interest in soils and manures. The traditional Olsen extractant consists of 0.5 M $NaHCO_3$ with a pH adjusted to 8.5 through addition of 1 M NaOH. The desired extractant:solid ratio for this alkaline extraction was 20 mL Olsen extractant:1 g manure dry matter (Olsen, 1954). The same approach described for the other extraction methods of mixing manure with "double-strength" extractant plus water was used for the Olsen extraction. Therefore, the alkaline Olsen solution prepared for this study consisted of 1 M $NaHCO_3$. Manure to be extracted was mixed with this double-strength Olsen extractant so as to achieve a ratio of 10 mL of 1 M $NaHCO_3$:1 g manure dry matter. Distilled deionized water was then added to the extraction tube to achieve a ratio of 10 mL water (added plus entrained):1 g manure dry matter. The final result was the desired ratio of 20 mL 0.5 M $NaHCO_3$:1 g manure dry matter.

The Olsen extraction procedure differed slightly from the other two extraction procedures. A quantity of manure equal to 3 g of dry matter was weighed into each Olsen extraction tube. After addition of extracting solution at a 20 mL extractant:1 g manure dry matter ratio, the Olsen extraction tubes were shaken for 30 min prior to centrifugation and filtration of supernatant. Olsen manure extracts were filtered using the SampleTek Vacuum Extractor as described previously. Olsen extraction was not conducted on any individual pen manure samples. Olsen extraction was performed on subsamples of wet and dry composite manures. The resulting alkaline extracts were acidified and diluted 25x prior to analysis. The extracts were acidified by adding to the dilution volumetric 1 mL of 1 M HNO_3 for each mL of Olsen extract added. The volumetric was then brought up to volume using distilled deionized water. The diluted samples were analyzed for total P, Cu, and Zn content by ICP spectrometry. The alkaline samples were neutralized in order to ensure compatability with the ICP instrument. Olsen manure extracts were not analyzed for molybdate-reactive orthophosphate.

2.2.4.d. Extraction of Feeds

All manures extracted in this study were produced by turkeys eating one of five feeds from the original feeding experiment. Subsamples of these five feeds were subjected to the same extraction procedures used on manure samples. This was done with the expectation that comparison of feed and manure extraction results would help to explain manure P, Cu, and Zn solubility patterns. Three subsamples of each of the five feeds was subjected to the each of the three extraction procedures. Therefore, a total of 45 feed extractions were performed. All feed extracts were filtered using the SampleTek Vacuum Extractor as described previously. Water extracts of feeds were not diluted prior to analysis. Mehlich III extracts of feeds were diluted 10x with distilled deionized water prior to analysis. Alkaline Olsen extracts of feeds were acidified with 1 M HNO_3 as described previously and diluted 10x prior to analysis. The total concentration of P, Cu, and Zn in the manure extracts was determined by ICP spectrometry.

2.2.4.e. Data Analysis

A total of 381 extractions were conducted on manure and feed samples. The total concentration of P, Cu, and Zn in each of the 381 manure and feed extracts was determined via ICP analysis. The total concentration on P, Cu, and Zn in all manures and feeds subjected to extraction was known. These numbers were used to calculate the percentage of total manure or feed P, Cu, and Zn solubilized in each of the 381 different extractions performed. The following equation was used in this calculation:

$$((A \times B \times C)/D) \times 100\% = E$$

where

A = mg L^{-1} element in extract or mg element per L of analyzed extract
B = dilution factor or mL analyzed extract per mL of extractant used
C = extractant:dry matter ratio or mL extractant used per g of dry matter extracted
D = mg kg^{-1} element in dry matter or mg element per kg of dry matter extracted
E = % of element in manure or feed solubilized by extractant

For the water extracts of individual pen manures, the equation above was used to determine the percentage of total manure P solubilized in orthophosphate form and organic form. The amount of organic P in these water extracts was calculated using the following equation:

$$F - G = H$$

where

F = mg L^{-1} total (ICP-detectable) dissolved P in extract
G = mg L^{-1} orthophosphate (molybdate-reactive) dissolved P in extract
H = mg L^{-1} organic dissolved P in extract.

The above calculations were preformed using Excel spreadsheets. All data and calculation results were then imported into SAS for statistical analysis. The individual pen manure extraction results, the wet and dry composite manure extraction results, and the feed extraction results were analyzed as three separate datasets. The data were subjected to analysis of variance (ANOVA) using the general linear model procedure (proc glm) of SAS. The principal tool used for comparing means was Duncan’s Multiple Range Test using the default significance level of alpha = 0.05.

2.3. RESULTS AND DISCUSSION

2.3.1. MANURE CHARACTERIZATION

2.3.1.a. Individual Pen Manure Samples

Results of analysis of manures collected from the 34 individual pens are presented in Table 4. Note that all results of manure analysis in this document are presented on an oven-dry-weight basis. The data are arranged into five groups by dietary treatment fed to the birds. For each analyzed parameter, the mean and sample standard deviation for the mean for all pens fed the same dietary treatment are provided. It should be noted that standard deviations for Zn means for the NPA, HAP, NPA+Phyt, and HAP+Phyt diets are much higher than for the NPA+P diet. This is because the NPA, HAP, and NPA+Phyt groups each contain one pen with unusually high total Zn content and the HAP+Phyt group contains two pens with unusually high total Zn content. It is sususpected that these five samples contained unusually high levels of Zn due to contamination from corroded metal excreta trays used to collect manures during the turkey feeding experiment. This issue of Zn contamination will be discussed in greater detail later in this section.

Table 4: Analysis of Manures Collected from Individual Pens

	Pen	Diet	Dry Matter	pH			mg kg^{-1}, dry basis	
			%		P	Ca	Zn	Cu
	Pen 10	NPA	17.8	4.58	0.828	0.783	303.8	23.8
	Pen 17	NPA	19.9	5.29	0.914	0.850	326.8	24.6
	Pen 28	NPA	16.1	4.78	0.945	0.883	250.3	29.3
	Pen 40	NPA	19.0	5.82	0.915	0.906	903.1	26.3
	Pen 58	NPA	20.2	5.81	0.966	0.839	296.0	32.3
	Pen 69	NPA	22.3	5.31	0.924	0.999	246.6	26.8
	Pen 79	NPA	19.3	4.92	0.928	0.929	253.9	34.2
	Pen 91	NPA	17.7	4.56	0.940	0.853	299.4	38.2
Mean		NPA	19.0	5.13	0.920	0.880	360.0	29.4
SD†			1.9	0.51	0.041	0.066	221.4	5.1
	Pen 09	HAP	18.2	4.70	0.886	0.824	276.5	31.3
	Pen 23	HAP	19.5	5.82	0.893	0.736	937.8	27.5
	Pen 35	HAP	18.9	5.56	0.894	0.828	257.3	30.9
	Pen 47	HAP	21.2	5.38	0.698	0.700	247.5	23.8
	Pen 55	HAP	21.6	5.80	0.842	0.769	193.1	31.5
	Pen 63	HAP	20.5	4.92	0.840	0.672	244.7	28.5
	Pen 74	HAP	17.3	5.57	0.928	0.708	307.8	35.5

	Pen 95	HAP	18.3	4.65	0.915	0.869	283.0	43.9
Mean		HAP	19.4	5.30	0.862	0.763	343.5	31.6
SD†			1.5	0.48	0.073	0.071	242.5	6.0
	Pen 46	NPA+P	21.8	5.31	1.387	1.463	264.8	28.1
	Pen 57	NPA+P	27.5	5.96	1.450	1.541	304.0	25.8
	Pen 66	NPA+P	21.3	5.39	1.492	1.763	216.6	39.0
	Pen 80	NPA+P	19.9	5.32	1.476	1.652	236.3	36.0
	Pen 87	NPA+P	21.7	5.03	1.495	1.605	296.9	22.9
Mean		NPA+P	22.5	5.4	1.460	1.605	263.7	30.3
SD†			2.9	0.3	0.045	0.113	37.7	6.9
	Pen 37	NPA+Phyt	19.3	4.73	0.857	0.760	241.7	30.1
	Pen 52	NPA+Phyt	21.9	5.48	0.841	0.643	207.4	29.2
	Pen 64	NPA+Phyt	17.6	4.98	0.961	0.770	1236.5	32.7
	Pen 81	NPA+Phyt	20.2	4.65	0.788	0.686	259.4	28.5
	Pen 92	NPA+Phyt	19.2	4.95	0.962	0.844	277.9	30.8
Mean		NPA+Phyt	19.6	5.0	0.882	0.741	444.6	30.3
SD†			1.6	0.3	0.077	0.078	443.4	1.6
	Pen 07	HAP+Phyt	20.2	4.94	0.814	0.636	183.8	32.5
	Pen 16	HAP+Phyt	21.6	5.40	0.921	0.666	256.0	30.1
	Pen 26	HAP+Phyt	20.6	5.71	0.775	0.918	230.2	27.9
	Pen 39	HAP+Phyt	20.2	5.40	0.829	0.737	744.0	27.6
	Pen 53	HAP+Phyt	22.8	5.42	0.779	0.638	195.8	28.6
	Pen 61	HAP+Phyt	21.6	5.29	0.889	0.625	262.9	31.4
	Pen 73	HAP+Phyt	21.7	5.36	0.861	0.643	653.5	29.2
	Pen 88	HAP+Phyt	19.9	5.01	0.886	0.719	212.2	28.3
Mean		HAP+Phyt	21.1	5.32	0.844	0.698	342.3	29.4
SD†			1.0	0.24	0.054	0.098	223.0	1.8

†SD = Sample standard deviation

For the reader’s convenience, treatment means for individual pen manure sample analyses from Table 4 are reprinted in Table 5. As demonstrated in Table 5, the mean total P content of the individual pen manures varied by dietary treatment. As expected, the NPA+P manure derived from the conventional commercial diet contained the greatest amount of total P at 1.46%. The NPA+Phyt manure derived from the phytase-amended diet contained only 0.88% total P. Therefore, replacing conventional defluorinated phosphate in the diet with the phytase enzyme caused a drop in total P excretion of approximately 40%. Achieving such a drop in manure P excretion is one of the principal goals of using phytase. However, in a typical commercial setting, the conversion to phytase feeding would be expected to produce a smaller decrease in total P excretion of 25% to 35% (Duval, 1996). This is because a commercial operator would probably formulate a phytase-amended diet with more defluorinated phosphate than was used in this experiment’s NPA+Phyt diet, as explained below.

Table 5: Analysis of Manures from Individual Pens, Means for Treatments

Diet	Dry Matter	pH	%, dry basis		$\mathrm{mg\ kg^{-1}}$, dry basis	
	%		P	Ca	Zn	Cu
NPA	19.0	5.13	0.920	0.880	360.0	29.4
HAP	19.4	5.30	0.862	0.763	343.5	31.6
NPA+P	22.5	5.40	1.460	1.605	263.7	30.3
NPA+Phyt	19.6	4.96	0.882	0.741	444.6	30.3
HAP+Phyt	21.1	5.32	0.844	0.698	342.3	29.4

As shown previously in Table 2, total nonphytate P represented 0.492% of the NPA+P diet, which was near the NRC-recommended level of 0.5 to 0.6% (feed concentrations in this paragraph are “as fed”). This 0.492% total nonphytate P in the NPA+P diet consisted of 0.345% supplemental inorganic P and 0.147% plant-derived P. Total nonphytate P supplied by the NPA+Phyt diet was estimated at only 0.382% and consisted of 0.135% supplemental inorganic P and 0.247% plant-derived P (0.147% native nonphytate P in grains, plus 0.100% phytate P converted to nonphytate forms by the 600 units phytase enzyme per kg feed). A commercial producer would probably boost this 0.382% total nonphytate P supplied by the phytase-amended feed in order to approach NRC-recommended levels. This would most likely be achieved by increasing inorganic P additions to the diet (Duval, 1996). Although supplemental inorganic P is nonphytate in form and is traditionally considered available to birds, only an estimated 40 to 50% of inorganic P in poultry diets is actually utilized (Kornegay, 1998). This accounts in part for the inefficiency of dietary P use and the high P excretion rates associated with the NPA+P diet. Therefore, if inorganic P levels in the NPA+Phyt diet were to be increased, one would anticipate a drop in total P use efficiency and an increase in total P excretion. In conclusion, the NPA+P and NPA+Phyt diets used in this experiment maximized the differences in feed composition and P excretion that one would expect to observe between conventional and phytase-amended diets under commercial conditions.

The NPA, HAP, and HAP+Phyt manures were similar to the NPA+Phyt manure in total P content (Table 5). The dramatic difference in manure P content between these four manures and the NPA+P manures can be related directly to total feed P contents. The NPA+P diet was formulated to contain 0.761% total P (as fed) while the other four diets were formulated to contain 0.551 or 0.556% total P (as fed) (Table 2). Increased P input into the birds as well as the inefficiency of P use associated with inorganic forms of dietary P explain the high P content of the NPA+P manure. The moderate differences in total P content between the NPA, HAP, NPA+Phyt, and HAP+Phyt manures can be also be related to feed P composition. Generally, as the percentage of total feed P in plant-derived nonphytate forms in these four diets increased, the amount of P excreted decreased. For example, comparing among these four diets only, the NPA feed was formulated to supply the least amount of nonphytate plant-derived P (0.147%, as fed) and it produced the manure with the highest total P content (0.92%). The HAP+Phyt diet was formulated to supply the highest amount of nonphytate plant-derived P (0.185% + 0.100% due to phytate conversion = 0.285%, as fed) and it produced the manure with the lowest total P content (0.84%). The HAP and NPA+P feeds were formulated to provide intermediate levels of nonphytate plant-derived P and they resulted in intermediate manure P contents.

There was a dramatic difference in total Ca content between the NPA+P manure (1.61%) and the other four manures (0.70% to 0.88%) (Table 5). This can be related to feed Ca content, which was 1.057% (as fed) for the NPA+P diet and between 0.633% and 0.667% (as fed) for the other four diets. Note that an important source of dietary Ca was the defluorinated phosphate inorganic P source. In general, a constant Ca:P ratio of 1.2 or 1.3:1 is desirable in poultry feed (Ensminger et al., 1990). For this reason, any increase in dietary P content (such as might be expected for the NPA+Phyt diet under commercial conditions) would likely mean an increase in dietary Ca content as well.

Total manure Cu contents were similar for all five manure types, ranging from 29.4 mg kg^{-1} to 31.6 mg kg^{-1}. This was expected, since all five diets were fomulated to contain 12.5 mg Cu kg^{-1} (as fed). It was also anticipated that all five manures would contain similar amounts of total Zn, since all diets were formulated to contain 95.0 mg kg^{-1} Zn (as fed). However, total Zn contents of the five manures showed notable differences and ranged from 263.7 mg kg^{-1} to 444.6 mg kg^{-1}. As stated previously, Zn contamination of the manure was suspected for five of the 34 pens used in the study. After exclusion of all data associated with the five pens suspected of Zn contamination (Table 6), mean Zn contents for the five manure types become much more consistent, ranging from 223.5 to 282.4 mg kg^{-1}. The large and variable standard deviations for Zn means of 37.7 to 443.4 mg kg^{-1} seen in Table 4 are replaced by standard deviations ranging only from 30.0 to 37.7 mg kg^{-1}. For the reader's convenience, the means for individual pen manure sample analyses from Table 6 are reprinted in Table 7.

Table 6: Analysis of Manures Collected from Selected Individual Pens

	Pen	Diet	Dry Matter	pH	%, dry basis		mg kg^{-1}, dry basis	
			%		P	Ca	Zn	Cu
	Pen 10	NPA	17.8	4.58	0.828	0.783	303.8	23.8
	Pen 17	NPA	19.9	5.29	0.914	0.850	326.8	24.6
	Pen 28	NPA	16.1	4.78	0.945	0.883	250.3	29.3
	Pen 58	NPA	20.2	5.81	0.966	0.839	296.0	32.3
	Pen 69	NPA	22.3	5.31	0.924	0.999	246.6	26.8
	Pen 79	NPA	19.3	4.92	0.928	0.929	253.9	34.2
	Pen 91	NPA	17.7	4.56	0.940	0.853	299.4	38.2
Mean		NPA	19.0	5.04	0.920	0.876	282.4	29.9
SD†			2.0	0.46	0.044	0.070	31.7	5.3
	Pen 09	HAP	18.2	4.70	0.886	0.824	276.5	31.3
	Pen 35	HAP	18.9	5.56	0.894	0.828	257.3	30.9
	Pen 47	HAP	21.2	5.38	0.698	0.700	247.5	23.8

	Pen 55	HAP	21.6	5.80	0.842	0.769	193.1	31.5
	Pen 63	HAP	20.5	4.92	0.840	0.672	244.7	28.5
	Pen 74	HAP	17.3	5.57	0.928	0.708	307.8	35.5
	Pen 95	HAP	18.3	4.65	0.915	0.869	283.0	43.9
Mean		HAP	19.4	5.23	0.858	0.767	258.6	32.2
SD†			1.7	0.46	0.078	0.076	36.4	6.3
	Pen 46	NPA+P	21.8	5.31	1.387	1.463	264.8	28.1
	Pen 57	NPA+P	27.5	5.96	1.450	1.541	304.0	25.8
	Pen 66	NPA+P	21.3	5.39	1.492	1.763	216.6	39.0
	Pen 80	NPA+P	19.9	5.32	1.476	1.652	236.3	36.0
	Pen 87	NPA+P	21.7	5.03	1.495	1.605	296.9	22.9
Mean		NPA+P	22.5	5.4	1.460	1.605	263.7	30.3
SD†			2.9	0.3	0.045	0.113	37.7	6.9
	Pen 37	NPA+Phyt	19.3	4.73	0.857	0.760	241.7	30.1
	Pen 52	NPA+Phyt	21.9	5.48	0.841	0.643	207.4	29.2
	Pen 81	NPA+Phyt	20.2	4.65	0.788	0.686	259.4	28.5
	Pen 92	NPA+Phyt	19.2	4.95	0.962	0.844	277.9	30.8
Mean		NPA+Phyt	20.1	5.0	0.862	0.733	246.6	29.6
SD†			1.2	0.4	0.073	0.088	30.0	1.0
	Pen 07	HAP+Phyt	20.2	4.94	0.814	0.636	183.8	32.5
	Pen 16	HAP+Phyt	21.6	5.40	0.921	0.666	256.0	30.1
	Pen 26	HAP+Phyt	20.6	5.71	0.775	0.918	230.2	27.9
	Pen 53	HAP+Phyt	22.8	5.42	0.779	0.638	195.8	28.6
	Pen 61	HAP+Phyt	21.6	5.29	0.889	0.625	262.9	31.4
	Pen 88	HAP+Phyt	19.9	5.01	0.886	0.719	212.2	28.3
Mean		HAP+Phyt	21.1	5.30	0.844	0.700	223.5	29.8
SD†			1.1	0.29	0.063	0.112	32.0	1.9

†SD = Sample standard deviation

It should be noted that excluding all data associated with the five pens suspected of Zn contamination did not notably affect means or standard deviations for pH or dry matter or for P, Ca, or Cu contents (compare Tables 4 and 5 versus Tables 6 and 7). This suggests that Zn contamination did not impact other manure characteristics important to this study. Therefore, analyses related to pH, P, Ca, or Cu in individual pen manures will generally include data from all 34 pens. Analyses related to Zn in individual pen manures will focus primarily on data from those 29 pens with no evidence of Zn contamination.

Table 7: Analysis of Manures from Select Individual Pens, Means for Treatments

Diet	Dry Matter	pH	%, dry basis		$mg\ kg^{-1}$, dry basis	
	%		P	Ca	Zn	Cu
NPA	19.0	5.04	0.920	0.876	282.4	29.9
HAP	19.4	5.23	0.858	0.767	258.6	32.2
NPA+P	22.5	5.40	1.460	1.605	263.7	30.3
NPA+Phyt	20.1	4.95	0.862	0.733	246.6	29.6
HAP+Phyt	21.1	5.30	0.844	0.700	223.5	29.8

2.3.1.b. Composite Manure Samples

Results of analysis of wet composite manure samples are presented in Table 8. As explained above, the wet composite manures were prepared by grouping all 34 individual pen manure samples by dietary treatment and then combining subsamples to form five composite manures. Since the composite manures were composed of subsamples from all 34 individual pens, it is not surprising that the wet composite manure analysis results (Table 8) are relatively similar to the means from analysis of all 34 individual pen manures (Table 5). The wet composites were the only manure samples analyzed for N content. The total N contents of the five different manures were relatively similar, ranging from 7.7 to 8.5%.

Table 8: Analysis of Wet Composite Manures

Diet	Dry Matter	pH	%, dry basis			$mg\ kg^{-1}$, dry basis	
	%		P	Ca	N	Zn	Cu
NPA	18.1	5.16	0.835	0.944	8.4	422.7	30.5
HAP	18.2	5.24	0.782	0.746	8.5	393.8	28.6
NPA+P	21.1	5.10	1.328	1.546	7.7	293.1	30.7
NPA+Phyt	18.9	4.84	0.793	0.792	7.8	483.0	30.7
HAP+Phyt	19.7	5.38	0.757	0.692	8.2	413.0	29.6

Results of analysis of the dried composite manures are presented in Table 9. Incubation of composite manures for 4 d in an oven maintained at 40 °C (“air-drying”) increased manure dry matter contents to roughly 90%. Air-drying also tended to increase the levels of P, Ca, and Zn measured in the composite manures. It is suggested that microbial respiration or volitalization resulted in minor losses of carbon and other components from the composite manure. This dry matter loss may explain why oven-dry-weight basis concentrations in the air-dried manures tend to be higher than oven-dry-weight basis concentrations in the wet manures.

Table 9: Analysis of Dried Composite Manures

Diet	Dry Matter	pH	%, dry basis		mg kg^{-1}, dry basis	
	%		P	Ca	Zn	Cu
NPA	88.6	4.86	0.896	0.904	410.0	32.4
HAP	86.1	4.87	0.923	0.778	403.3	34.9
NPA+P	91.9	5.45	1.524	1.707	304.0	33.0
NPA+Phyt	88.0	5.64	0.900	0.818	506.1	34.0
HAP+Phyt	89.2	6.04	0.876	0.758	492.9	33.4

2.3.2. FEED P EXTRACTION

The first extraction data presented are the results of P extraction from feed samples. Although the primary goal of this study was evaluation of manure P solubility, an initial review of feed extraction results provides a useful starting point for later interpretation of manure extraction data. This is because detailed information is available about the forms of P in the feeds. This information can be used not only to explain feed P solubility results, but also to gain insight about which forms of P were soluble in the three different extractants.

2.3.2.a. $CaCl_2$ Extraction of Feed P

Results from extraction of feeds with 0.01 M $CaCl_2$ are presented in Table 10. The amounts of total and soluble P in the five feeds are shown, as well as percentages of total P that were soluble. Three subsamples of feed were extracted for each of the five diets. The five diets all differed statistically from each other in percentage of total feed P soluble in the water extractant. The observed differences in feed P water solubility can be related to differences in feed composition presented in Table 2. It is suggested that the forms and amounts of P in the feeds during the water extraction were most similar to the “Predicted after Phytase Activity” values in Table 2. This is because the water extraction involved shaking a subsample of feed in 0.01 M $CaCl_2$ at room temperature for 1 h, which provided appropriate moisture and temperature conditions for activation of the phytase enzyme in the NPA+Phyt and HAP+Phyt feeds (Coelho, 1996b). Therefore, the NPA+P feed contained the lowest percentage of total feed P in plant-derived nonphytate form (19.3%) and the HAP+Phyt feed the highest (51.2%). When these percentages of total feed P in plant-derived nonphytate form from Table 2 are plotted versus the percentages of total feed P soluble in water from Table 10, a linear relationship with an r^2 value of 0.96 is revealed, as shown in Figure 2.

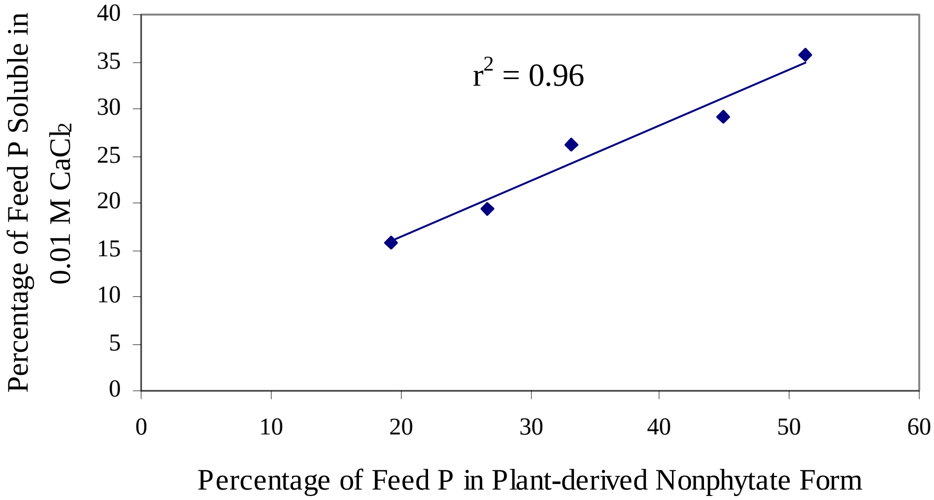
Table 10: Water Solubility of P in Feeds

Diet	Total P	Soluble P	
	mg kg^{-1}	mg kg^{-1}	% of total
HAP+Phyt	6210	2216	35.7 a†
NPA+Phyt	6110	1778	29.1 b
HAP	6170	1617	26.2 c
NPA	6090	1182	19.4 d
NPA+P	8270	1310	15.8 e

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

The direct relationship between the percentage of feed P in plant-derived nonphytate form and the water solublility of feed P can be readily explained. The three principal forms of P in the feeds were phytate P derived from plants, nonphytate inorganic P derived from defluorinated calcium phosphate, and plant-derived nonphytate P (Table 2). Since phytate P is considered nutritionally unavailable to poultry and is known to form highly insoluble complexes, it is not surprising that this form of P was not soluble in the water extractant (Coelho, 1996a). Feed P derived from defluorinated calcium phosphate is nonphytate in form and is therefore considered digestible. However, it is also logical that this form of P was relatively insoluble in the water extractant. Calcium phosphate solubility is favored by acidic conditions, which explains why little of it dissolved in the 0.01 M $CaCl_2$ solution of pH 5.6 (Li and Shuman, 1996a). Finally, since plant-derived nonphytate P is considered highly digestible, it is not surprising that this form of feed P was the one most readily dissolved in the water extract. These results suggest that the 0.01 M $CaCl_2$ solution was a relatively effective extractant of plant-derived nonphytate P and relatively poor extractant of phytate and calcium phosphate P.

Figure 2: Water Solubility of Feed P versus Percentage of Feed P in Plant-derived Nonphytate Form



2.3.2.b. Mehlich III Extraction of Feed P

The preceding discussion strongly suggests that phytate and inorganic calcium phosphate were the principal compounds controlling the water solubility of feed P. One would expect both of these forms of P to be more soluble in Mehlich III solution than in 0.01 M $CaCl_2$, for the following reasons. The insolubility of the phytate molecule is related to the manner in which its anionic phosphate functional groups readily chelate metals and other multivalent cations to form insoluble complexes (Coelho, 1996a). In the pH 2.0 Mehlich III solution, one would expect the chelating ability of the phytate molecule to be reduced due to a tendency toward hydrogenation of phosphate groups. One would also expect the formation of insoluble phytate complexes to be further reduced through complexation of multivalent cations by the Mehlich III solution's EDTA component. The low pH as well as the Ca-chelating properties of the Mehlich III solution would also be expected to enhance dissolution of calcium phosphate compounds.

The results of feed extraction with Mehlich III are presented in Table 11. As anticipated, certain key differences in feed P solubility observed in the water extracts were not observed in the Mehlich III extracts. First, recall that the NPA and NPA+P feeds differed only in that the latter was formulated to contain more than twice the inorganic P found in the former (Table 2). The similarity in Mehlich III P solubility between these two feeds strongly suggests that inorganic calcium phosphate feed components were soluble in the Mehlich III extractant. A second key observation is that Mehlich III solubility of feed P could not be related to phytate P and/or plant-derived nonphytate P contents of the feeds. For example, the NPA+Phyt feed differed from the HAP+Phyt feed only in that the latter contained less phytate P and more plant-derived nonphytate P than the former (Table 2). Note that this holds true regardless of the degree to which phytase enzyme was activated during the Mehlich III extraction. The P in the NPA+Phyt feed was significantly more Mehlich III soluble than was the P in the HAP+Phyt feed (71.2% for NPA+Phyt versus 66.9% for HAP+Phyt). This suggests that Mehlich III solubility of feed P was not enhanced by increasing plant-derived nonphytate P content, in contrast with

$CaCl_2$ solubility. Other comparisons among the feeds reveal this same pattern. Note also that the Mehlich III solution was a more effective overall extractant of feed P than was 0.01 M $CaCl_2$. All of these results suggest that the Mehlich III solution was an effective extractant of both calcium phosphate and phytate forms of P.

Table 11: Mehlich III Solubility of P in Feeds

Diet	Total P mg kg^{-1}	Soluble P	
		mg kg^{-1}	% of total
NPA+Phyt	6110	4352	71.2 a†
NPA	6090	4289	70.4 ab
NPA+P	8270	5823	70.4 ab
HAP	6170	4212	68.3 bc
HAP+Phyt	6210	4152	66.9 c

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

2.3.2.c. Olsen Extraction of Feed P

The results of feed extraction with alkaline Olsen solution are shown in Table 12. The five feeds all differed significantly from each other in Olsen P solubility. Extraction with the pH 8.5 $NaHCO_3$ solution produced feed P solubility results similar to those obtained by water extraction, with only one difference in P solubility ranking among the diets observed between Tables 10 and 12. It is therefore suggested that similar mechanisms controlled water and Olsen solubility of feed P.

Table 12: Olsen Solubility of P in Feeds

Diet	Total P mg kg^{-1}	Soluble P	
		mg kg^{-1}	% of total
HAP+Phyt	6210	1949	31.4 a†
HAP	6170	1593	25.8 b
NPA+Phyt	6110	1455	23.8 c
NPA	6090	1153	18.9 d
NPA+P	8270	1209	14.6 e

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

Olsen solubility of feed P was not directly proportional to the percentage of feed P in plant-derived nonphytate form, but $CaCl_2$ solubility of feed P was, as shown in Figure 2. One factor contributing to this difference between Olsen and $CaCl_2$ solubility patterns may be variation in the degree to which the two extraction procedures triggered phytase enzyme activity. Phytase enzyme in the NPA+Phyt and HAP+Phyt feeds was probably fully activated during the water extraction. Phytase activity is significantly reduced by alkaline conditions and was probably minimal during the Olsen extraction (Coelho, 1996a). Therefore, at the end of the Olsen extraction, the forms and amounts of P in the feeds were probably more similar to those “Prior to Phytase Activity” in Table 2 than those “Predicted after Phytase Activity.” This may account for some of the differences between the water and Olsen solubility results. The HAP+Phyt and HAP feeds contained the most plant-derived nonphytate P in the absence of phytase activity and these feeds yielded the most P to the alkaline extractant. The NPA+Phyt and NPA feeds contained lower levels of plant-derived nonphytate P in the absence of phytase activity and these feeds yielded less P to the alkaline extractant. Note that some phytase activity may have occurred during the alkaline extraction, which would explain why Olsen P solubility was greater for HAP+Phyt than HAP and greater for NPA+Phyt than NPA.

2.3.2.d. Summary

The feed P extraction results provide useful insight about the forms of P that were soluble in the three different extractants. The results suggest that both the water and Olsen solutions were relatively poor extractants of P in calcium phosphate form, while the Mehlich III solution was much more effective at solubilizing this form of P. The results also suggest that the 0.01 M $CaCl_2$ extraction procedure was a reliable method for distinguishing between plant-derived nonphytate P and phytate P, while the Mehlich III extractant was not. These concepts will be used to help explain manure P solubility patterns.

2.3.3. MANURE P EXTRACTION

2.3.3.a. $CaCl_2$ Extraction of Manure P

<u>Individual Pen Manures</u>

Water extracts of wet manures from individual pens were analyzed for both ICP-detectable (total) dissolved P and molybdate-reactive orthophosphate (inorganic) dissolved P. For each extract, the difference between total dissolved P and orthophosphate dissolved P was considered to be organic dissolved P. The results of ICP (total) P analysis conducted on water extracts of individual pen manures are summarized in Table 13. All individual pen manures were extracted wet (i.e., at the point of excretion). For each manure type, total manure P, total soluble manure P, and percentage of total manure P that was soluble are presented. Each value shown for the NPA, HAP, and HAP+Phyt manures is a mean for extraction data from eight replicate pens, three subsamples extracted per pen. Each value presented for the NPA+P and NPA+Phyt manures is a mean for extraction data from five replicate pens, three subsamples extracted per pen.

The HAP+Phyt, HAP, and NPA+Phyt manures contained the most water-soluble form of P (84.3%, 79.3%, and 78.5% of total manure P solubilized, respectively). These three manures did not differ statistically from each other in P solubility percentage. The NPA manure contained P of intermediate water solubility (69.1%) and differed significantly from the four other manures. The NPA+P manure was also statistically different from all other manures and contained the least soluble form of P, with only 51.8% of total manure P dissolving in water. Note that, despite its low solubility percentage, the NPA+P manure released the greatest overall quantity of P to the $CaCl_2$ extractant on an equal-manure-weight basis (7568 mg P per kg manure).

Table 13: Water Solubility of Total (ICP-detectable) P in Manures from Individual Pens

Manure Type	Total P	Soluble P	
	mg kg^{-1}	mg kg^{-1}	% of total
HAP+Phyt	8443	7118	84.3 a†
HAP	8620	6836	79.3 a
NPA+Phyt	8822	6929	78.5 a
NPA	9197	6352	69.1 b
NPA+P	14604	7568	51.8 c

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

The results of orthophosphate analysis conducted on the water extracts of individual pen manures are summarized in Table 14. The ortho P solubility pattern among the five manure types was similar to the total P solubility pattern of Table 13. Statistically as well as numercially, the HAP+Phyt manure contained the greatest percentage of manure P solubilized to orthophosphate form by water (75.0%). The NPA+Phyt and HAP manures did not differ statistically in percentage of total manure P solubilized to orthophosphate form (67.9% and 67.5%, respectively). The NPA manure contained P of intermediate solubility, with 59.5% of total manure P dissolving into orthophosphate form in 0.01 M $CaCl_2$. Finally, the NPA+P manure contained the least soluble P, with only 43.6% of total manure P dissolving in $CaCl_2$ into orthophosphate form. Both the NPA and NPA+P manures differed statistically in ortho P water solubility from all other manures. Note that the NPA+P manure still released the greatest overall quantity of orthophosphate (6366 mg ortho P per kg manure).

Table 14: Water Solubility of Orthophosphate (Molybdate-reactive) P in Manures from Individual Pens

Manure Type	Total P	Soluble P	
	mg kg^{-1}	mg kg^{-1}	% of total
HAP+Phyt	8443	6330	75.0 a†
NPA+Phyt	8822	5988	67.9 b
HAP	8620	5818	67.5 b
NPA	9197	5468	59.5 c
NPA+P	14604	6366	43.6 d

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

Results of organic P analysis of the $CaCl_2$ extracts of individual pen manures are summarized in Table 15. The organic P solubility pattern obtained was very different from the total P and ortho P solubility patterns observed in Tables 13 and 14. There were no statistically significant differences in organic P solubililty between any of the five manures. For the HAP manure, 11.8% of total manure P dissolved into organic forms in 0.01 M $CaCl_2$. The percentages for NPA+Phyt, NPA, and HAP+Phyt were 10.7%, 9.6%, and 9.3%, respectively. Numerically, the NPA+P manure presented the lowest organic P solubility percentage, with 8.2% of total manure P dissolved in $CaCl_2$ in organic forms.

Table 15: Water Solubility of Organic P in Manures from Individual Pens

Manure Type	Total P	Soluble P	
	mg kg^{-1}	mg kg^{-1}	% of total
HAP	8443	997	11.8 a†
NPA+Phyt	8822	941	10.7 a
NPA	8620	828	9.6 a
HAP+Phyt	9197	858	9.3 a
NPA+P	14604	1201	8.2 a

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

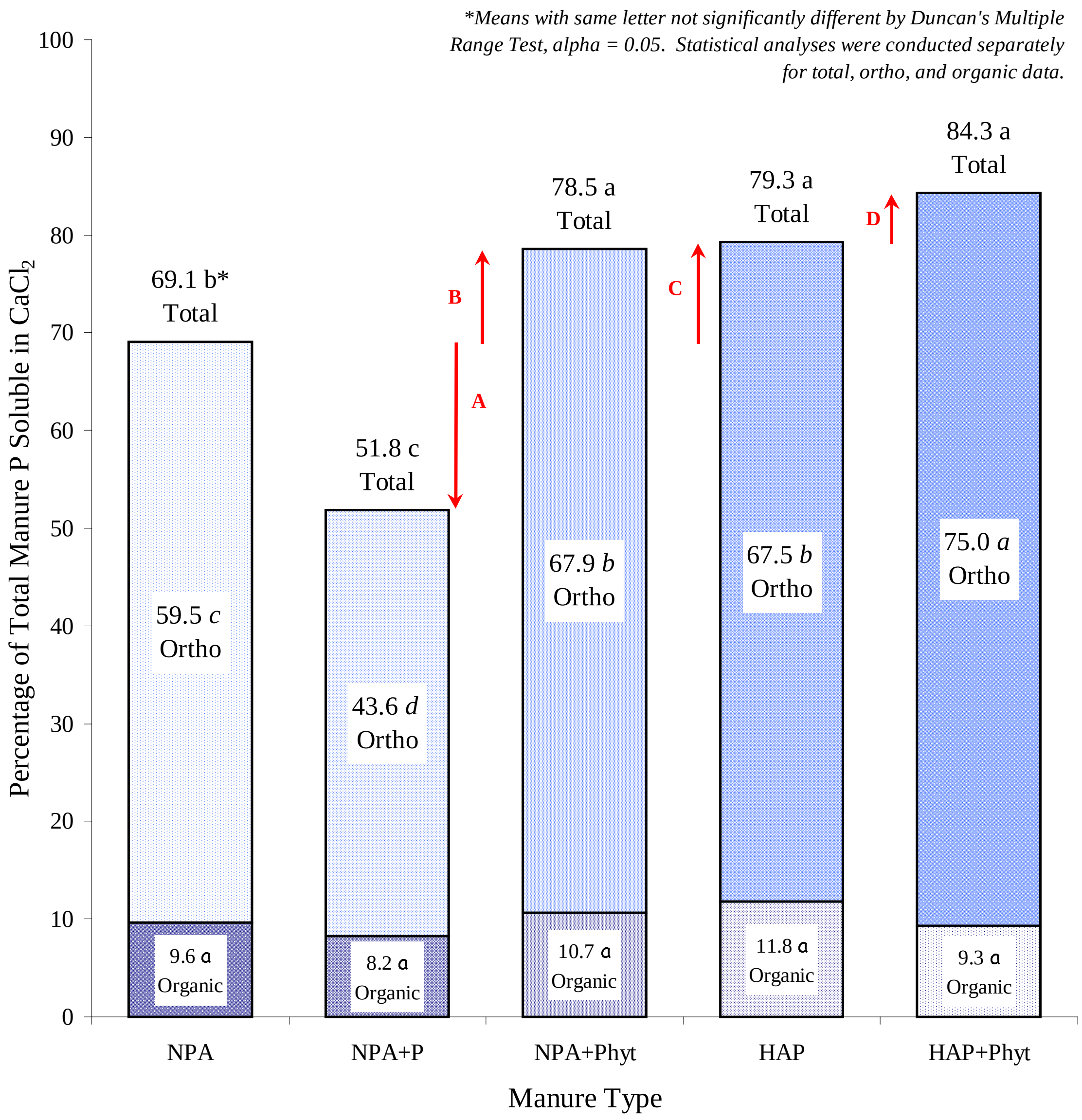
Data from Tables 13, 14, and 15 are combined in Figure 3 to present in graphic form all P solubility results from $CaCl_2$ extraction of individual pen manure samples. Figure 3 will be used as the main point of reference for the interpretative discussion that follows. The first issue to consider is the difference in P water solubility between the NPA+P and NPA+Phyt manures. In this experiment, switching from the conventional NPA+P diet to the alternative NPA+Phyt feed resulted in a statistically significant 1.52-fold increase in overall water solubility of manure P at the point of excretion (51.8% for NPA versus 78.5% for NPA+Phyt). This difference in overall solubility of P is graphically represented by the combined length of line segments A and B in Figure 3. The percentage of total manure P that was solubilized into dissolved organic form was not statistically different for the two manures (8.2% for NPA+P versus 10.7% for NPA+Phyt). The percentage of total manure P that was solubilized into dissolved orthophosphate form differed significantly between the two manures (43.6% for NPA+P versus 67.9% for NPA+Phyt) and accounted for most of the difference in overall P water solubility.

The NPA manure derived from the negative control dietary treatment provides a key baseline against which other manures can be compared. Recall that the NPA and NPA+P diets differed only in that the NPA+P feed was formulated to contain an additional 0.21% supplemental inorganic P (as fed). Adding calcium phosphate to the baseline NPA diet caused water solubility of manure P to drop by a factor of 0.75 (69.1% for NPA versus 51.8% for NPA+P). This decrease is represented graphically by line segment A in Figure 3. The NPA and NPA+P manures did not differ significantly in the percentage of total P dissolved into organic forms. Therefore, differences in overall P solubility between the two manures were related to differences in ortho P solubility. The NPA and NPA+Phyt diets differed only in that 600 units of phytase enzyme per kg of feed was added to the latter diet. The phytase addition caused a 1.14-fold increase in manure P solubility (69.1% for NPA versus 78.5% for NPA+Phyt). This phytase-induced increase in manure P solubility is represented by line segment B in Figure 3. The NPA and NPA+Phyt manures did not differ significantly in the percentage of total P dissolved into organic forms. Differences in overall P solubility between these two manures therefore were related to differences in ortho P solubility. Comparisons involving the baseline NPA manure therefore suggest that two mechanisms contributed to differences in P water solubility between the NPA+P and NPA+Phyt manures: a significant depression in manure P water solubility caused by the supplemental defluorinated phosphate in the NPA+P diet (line segment A) and a less important but statistically significant increase in manure P water solubility caused by the supplemental phytase in the NPA+Phyt diet (line segment B).

Further comparisons show the effects of dietary HAP corn on manure P solubility. Recall that the NPA and HAP feeds differed only in the type of corn used in their formulation. Substituting HAP corn for NPA corn resulted in a 1.15-fold increase in the water solubility of manure P (69.1% for NPA versus 79.3% for HAP), represented graphically in Figure 3 by line segment C. Comparison of the HAP and HAP+Phyt manures shows that phytase addition to the HAP diet caused a 1.06-fold increase in manure P water solubility. This phytase-induced solubility increase is represented by line segment D. The combined effect of HAP corn substitution and phytase enzyme addition to the baseline NPA diet resulted in a significant increase in manure P solubility in water (69.1% for NPA versus 84.3% for HAP+Phyt). Note that phytase addition to the baseline NPA diet produced a greater increase in manure P water solubility (line segment B) than did phytase addition to the HAP diet (line segment D). Therefore, the solubility increase produced by feeding HAP corn plus phytase was not an additive effect. Again, the percentage of total manure P solubilized in organic forms did not differ between the NPA, HAP, and

HAP+Phyt manures. Differences in overall manure P solubility between these manures was related to differences in orthophosphate P water solubility.

Figure 3: Comparison of Total, Orthophosphate, and Organic Water-soluble P in Manures from Individual Pens



What underlying mechanisms produced the differences in water solubility of P between manures derived from the five different diets? The P solubility pattern illustrated in Figure 3 is strikingly similar to the pattern obtained by extracting the five feeds with water. These similarities between feed and manure $CaCl_2$ extraction results suggest that certain common mechanisms controlled P water solubility in both materials. It is proposed that one such mechanism was a reduction in P solubility associated with increasing calcium phosphate levels in manure. The NPA+P feed contained almost twice as much defluorinated calcium phosphate as any of the other diets (Table 2). As explained previously, poultry typically do not digest all calcium phosphate components of their feeds, especially as the amount of inorganic P fed increases (Kornegay, 1998). This supports the hypothesis that the turkey manure derived from the NPA+P feed was the manure containing the largest proportion of P in calcium phosphate form. A key question tied to this hypothesis involves the extent to which the original calcium phosphate compounds in the NPA+P feed passed through the turkeys unchanged. One would expect at least some solubilization of feed-derived calcium phosphate compounds during passage through the turkey's digestive system. However, near the end of the bird's digestive system, pH levels rise into the basic range (Kornegay and Yi, 1996). In this alkaline environment, one would expect dissolved Ca and P to reprecipitate, possibly resulting in subsequent excretion of calcium phosphates. This suggests that not all undigested P of calcium phosphate origin passed through the turkeys unmodified. Nevertheless, it is hypothesized that the high calcium phosphate levels in the NPA+P feed caused the NPA+P manure to contain a high proportion of P in calcium phosphate form, leading to low $CaCl_2$ extractability of P.

Differences in water solubility of P among the NPA, HAP, NPA+Phyt, and HAP+Phyt manures were not likely due to differences in calcium phosphate content. This is because all four manures were derived from feeds contaning identical amounts of defluorinated calcium phosphate. It is proposed instead that P solubility differences among the four manures were related to differences in manure phytate P content. Recall that NPA, HAP, NPA+Phyt, and HAP+Phyt feeds differed from each other only in that they contained different amounts of phytate P versus plant-derived nonphytate P. It is logical to suggest that such differences in phytate P levels in feeds translated into differences in phytate P levels in manures, at least at the point of excretion. This is because the phytate molecule is known to be highly resistant to digestion by poultry. The low digestibility of the phytate molecule implies that at least some portion of dietary phytate passed unchanged through turkeys during the feeding trial. The swine research of Baxter et al., (1998) supports this hypothesis. Results of water extraction of feed P have already been presented which suggest that the $CaCl_2$ solution was a relatively poor extractant of phytate P, but an effective extractant of plant-derived P in nonphytate form. All of these factors support the theory that differences in the proportion of feed P in phytate versus plant-derived nonphytate form carried through to the manures and contributed to differences in water-soluble P percentages among the NPA, HAP, NPA+Phyt, and HAP+Phyt manures.

A strong direct correlation was observed between $CaCl_2$ extractability of feed P and percentage of feed P in plant-derived nonphytate form (Figure 2). Since the forms of P in the manures were not analyzed, it was not possible to plot extractability of manure P versus percentage of manure P in various forms. However, given the preceding argument that feed P in phytate form was not digested by the turkeys, the reader may ask why a linear relationship cannot be plotted between $CaCl_2$ extractability of P in the NPA, HAP, NPA+Phyt, and HAP+Phyt manures and percentage of feed P in plant-derived nonphytate form. The explanation proposed is that phytate P passed through the turkeys unused, but not unchanged, as discussed in detail below.

Native phytase activity is negligible in the initial and most nutritionally important parts of the turkey's digestive system. This is why the addition of phytase enzyme to feed can improve the efficiency with which poultry use dietary P of plant origin (Kornegay and Yi, 1996). The final portion of the bird’s digestive system, the large intestine or hindgut, typically contains a microbial population in which phytase activity can be detected. The nutritional availability of P cleaved from phytate in the hindgut is generally considered negligible. However, material that passes through the entire poultry digestive system is subjected to some native phytase activity (Cromwell, 1996). It is hypothesized that, in the initial portion of the turkey digestive tract, conversion of dietary phytate P to more water-soluble nonphytate form occurred with the NPA+Phyt and HAP+Phyt diets, but not with the NPA and HAP diets. As feed material moved through the digestive system, the supplemental phytase enzyme in the NPA+Phyt and HAP+Phyt feeds was degraded by microbial flora (Kornegay and Yi, 1996). At the same time, native hindgut phytase activity began converting phytate P to nonphytate form in birds on all diets. This native phytase activity probably had the greatest impact on the feed material containing the greatest amount of phytate substrate (NPA). It is therefore suggested that this native phytase activity had an equalizing effect, reducing differences in plant-derived nonphytate P content that would have otherwise existed between diets with and without supplemental phytase enzyme.

For example, the addition of phytase to the baseline NPA diet caused a 1.5-fold increase in feed P water solubility, as shown in Table 10 (19.4% for NPA versus 29.1% for NPA+Phyt). However, P in the NPA+Phyt manure was only 1.14 times more water soluble than P in the NPA manure (69.1% for NPA versus 78.5% for NPA+Phyt; line segment B in Figure 3). A similar pattern was observed with the HAP and HAP+Phyt treatments. Adding phytase to the HAP diet produced a statistically significant 1.36-fold increase in water solubility of feed P (26.2% for HAP versus 35.7% for HAP+Phyt). The HAP and HAP+Phyt manures did not differ statistically in water solubility of P, with dietary phytase addition causing only a 1.06-fold rise (line segment D in Figure 3) in the amount of manure P extracted. Native phytase activity in the birds was probably one of a number of digestive processes that minimized differences in P solubility between the different manure types.

A final point concerning the results of water extraction of individual pen manures relates to suspected Zn contamination of manures. As mentioned previously, five of the 34 individual pen manure samples used in the study contained unusually high levels of Zn, suggesting contamination during the collection process. Individual pen extraction results after removal of all data associated with the five pens suspected of Zn contamination are presented in Table 16. Numerically and statistically, these results are very similar to the results in Table 13 that include data from all 34 individual pen manure samples. This comparison supports the argument that Zn contamination of the manures did not notably affect P solubility.

Table 16: Water Solubility of Total (ICP-detectable) P in Manures from Selected Individual Pens

Manure Type	Total P	Soluble P	
	mg kg^{-1}	mg kg^{-1}	% of total
HAP+Phyt	8440	6972	82.6 a†
NPA+Phyt	8623	6727	78.0 a
HAP	8576	6669	77.8 a
NPA	9203	6056	65.8 b
NPA+P	14604	7568	51.8 c

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

Composite Manures

As explained previously, five composite manures were prepared by combining samples of manures from all 34 individual pens. Water extracts of wet and air-dried composite manures were analyzed only for total P by ICP spectrometry. Results of P extraction from the wet composite manures are summarized in Table 17. Each value shown in Table 17 represents a mean for data obtained from extraction of four subsamples of a single composite manure sample. As expected, the P solubility pattern obtained was very similar to that observed in Table 13 for the wet individual pen manures. The HAP+Phyt, HAP, and NPA+Phyt manures contained the most $CaCl_2$-soluble P (89.1%, 86.6%, and 86.1% of total manure P solubilized, respectively). These three manures did not differ statistically in the percentage of total manure P solubilized. The NPA manure contained P of intermediate solubility (78.2%) and differed statistically from the four other manures. The NPA+P manure was also statistically different from all other manures and contained the least $CaCl_2$-soluble form of P, with only 60.0% of total manure P dissolving in water. Note that, despite its low solubility percentage, the NPA+P composite manure released the greatest overall quantity of P to the $CaCl_2$ extractant on an equal-manure-weight basis (7973 mg P per kg manure).

Table 17: Water Solubility of P in Wet Composite Manures

Manure Type	Total P	Soluble P	
	mg kg^{-1}	mg kg^{-1}	% of total
HAP+Phyt	7570	6747	89.1 a†
HAP	7820	6773	86.6 a
NPA+Phyt	7930	6824	86.1 a
NPA	8350	6529	78.2 b
NPA+P	13280	7973	60.0 c

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

The results of $CaCl_2$ extraction of P from dry composite manures are shown in Table 18. Drying the composite manures at 40 °C produced important shifts in the P solubility pattern observed at the point of excretion. After drying, the percentage of total manure P solubilized in 0.01 M $CaCl_2$ was similar for the HAP, NPA, HAP+Phyt, and NPA+Phyt composite manures. Solubility percentages from these composite manures ranged from 81.1% to 75.8%, although certain statistically significant differences did exist within the group. The dry NPA+P composite manure differed dramatically from the other four manures in percentage of total P soluble in $CaCl_2$. Only 34.1% of total P in the air-dried NPA+P composite manure was solubilized by the water extractant. After drying, the NPA+P composite manure also released the smallest overall quantity of P to the $CaCl_2$ extractant on an equal-manure-weight basis (5193 mg P per kg manure).

Table 18: Water Solubility of P in Dry Composite Manures

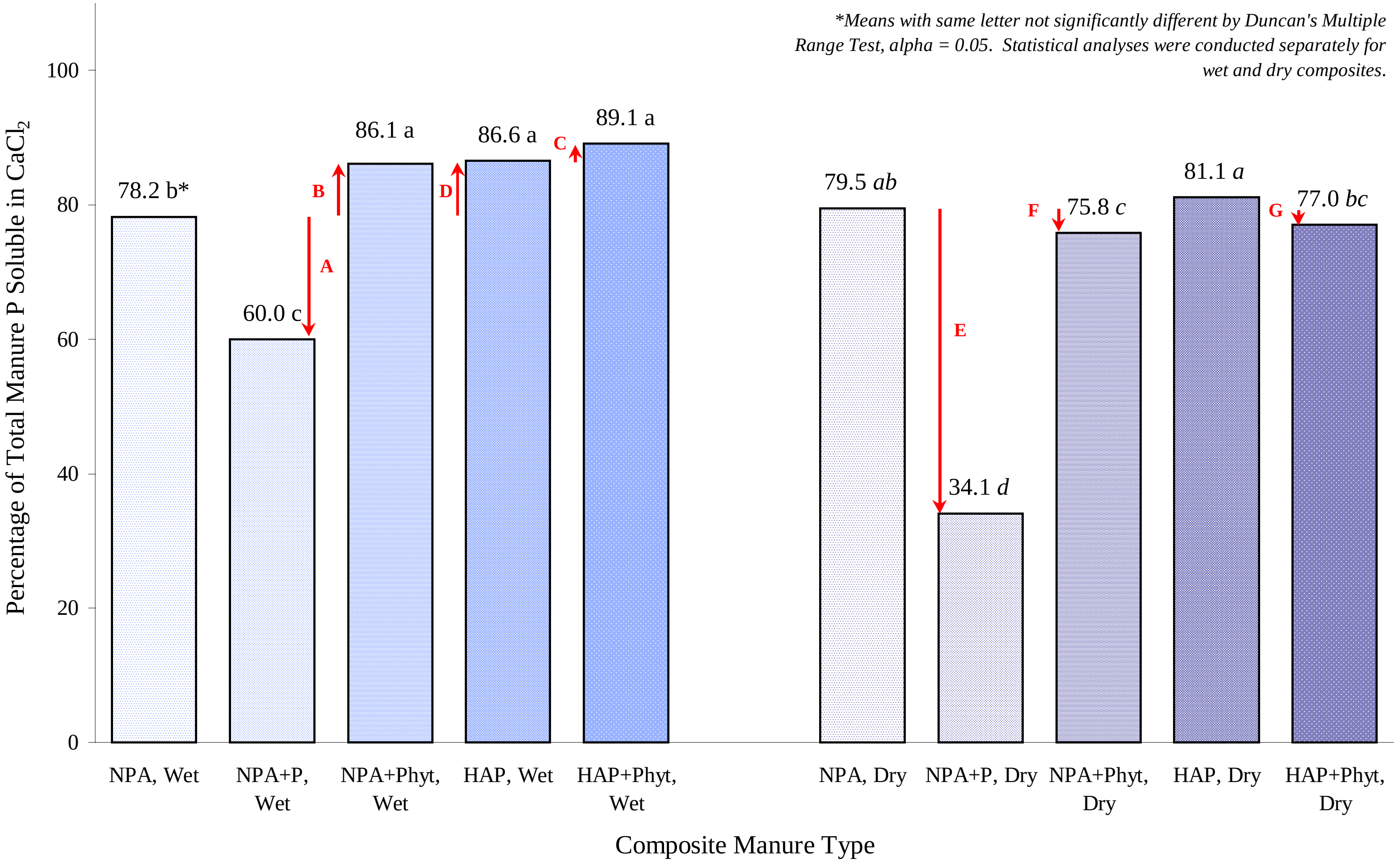
Manure Type	Total P	Soluble P	
	mg kg^{-1}	mg kg^{-1}	% of total
HAP	9230	7490	81.1 a†
NPA	8960	7124	79.5 ab
HAP+Phyt	8760	6749	77.0 bc
NPA+Phyt	9000	6826	75.8 c
NPA+P	15240	5193	34.1 d

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

The wet and dry composite manure P solubility results from Tables 17 and 18 are compared graphically in Figure 4. As anticipated, the P solubility pattern for the wet composite manure samples was essentially the same as that shown in Figure 3 for the wet individual pen manure samples. The same hypotheses presented previously to explain the individual pen manure P water solubility results apply to the wet composite manures. Important differences are revealed by comparison of the wet and dry composite manure solubility patterns in Figure 4. A key consequence of drying the composite manues at 40 °C was promotion of the solubility-reducing effect of supplemental dietary calcium phosphate. Addition of supplemental defluorinated phosphate to the baseline NPA diet caused composite manure P solubility at the point of excretion (i.e., prior to drying) to drop from 78.2% to 60.0%, a reduction represented graphically in Figure 4 by line segment A. Manure drying almost doubled this solubility-reducing dietary calcium phosphate effect, producing an overall drop in solubility from 79.5% for NPA to 34.1% for NPA+P (represented graphically by line segment E).

Manure drying also changed the impact of dietary phytase supplementation on the $CaCl_2$ solubility of manure P. Adding phytase enzyme to the NPA and HAP diets enhanced the water solubility of composite manure P at the point of excretion, as represented by line segments B and D, respectively. Drying of composite manures reversed this effect of dietary phytase addition. Phytase modification of the baseline NPA diet significantly reduced P solubility in the dry manures from 79.5% to 75.8%, as represented by line segment F. Similarly, phytase addition to the HAP diet significantly reduced $CaCl_2$ solubility of P in dry composite manures from 81.1% to 77.0%, as represented by line segment G. In both wet and dry composite manures, dietary HAP corn substitution enhanced water solubility of manure P. This HAP-induced increase in water extractability of P was not statistically significant for the dried composites, however. No specific mechanisms are proposed to explain these effects of manure drying. These results clearly suggest, however, that important changes in the form and solubility of manure P may occur between the point of excretion and the point of land disposal.

Figure 4: Water Solubility of P in Wet and Dried Composite Manures



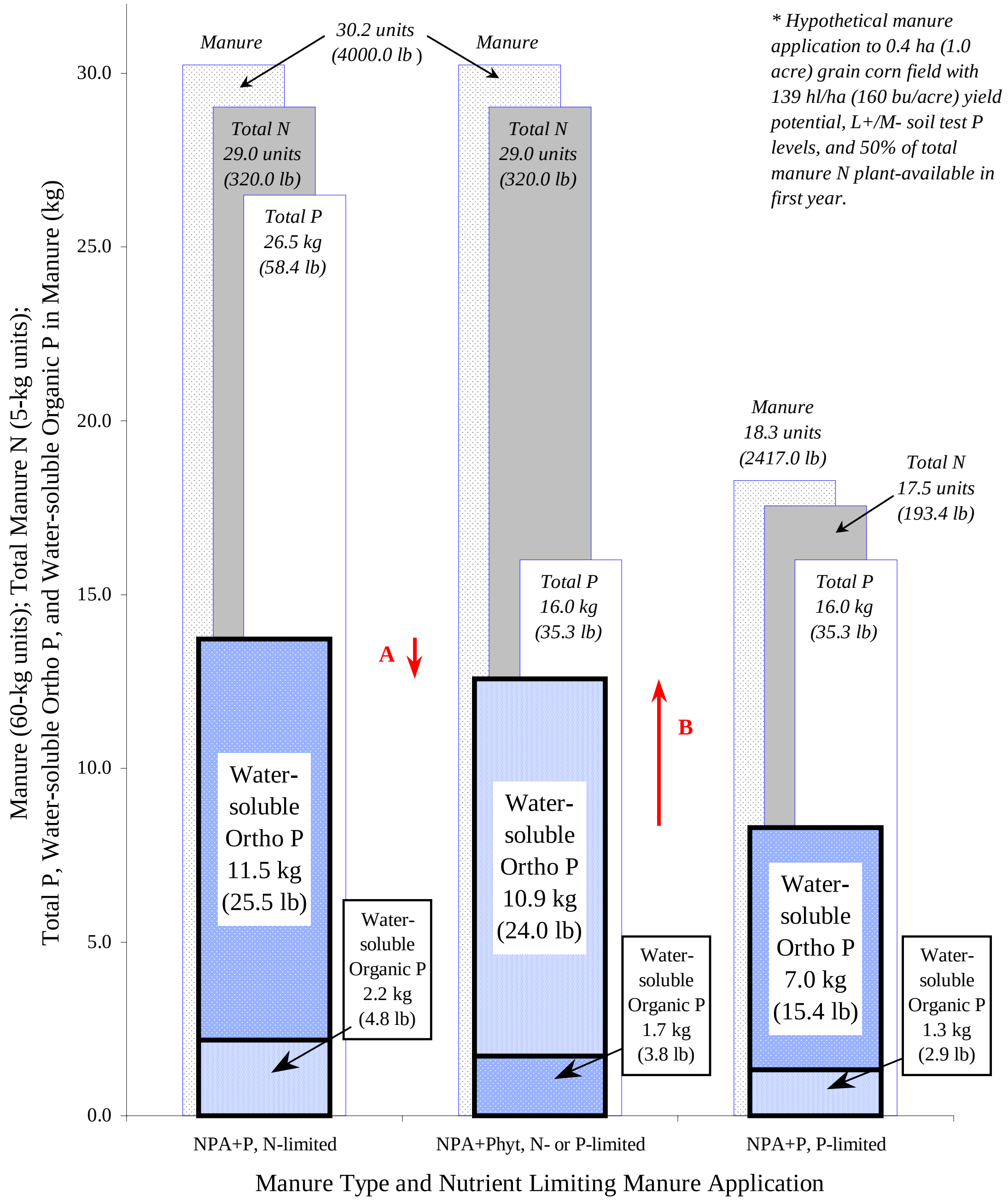
Implications for Land Application

Extraction with 0.01 M $CaCl_2$ revealed differences in P solubility between conventional and alternative manure types. What are the implications of these differences? Given these findings, how might the transition from conventional to alternative rations affect the amount of readily soluble manure P ultimately placed on the land? When assessing the land application implications of the manure solubility data, attention should focus on the results of extraction of NPA+P and NPA+Phyt manures. This is because the NPA+P and NPA+Phyt diets were the experimental feeds most similar to conventional and alternative diets currently in use in the poultry industry. The following pages present the first of a number of examples showing what would occur if the NPA+P and NPA+Phyt manures from this experiment were used to fertilize a crop of grain corn. These examples are designed to illustrate how switching from a conventional to an phytase-amended poultry diet changes the overall quantity of water-soluble manure P applied to the land. Each example explores two manure application scenarios: limitation of manure spreading based on manure N content and limitation of manure spreading based on manure P content. In these hypothetical examples, the field receiving manure is imaginary. The next chapter will present results from application of manures to actual soils.

For the hypothetical examples in this chapter, crop demands and soil fertility levels were chosen to allow convenient comparison of N- versus P-based manure management scenarios using the same figure. They were not necessarily chosen in order to illustrate typical field conditions in Virginia’s animal intensive regions. Furthermore, these examples show P-based limitations on manure application for soils with moderate soil test P levels. This is not particularly realistic, since restrictions on application of manure P to soils typically occur when soils are high in soil test P. The reader is therefore cautioned to keep in mind the intent and limitations of these hypothetical land application examples. Note that an identical combination of crop nutrient needs, soil fertility levels, and other factors will be used in all hypothetical manure application examples presented in this chapter. Finally, it must be emphasized that the ultimate solubility of manure P applied to the land may be dramatically altered by biological and chemical reactions in soils. These hypothetical manure application examples are not intended to address this issue. They reflect the results of extraction of raw manures only. Chapter 3 will investigate how reactions with soil modified the solubility of manure P compounds.

The amounts of NPA+P and NPA+Phyt manure that would be used to fertilize a hypothetical 0.405 ha or one acre field of grain corn are illustrated in Figure 5. Background columns in the figure represent quantities of NPA+P and NPA+Phyt manure allocated for land application, as well as the amounts of total N and total P contained in those manures. The foreground columns show the quantity of water-soluble P (total, orthophosphate, and organic) applied to the land. Two nutrient management scenarios are presented. The N-based comparison is illustrated by the left-hand and center groups of columns, while the P-based comparison is illustrated by the right-hand and center groups of columns. All P values in Figure 5 were derived from the results of total P analysis and 0.01 M $CaCl_2$ extraction conducted on wet individual pen manure samples (Tables 5, 13, 14, and 15). Since total N analysis was not conducted on individual pen manure samples, total manure N contents were estimated from analysis of wet composite manures (Table 8). For purposes of these examples, the wet NPA+P and NPA+Phyt manures from individual pens are considered to contain 8.0% total N.

Figure 5: Water-soluble P Loadings from N- and P-based Land Application of Wet NPA+P and NPA+Phyt Manures †



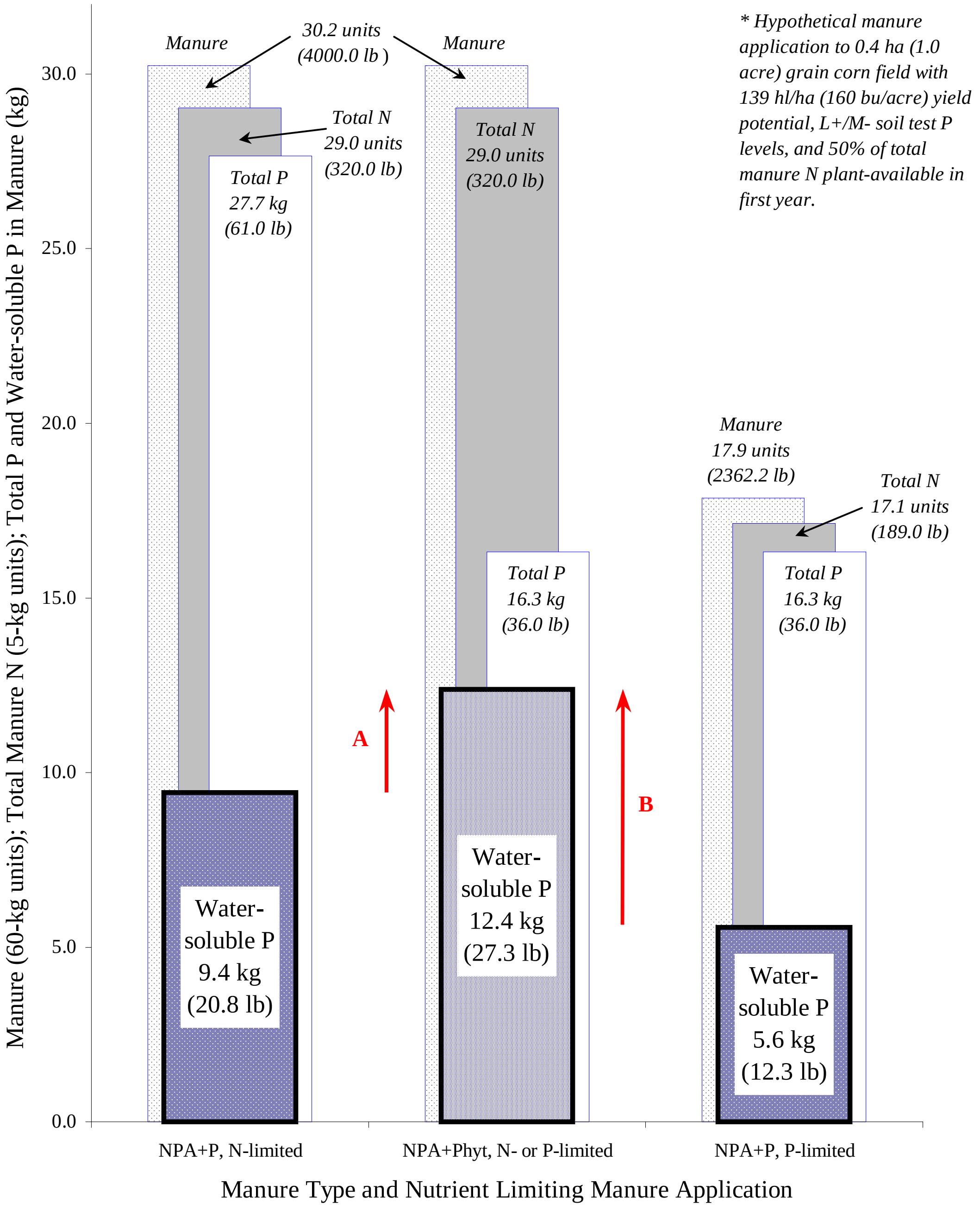
The first issue to consider is the N-limited manure application comparison in Figure 5. In this scenario, the goal is to spread manure so as to supply the plant-available N required by 0.4 ha (1.0 acre) of corn with a yield potential of 10 Mg ha^{-1} (160 bu acre^{-1}). Virginia Cooperative Extension recommends that such a crop be fertilized annually with 179 kg ha^{-1} (160 lb acre^{-1}) of plant-available N (Donohue and Heckendorn, 1994). In order to provide this N, 1814 kg (4000 lb) of either NPA+P manure or NPA+Phyt manure can be spread on this unit of ground. This provides 145 kg (320 lb) of total N and 72.5 kg (160 lb) of plant-available N (Virginia nutrient management guidelines predict that about 50% of total N in these particular manures would be plant-available in the first season following no-till application) (Nagle et al., 1998). In this N-based scenario, feeding phytase dramatically affects the amount of total manure P applied to the land. The 1814 kg (4000 lb) of conventional NPA+P manure contains 26.5 kg of total P (58.4 lb P or 133.7 lb P_2O_5), all of which is considered available to the corn crop. An annual P application of this magnitude on 0.4 ha (1.0 acre) would exceed Virginia Cooperative Extension single-season needs for corn in the vast majority of cases, since only 134.4 kg ha^{-1} (120 lb $acre^{-1}$) P_2O_5 are recommended annually on land with extremely low (L-) soil test P levels (Donohue and Heckendorn, 1994). The 1814 kg (4000 lb) of alternative NPA+Phyt manure contains only 16.0 kg of total P (35.3 lb P or 80.8 lb P_2O_5). Such a P application is recommended annually in Virginia for grain corn planted on 0.4 ha (1.0 acre) of land with low to medium (L-/M+) soil test P levels. This illustrates how, under N-limited manure management, phytase feeding can significantly reduce total manure P applications to levels more consistent with crop needs.

Under N-based manure management, conversion from the NPA+P to the NPA+Phyt feed produces a 40% drop in the amount of total manure P applied to the land. However, the greater solubility of P in the NPA+Phyt manure means that use of the alternative feed produces a mere 8% decrease in the total (sum of ortho plus organic) amount of water-soluble manure P applied to soil (from 13.7 kg or 30.3 lb for NPA+P to 12.6 kg or 27.7 lb for NPA+Phyt). This 8% decrease is illustrated in Figure 5 by line segment A. This drop in water-soluble P loading has both an orthophosphate and an organic P component, with a 6% decrease in soluble ortho P applied (from 11.5 kg or 25.5 lb for NPA+P to 10.9 kg or 24.0 lb for NPA+Phyt) and a 22% decrease in soluble organic P applied (from 2.2 kg or 4.8 lb for NPA+P to 1.7 kg or 3.8 lb for NPA+Phyt). Therefore, if the conventional and phytase-derived manures from this experiment were applied to land on an N basis immediately after excretion, use of phytase technology would decrease the overall amount of readily soluble manure P applied to soils.

Consider next the P-based manure management comparison illustrated in Figure 5 (center and right-hand groups of columns). Under P-limited management, manure is allocated so as to meet but not exceed the P needs of the crop to be grown. For purposes of these hypothetical examples, P applications are limited to crop needs even though soil P has not been built up to high levels. On soils such as these testing low to medium (L+/M-) in P, Virginia Cooperative Extension recommends an application of about 16.0 kg P ha^{-1} (35.3 lb P $acre^{-1}$ or 80.8 lb P_2O_5 $acre^{-1}$) (Donohue and Heckendorn, 1994). Note how, in order to limit P loading to this level, the original N-based NPA+P manure application must be reduced by 40%, which leads to a shortfall in plant-available N of 28.7 kg or 63.3 lbs. This underlines the combination of reduced manure disposal capacity and plant-available N shortage typically seen when P-based limits on manure disposal are imposed in poultry-producing regions. More important is the impact of switching from the conventional NPA+P diet to the alternative NPA+Phyt diet under P-based manure management, illustrated in Figure 5. In this situation, switching from the NPA+P to the NPA+Phyt feed increases by 40% the quantity of manure and plant-available N available for land application. Although use of the alternative diet does not change the total (sum of ortho plus organic) amount of P applied to soil, it does produce a 52% increase in the total amount of water-soluble manure P applied to the land (from 8.3 kg or 18.3 lb for NPA+P to 12.6 kg or 27.7 lb for NPA+Phyt). This 52% increase is illustrated by line segment B in Figure 5. Loadings of water-soluble manure P in ortho form increase by 56% (from 7.0 kg or 15.4 lb for NPA+P to 10.9 kg or 24.0 lb for NPA+Phyt), while manure P readily solubilized into dissolved organic forms increases by 30% (from 1.3 kg or 2.9 lb for NPA+P to 1.7 kg or 3.8 lb for NPA+Phyt).

Figure 5 was presented to illustrate what might happen if the NPA+P and NPA+Phyt manures used in this study were land applied wet (i.e., immediately after excretion). The same example is shown in Figure 6, except that total and soluble P values were derived from analysis and extraction of composite NPA+P and NPA+Phyt manures that were dried at 40 °C for 4 d (Tables 9 and 18). Note that the N content of both dried manure types was still taken to be 8.0%, since the dried composite manures were not analyzed for N. As indicated previously, drying dramatically depressed the water solubility of P in the NPA+P composite manure. The implications of this drying-related solubility shift are illustrated in Figure 6. Under N-limited manure management, switching from the conventional NPA+P feed to the alternative NPA+Phyt diet produces a 61% decrease in the total amount of manure P loaded on soils (from 27.7 kg or 61.0 lb for NPA+P to 16.3 kg or 36.0 lb for NPA+Phyt). At the same time, the amount of water-soluble manure P loaded onto soils increases by 31% (from 9.4 kg or 20.8 lb for NPA+P to 12.4 kg or 27.3 lb for NPA+Phyt). This 31% increase is represented by line segment A in Figure 6. Under P-limited manure management, converting from NPA+P feed to NPA+Phyt feed does not change the total amount of manure P applied to the soil, but increases the amount of water-soluble P applied to the land by 122% (from 5.6 kg or 12.3 lb for NPA+P to 12.4 kg or 27.3 lb for NPA+Phyt), as represented by line segment B.

Figure 6: Water-soluble P Loadings from Land Application of Dry NPA+P and NPA+Phyt Manures, N- and P-limited Applications



Summary

Extracting manures at the point of excretion (i.e., wet) with 0.01 M $CaCl_2$ showed that P in the alternative NPA+Phyt manure was up to 1.5 times more water soluble than P in the conventional NPA+P manure. This statistically significant difference in solubility was caused in large part by the greater quantity of defluorinated calcium phosphate in the conventional feed. It is hypothesized that, because of the higher levels of calcium phosphate in the NPA+P feed, a greater proportion of manure P was in water-insoluble calcium phosphate forms. Although P in the conventional NPA+P manure was less soluble on a percentage basis, a greater total amount of P was extracted from the wet NPA+P manure than from an equal quantity of wet NPA+Phyt manure. The solubility-depressing calcium phosphate effect was strongly enhanced by drying manures at moderate temperature prior to $CaCl_2$ extraction. Therefore, drying prior to extraction increased differences in water solubility of manure P between the conventional NPA+P and alternative NPA+Phyt manures.

It appears that phytase enzyme in the alternative feed contributed to the differences in P water solubility between wet NPA+P and NPA+Phyt manures. It is hypothesized that adding phytase to the baseline corn and soybean meal ration reduced phytate P excretion, which translated into increased water solubility of manure P at the point of manure excretion. Note that this phytase-related enhancement of manure P solubility did not persist after drying of manures at moderate temperature for 4 d.

Feeding HAP corn as well as HAP corn plus phytase enzyme also increased solubility of P in wet manures. Drying manures eliminated the HAP-plus-phytase effect, but not the solubility-enhancing effect of feeding HAP corn alone. The P in the HAP and HAP+Phyt manures was significantly more water soluble than P in the conventional NPA+P manure both at the point of excretion and after drying, again in large part due to the calcium phosphate effect described previously.

Total analysis and extraction of manures suggest that, if the NPA+P and NPA+Phyt manures were land-applied immediately after excretion (i.e., wet) on an equal-N basis, the alternative manure would load 8% less water-soluble P on the soil than would the conventional manure. The outcome changes when results of extraction after manure drying are considered. If the dried NPA+P and NPA+Phyt manures were land applied on an N-basis, the alternative manure would load 41% less total manure P, but 31% more water-soluble manure P on the soil than would the conventional manure.

If the NPA+P and NPA+Phyt manures were land applied on a P-basis immediately after excretion, the phytase-derived NPA+Phyt manure would load the same amount of total P, but 52% more water-soluble manure P on soils than the conventional NPA+P manure. The amount of land-applied manure P readily dissolved into orthophosphate form would increase by 56%. These trends would be magnified if dried manures were land applied. If the dried NPA+P and NPA+Phyt manures were spread on an equal-P basis, the alternative manure would load the same amount of total manure P, but 122% more water-soluble manure P on soils than would the conventional manure.

2.3.3.b. Mehlich III Extraction of Manure P

Individual Pen Manure Samples

Results of ICP (total) P analysis conducted on the Mehlich III extracts of wet manures from individual pens are summarized in Table 19. Each value shown for the NPA, HAP, and HAP+Phyt manures is a mean for extraction data from eight replicate pens, three subsamples extracted per pen. Each value presented for the NPA+P and NPA+Phyt manures is a mean for extraction data from five replicate pens, three subsamples extracted per pen. Statistically as well as numercially, the HAP+Phyt manure contained the most Mehlich III-soluble forms of P, with 87.0% of total manure P dissolved in the acidic extractant. The HAP, NPA+Phyt, and NPA manures were next in terms of Mehlich III solubility of manure P, with 77.2%, 73.2%, and 69.6% of total manure P dissolved. There were no statistically significant differences in solubility between these three manure types. Numerically, the NPA+P manure contained the least acid-soluble forms of P, with 67.6% of total manure P dissolved in the Mehlich III extractant. However, the NPA+P manure did not differ statistically from the NPA or NPA+Phyt manures in P solubility percentage at the point of excretion. Note that the Mehlich III solution extracted the greatest total amount of manure P (on an equal-manure-weight basis) from the NPA+P manure. Therefore, despite a few key statistical differences, the overall P solubility pattern produced by Mehlich III extraction of wet manures was relatively similar to that obtained by extraction with $CaCl_2$.

Table 19: Mehlich III Solubility of P in Manures from Individual Pens

Manure Type	Total P	Soluble P	
	mg kg^{-1}	mg kg^{-1}	% of total
HAP+Phyt	8443	7347	87.0 a†
HAP	8591	6636	77.2 b
NPA+Phyt	8822	6455	73.2 bc
NPA	9199	6405	69.6 bc
NPA+P	14604	9868	67.6 c

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

The Mehlich III extraction results from Table 19 are presented graphically in Figure 7. This figure illustrates that switching from the conventional NPA+P diet to the alternative NPA+Phyt diet increased the Mehlich III extractability of manure P at the point of excretion by a factor of 1.08 (67.6% for NPA+P versus 73.2% for NPA+Phyt). This solubility enhancement, represented by the combined length of line segments A and B in Figure 7, was not statistically meaningful. Why did the NPA+P and NPA+Phyt manures, which differed in water solubility of P, not differ significantly in Mehlich III extractability of P? As explained below, it is hypothesized that water-insoluble calcium phosphate and phytate compounds which controlled $CaCl_2$ solubility of manure P at the point of excretion were more readily dissolved by the acidic Mehlich III extractant.

Consider first the effect of feeding supplemental defluorinated phosphate on the percentage of total manure P soluble in the Mehlich III extractant. Adding calcium phosphate to the baseline NPA diet produced an insignificant decrease in Mehlich III P solubility from 69.6% to 67.6% (represented by line segment A). It is hypothesized that the NPA+P manure contained more P in calcium phosphate form than did the other manures, but that the Mehlich III solution was relatively effective at dissolving this form of P. One would indeed expect calcium phosphate compounds to be more soluble in the pH 2.0, chelate-containing Mehlich III extractant than in the pH 5.6 $CaCl_2$ solution. Recall that a similar phenomenon was observed when feed P was extracted with $CaCl_2$ and Mehlich III solutions. These results therefore lend further support to the hypothesis that differences in calcium phosphate content controlled $CaCl_2$ extractability of P in the NPA+P manure.

Consider next the fact that addition of phytase enzyme to the baseline NPA diet produced a insignificant increase in Mehlich III P solubility at the point of excretion, from 69.6% to 73.2% (represented by line segment B in Figure 7). Remember that adding phytase to the baseline NPA diet produced a statistically meaningful increase in $CaCl_2$ solubility of wet manure P. It was suggested that this solubility difference occurred because the baseline NPA manure contained a higher proportion of P in phytate form at the point of excretion than did the manures derived from alternative feeds. Building on this initial hypothesis, it is proposed here that the Mehlich III solution was more effective than the $CaCl_2$ extractant in solubilizing phytate P compounds. Again, a similar phenomenon was observed with solubility of feed P.

Figure 7: Comparison of Mehlich III Solubility of P in Manures from All Individual Pens

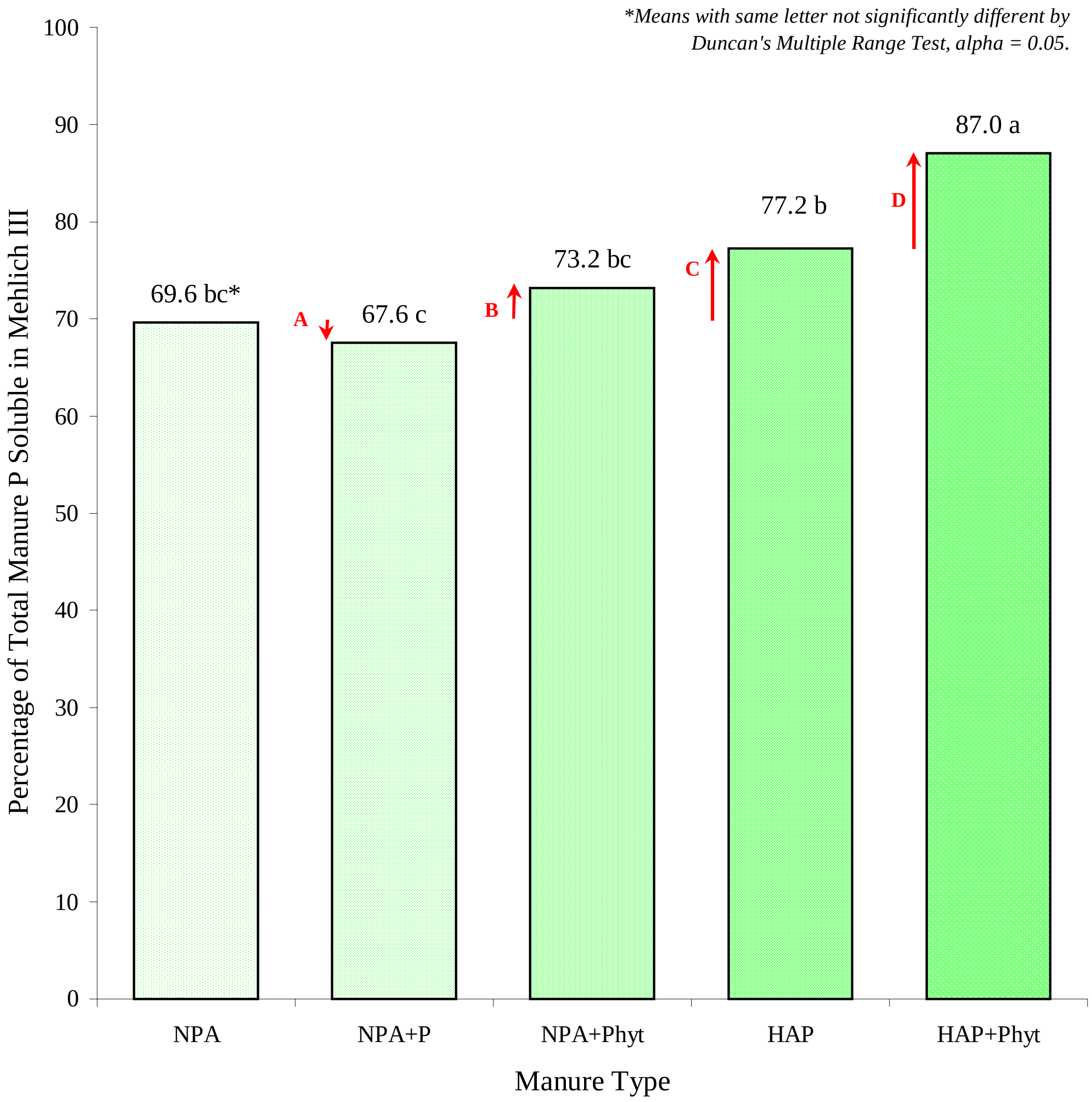


Figure 7 illustrates that substituting HAP corn in the baseline NPA diet caused an increase in Mehlich III extractability of manure P, from 69.6% for NPA to 77.2% for HAP. This increase, represented by line segment C, was dramatic, but not statistically significant. Modifying the baseline NPA diet with HAP corn plus phytase produced the only statistically significant increase in Mehlich III extractability of manure P at the point of excretion, from 69.6% for NPA to 87.0% for HAP+Phyt (represented by the combined length of line segments C and D in Figure 7). The P in both the HAP and HAP+Phyt manures was significantly more acid soluble than P in the conventional NPA+P manure.

Composite manure samples

The results of Mehlich III extraction of P from wet composite manures are presented in Table 20. Each value in Table 20 is a mean from extraction of four subsamples of a single composite manure sample. These wet composite manure extraction results differed slightly from the wet individual pen manure extraction results discussed above. Among the wet composites, the HAP+Phyt, NPA+Phyt, HAP, and NPA manures did not differ statistically from each other in Mehlich III extractability of P. The percentage of total manure P solubilized for these four treatments was 91.8%, 90.0%, 87.7%, and 82.2%, respectively. The NPA+P manure contained the least acid-soluble forms of P, with 75.0% of total P dissolved by the Mehlich III extractant. However, the NPA+P and NPA treatments did not differ statistically in Mehlich III solubility of manure P. Despite the low solubility percentage of the NPA+P manure, the Mehlich III solution dissolved more P from the wet composite NPA+P sample (on an equal-manure-weight basis) than from any other manure (9956 mg P per kg manure).

Table 20: Mehlich III Solubility of P in Wet Composite Manures

Manure Type	Total P	Soluble P	
	mg kg^{-1}	mg kg^{-1}	% of total
HAP+Phyt	7570	6950	91.8 a†
NPA+Phyt	7930	7137	90.0 a
HAP	7820	6861	87.7 a
NPA	8350	6861	82.2 ab
NPA+P	13280	9956	75.0 b

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

Results of Mehlich III extraction of P from dried composite manures are summarized in Table 21. After drying at moderate temperature, the HAP+Phyt composite manure contained P that was significantly more Mehlich III-soluble (87.5%) than P in the other four manure types. The dry NPA, NPA+Phyt, and HAP composite manures did not differ significantly in the percentage of total manure P soluble in Mehlich III, with 84.0%, 83.8%, and 82.8% of total manure P dissolved, respectively. Finally, the dried NPA+P composite contained the lowest Mehlich III P solublity percentage (62.6%), both statistically and numerically.

Table 21: Mehlich III Solubility of P in Dry Composite Manures

Manure Type	Total P	Soluble P	
	mg kg^{-1}	mg kg^{-1}	% of total
HAP+Phyt	8760	7664	87.5 a†
NPA	8960	7525	84.0 b
NPA+Phyt	9000	7543	83.8 b
HAP	9230	7645	82.8 b
NPA+P	15240	9543	62.6 c

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

The results of Mehlich III extraction of wet and dry composite manures from Tables 20 and 21 are presented graphically in Figure 8. Figure 8 shows that switching from the conventional NPA+P diet to the alternative NPA+Phyt diet produced a statistically significant increase in Mehlich III solubility of composite manure P at the point of excretion (from 75.0% for NPA+P to 90.0% for NPA+Phyt). This solubility increase is represented by the combined length of line segments A and B. Adding defluorinated phosphate to the baseline NPA diet resulted in an insignificant decrease in Mehlich III P solubility, from 82.2% for NPA to 75.0% for NPA+P (represented by line segment A). Adding phytase to the baseline NPA diet produced a statistically insignificant increase in Mehlich III solubility of manure P, from 82.2% for NPA to 90.0% for NPA+Phyt (represented by line segment B). Therefore, Mehlich III extraction of the wet composite manures produced greater differences in P solubility among the NPA, NPA+P, and NPA+Phyt treatments than were observed with Mehlich III extraction of the individual pen manures. Conversely, Mehlich III extraction of the wet composite manures revealed no significant differences in P solubility between the NPA, HAP, and HAP+Phyt manures. Recall that individual pen manure extraction results showed statistically significant differences among these manure types.

Figure 8: Mehlich III Solubility of P in Wet and Dry Composite Manures

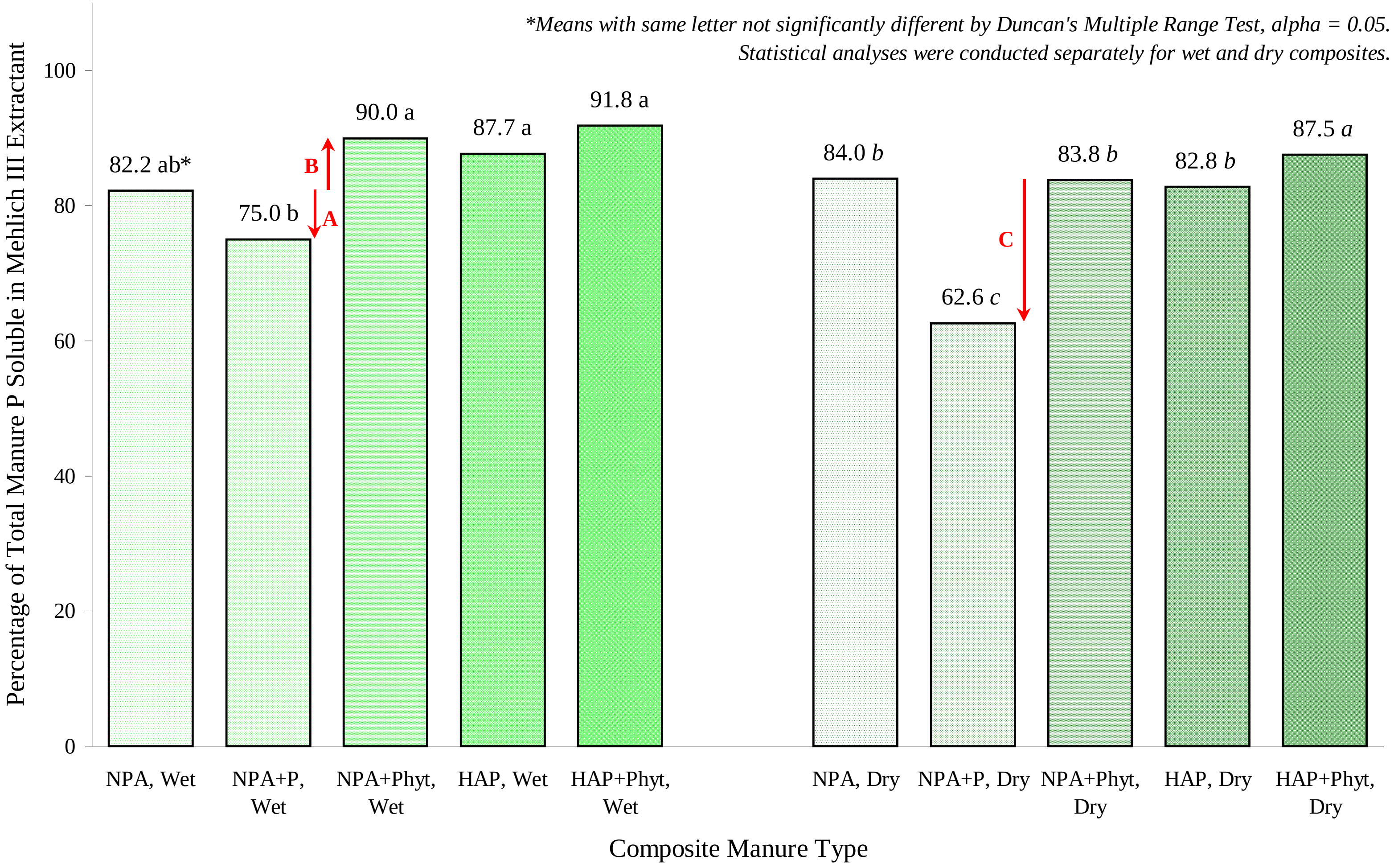


Figure 8 shows that drying the composite manures prior to extraction changed Mehlich III solubility patterns in the much the same way that it affected $CaCl_2$ solubility patterns. Drying enhanced the solubility-reducing effect of supplemental calcium phosphate in feed. Addition of inorganic P to the baseline NPA diet caused Mehlich III solubility of dry composite manure P to drop by a factor of 0.75, from 84.0% for NPA to 62.6% for NPA+P (represented by line segment C). Therefore, after drying, P in the composite NPA+P manure was significantly less Mehlich III-soluble than P in the four other manure types. These results as well as the results of $CaCl_2$ extraction of composite manures strongly suggest that manure drying enhanced bonding and reduced solubility of calcium phosphate compounds in the manure. Note that drying reduced the differences in Mehlich III P solubility percentage between the NPA, NPA+Phyt, and HAP composite manures. At the same time, a statistically significant difference in Mehlich III P solubility was observed between the HAP+Phyt and the other four manures after drying. This difference was not observed with Mehlich III extraction of the wet composite manures.

Implications for Land Application

What are the implications for land application of the Mehlich III extraction results? Results of Mehlich III extraction of manure P are presented in Figures 9 and 10. The same hypothetical land application scenario utilized in previous examples is used in Figures 9 and 10. These figures show the amounts of manure and manure components, including acid-soluble manure P, that would be loaded on the land if the NPA+P and NPA+Phyt manures from the experiment were used to fertilize a 0.4 ha (1.0 acre) field of corn. Results from extraction of wet manures are presented in Figure 9. Under N-based manure management, a shift from the conventional NPA+P to the alternative NPA+Phyt diet causes a 40% decrease in total manure P loadings to soil, along with a 35% drop in Mehlich III-soluble P loadings (from 17.9 kg to 11.7 kg, represented by line segment A in Figure 9). Under P-based manure management, switching to the alternative feed does not change total P loadings, but does increase the amount of Mehlich III-extractable manure P applied by 8% (from 10.8 kg to 11.8 kg, represented by line segment B in Figure 9).

Figure 10 shows what might occur if the dried NPA+P and NPA+Phyt manures from the experiment were applied to the land. Recall that drying for 4 d at moderate temperature enhanced differences in Mehlich III solubility of P between the NPA+P and NPA+Phyt manures. As a result, under equal-N basis manure management, switching from the conventional to the NPA+Phyt diet reduces acid-soluble P loading by only 21% (from 17.3 kg to 13.7 kg, represented by line segment A in Figure 10). Application of dried manures on an equal-P basis results in a 34% increase in water soluble-P loading (from 10.2 kg to 13.7 kg, represented by line segment B in Figure 10).

Figure 9: Mehlich III-soluble P Loadings from Land Application of Wet NPA+P and NPA+Phyt Manures, N- and P-limited Applications†

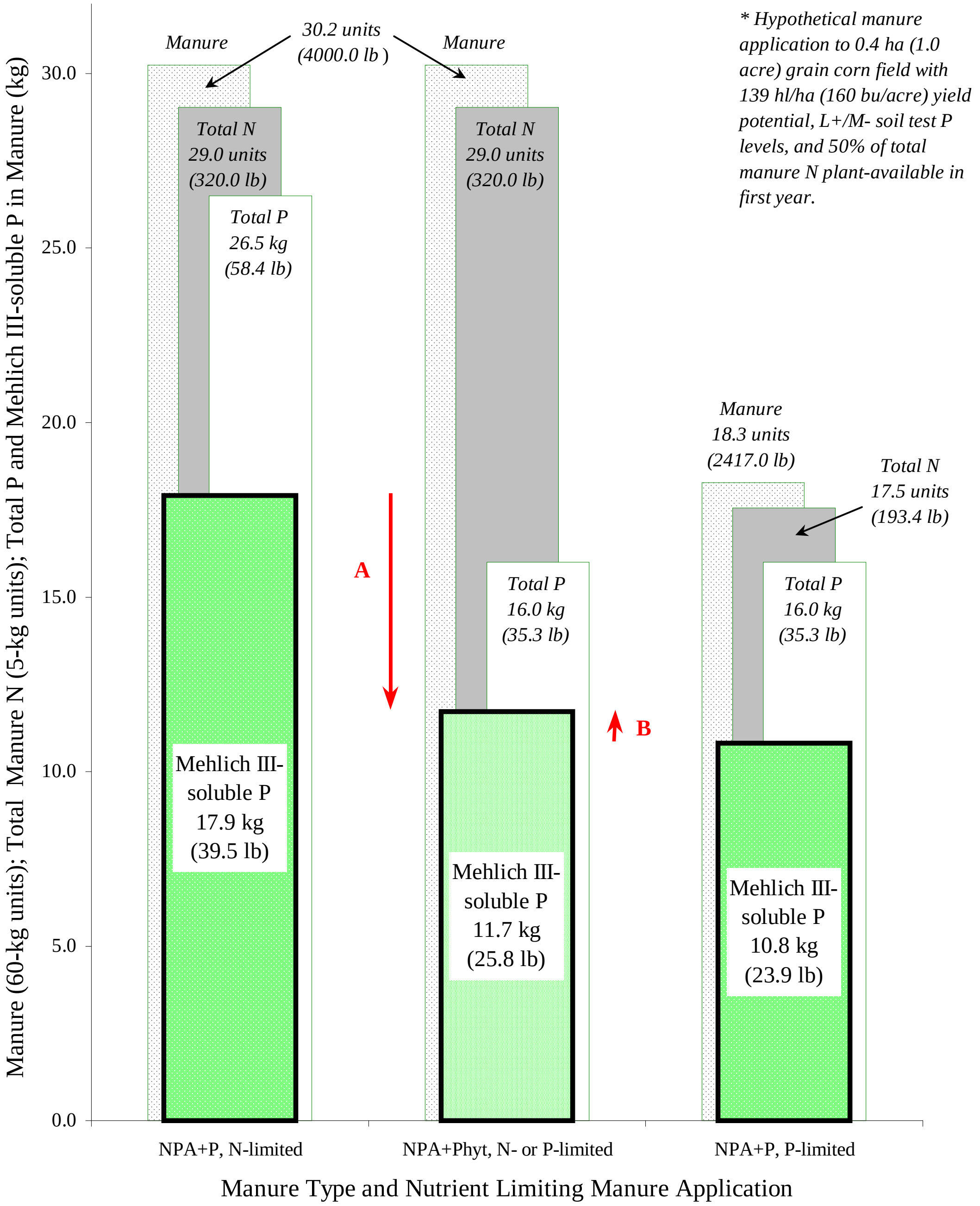
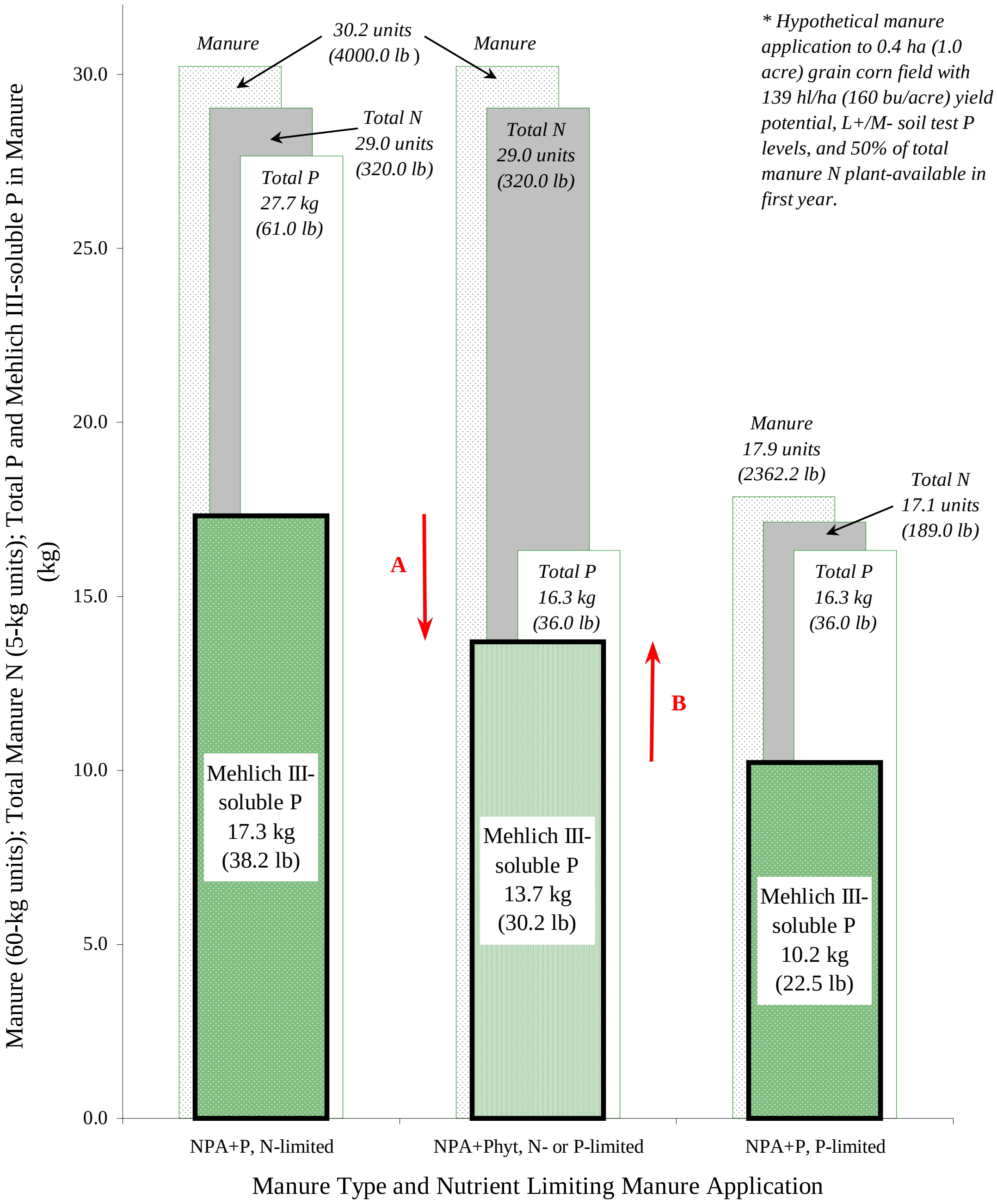


Figure 10: Mehlich III-soluble P Loadings from Land Application of Dry NPA+P and NPA+Phyt Manures, N- and P-limited Applications†



Summary

Mehlich III extraction revealed fewer differences in P solubility between the wet NPA+P and NPA+Phyt manures than were seen with the $CaCl_2$ extractant. At the point of excretion, P in the alternative NPA+Phyt manure was only 1.1 times more acids oluble than P in the conventional NPA+P manure. This difference was not statistically significant. After manures were combined to make composites, a significant Mehlich III solubility difference between these two manure types was observed. In this case, P in the NPA+Phyt manure was 1.2 times more Mehlich III-soluble than P in the conventional NPA+P manure. However, neither the solubility-depressing effects of supplemental calcium phosphate nor the solubility-enhancing effect of supplemental phytase were significant individually. These results are consistent with the hypothesis that water-insoluble, acid-soluble calcium phosphate compounds were largely responsible for differences in $CaCl_2$ solubility of P among the conventional and alternative manures.

Drying manures at moderate temperature for 4 d increased differences in Mehlich III solubility of P between the NPA+P and NPA+Phyt manures. This was apparently the result of a drying-related enhancement of the solubility-reducing influence of supplemental calcium phosphate in the NPA+P feed. Extraction with $CaCl_2$ revealed the same reduction in solubility of manure calcium phosphate compounds with drying. These results suggest that drying or other modifications of manure between the point of excretion and land application have the potential to strongly influence the persistence of manure P solubility differences.

Amending the baseline NPA diet with phytase enzyme or HAP corn (in the absence of dietary defluorinated calcium phosphate adjustment) did not significantly alter Mehlich III solubility of P in either wet or dry manures. Acid-solubility of P in both wet and dry manure generally increased when HAP corn and phytase enzyme were fed concurrently without changing dietary calcium phosphate levels.

2.3.3.c. Olsen Extraction of Manure P

Composite Manure Samples

Only composite manure samples were extracted with the alkaline $NaHCO_3$ Olsen solution. Results of ICP (total) P analysis of Olsen extracts of wet composite manures are presented in Table 22. The NPA+Phyt manure contained the most Olsen-soluble forms of P, with 74.6% of total manure P dissolved. The HAP treatment contained the next most Olsen-soluble forms of P, with 70.6% of total manure P dissolved in the Olsen extractant. Both the NPA+Phyt and HAP manures differed statistically from all other manures in the percentage of total manure P soluble in the Olsen extractant. The HAP+Phyt and NPA manures were statistically similar in alkaline extractability of manure P, with 65.8% and 65.0% of total manure P solubilized, respectively. Finally, P in the NPA+P manure was the least Olsen-extractable, with only 43.2% of total manure P solubilized.

Table 22: Olsen-soluble P in Wet Composite Manures

Manure Type	Total P	Soluble P	
	mg kg^{-1}	mg kg^{-1}	% of total
NPA+Phyt	7930	5917	74.6 a
HAP	7820	5520	70.6 b
HAP+Phyt	7570	4980	65.8 c
NPA	8350	5424	65.0 c
NPA+P	13280	5734	43.2 d

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

Results of extracting P from dried composite manures with Olsen solution are summarized in Table 23. The dry HAP and NPA composite manures contained the most Olsen-soluble forms of P, with 72.7% and 72.5% of total manure P dissolved, respectively. There were no statistically significant differences between these manures in the percentage of total manure P soluble in the Olsen extractant. The dry NPA+Phyt and HAP+Phyt manures were statistically similar in Olsen P extractability, with 66.0% and 64.7% of total manure P dissolved. The dry NPA+P composite manure contained the least base-soluble forms of P, with 31.0% of total manure P dissolved in Olsen extractant.

Table 23: Olsen-soluble P in Dry Composite Manures

Manure Type	Total P	Soluble P	
	mg kg^{-1}	mg kg^{-1}	% of total
HAP	9230	6710	72.7 a†
NPA	8960	6496	72.5 a
NPA+Phyt	9000	5940	66.0 b
HAP+Phyt	8760	5668	64.7 b
NPA+P	15240	4724	31.0 c

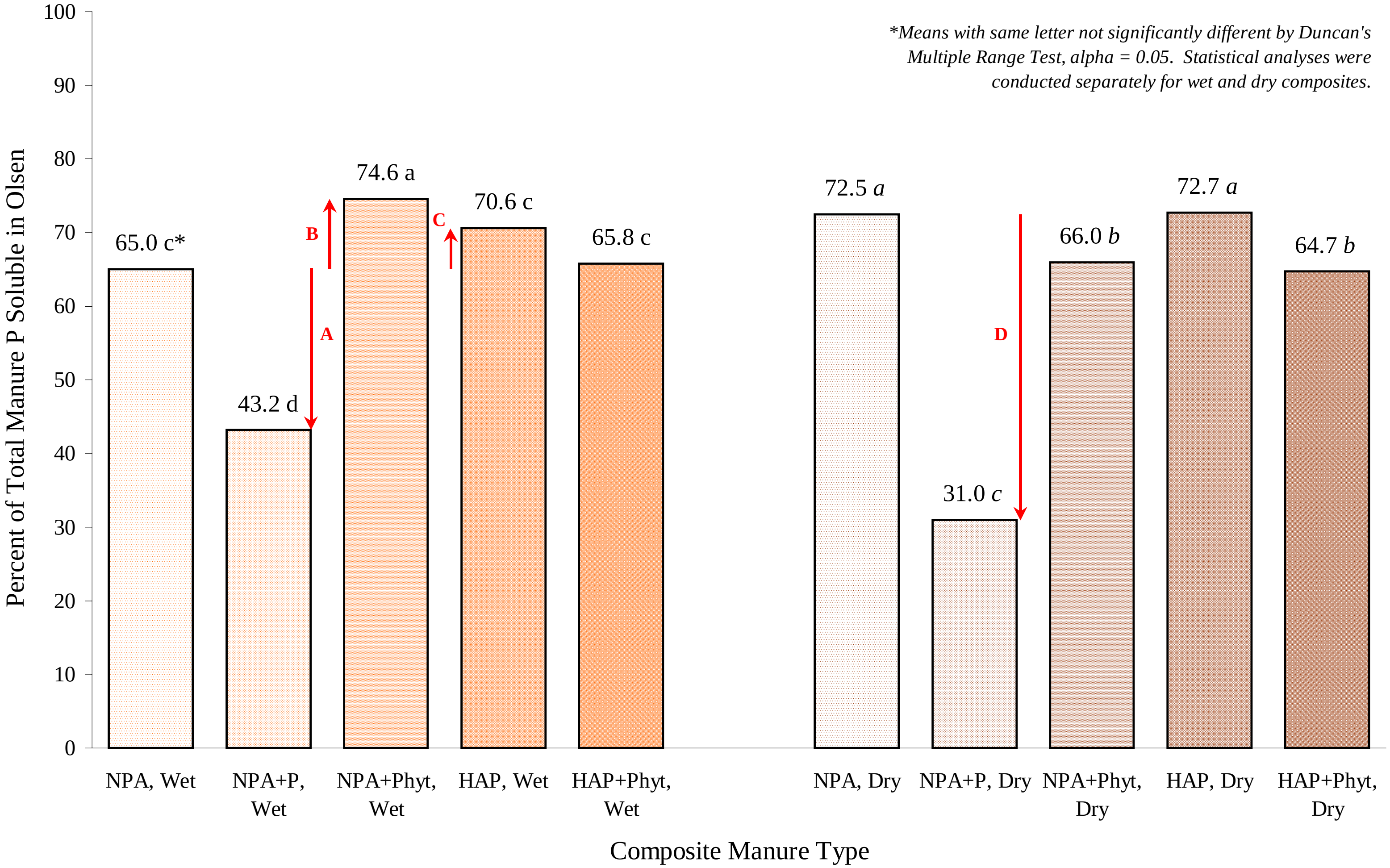
†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

Results of Olsen extraction of wet and dry composite manures from Tables 22 and 23 are compared graphically in Figure 11. The wet NPA+P and NPA+Phyt manures differed significantly in Olsen P solubility, with conversion from the conventional to the alternative diet producing a 1.73-fold increase in manure P extractability (43.2% for NPA versus 74.6% for

NPA+Phyt; represented by the combined length of line segments A and B in Figure 11). Addition of defluorinated calcium phosphate to the baseline NPA diet significantly depressed alkaline extractability of wet manure P, causing a 0.66-fold drop from 65.0% for NPA to 43.2% for NPA+P (represented by line segment A). These results are consistent with the hypothesis that the NPA+P manure contained more P in calcium phosphate forms than the other four manures, since one would expect to see calcium phosphate solubility significantly decreased in a solution with pH buffered at 8.5 such as the Olsen extractant. The addition of phytase enzyme to the baseline NPA diet significantly increased Olsen solubility of wet manure P by a factor of 1.15, from 65.0% for NPA to 74.6% for NPA+Phyt (represented by line segment B). Substitution of HAP corn for conventional corn in the baseline NPA diet also significantly increased alkaline solubility of wet manure P, from 65.0% for NPA to 70.6 % (represented by line segment C). However, the combined effect of HAP substitution and phytase addition to the NPA diet did not significantly increase wet manure P solubility. This suggests that differences in Olsen P solubility between the NPA, HAP, NPA+Phyt, and HAP+Phyt wet composite manures are not solely related to variations in phytate versus plant-derived nonphytate P levels. Therefore, no specific mechanism is proposed here to explain the Olsen P solubility differences between these four wet manures.

Figure 11 shows that drying the composite manures at 40 °C dramatically decreased the Olsen solubility of P in the NPA+P manure (from 43.2% for NPA+P wet to 31.0% for NPA+P dry). At the same time, drying increased the Olsen extractability of P in the NPA composite manure (65.0% for NPA wet versus 72.5% for NPA dry). Therefore, drying heightened the effect of adding defluorinated phosphate to the baseline NPA diet. In conjunction with manure drying, the extra inorganic P in the NPA diet led to a 0.43-fold reduction in Olsen solubility of manure P (72.5% for NPA versus 31.0% for NPA+P, represented by line segment D). Drying of composite manures increased the Olsen solubility of P in the NPA and HAP composite manures while decreasing the Olsen solubility of P in the NPA+Phyt and HAP+Phyt composite manures. As a result, P in the NPA and HAP manures was significantly more soluble (72.5% and 72.7%, respectively) than P in the NPA+Phyt and HAP+Phyt manures (66.0% and 64.7%, respectively). No specific mechanism is proposed to explain the Olsen P solubility differences between these four dry manures.

Figure 11: Olsen Solubility of P in Wet and Dry Composite Manures



Summary

Extraction of wet and dried composite manures with alkaline Olsen solution demonstrated the critical influence of feed inorganic calcium phosphate levels on base solubility of manure P. Simple addition of supplemental defluorinated phosphate to the baseline NPA diet caused the percentage of total manure P soluble in $NaHCO_3$ to drop from 65.0% to 43.2% at the point of excretion and from 72.5% to 31.0% after drying. Since none of the three alternative manures contained supplemental inorganic P, the solubility-depressing effect of defluorinated phosphate in the NPA+P feed was apparently the principal reason that P in the alternative NPA+Phyt, HAP, and HAP+Phyt manures was significantly more base soluble than P in the conventional manures, both at the point of excretion and after manure drying. At the point of excretion, both phytase addition and HAP corn addition to the baseline NPA diet caused a statistically significant increase in alkaline solubility of manure P. These individual phytase and HAP effects therefore contributed to the differences in base solubility of P between the wet NPA+P and wet NPA+Phyt and HAP manures. The combined effect of HAP-plus-phytase feeding on Olsen extractability of P from wet manures was not statistically meaningful.

2.3.4. FEED CU EXTRACTION

2.3.4.a. $CaCl_2$ Extraction of Feed Cu

The results of Cu analysis of water extracts of the five rations are presented in Table 24. The 0.01 M $CaCl_2$ solution dissolved about one third of total Cu in the feed samples. Since Cu solubility was very similar for the NPA and NPA+P feeds, it appears that the supplemental calcium phosphate in the NPA+P feed did not influence the solubility of Cu in the $CaCl_2$ extractant. The addition of phytase and/or HAP corn to the baseline NPA diet did affect water solubility of feed Cu, however. Significantly less Cu was extracted from diets supplemented with either of the alternative ingredients than from the NPA diet. This result was somewhat unexpected. As explained earlier, metals like Cu are strongly chelated by phytate into highly insoluble complexes. Feeding phytase is known to promote release and increased availability of these metals through breakdown of the phytate molecule. Although the phytase enzyme in the NPA+Phyt and HAP+Phyt feeds was probably activated during the 1-h $CaCl_2$ extraction, significantly less Cu was extracted from these feeds than from the NPA feed. No mechanism is proposed to explain these findings.

Table 24: Water Solubility of Cu in Feeds

Diet	Total Cu	Soluble Cu	
	mg kg^{-1}	mg kg^{-1}	% of total
NPA	13.8	5.3	38.3 a†
NPA+P	13.7	5.1	36.9 a
NPA+Phyt	13.7	3.9	28.8 b
HAP	13.8	3.9	28.5 b
HAP+Phyt	13.8	3.8	27.8 b

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

2.3.4.b. Mehlich III Extraction of Feed Cu

Results of Cu analysis of Mehlich III extracts of feeds are shown in Table 25. The acidic Mehlich III solution extracted about the same amount of Cu from the feeds as did the $CaCl_2$ extractant. The negative control NPA feed again released the greatest total amount and percentage of Cu during the Mehlich III extraction. In this case, all modifications of the baseline NPA diet, including addition of supplemental defluorinated calcium phosphate and addition of phytase enzyme, appeared to significantly reduce the extractability of feed Cu. In fact, as the percentage of P in phytate form in the feeds increased (Table 2), the Mehlich III solubility of feed Cu also increased (r^2 of 0.90, plot not shown). Given the phytate molecule’s strong chelating properties, this result was unexpected. Again, no specific mechanisms are proposed to explain these results.

Table 25: Mehlich III Solubility of Cu in Feeds

Diet	Total Cu	Soluble Cu	
	mg kg^{-1}	mg kg^{-1}	% of total
NPA	13.8	5.1	37.0 a†
HAP	13.8	4.4	31.8 b
NPA+P	13.7	4.3	31.7 b
HAP+Phyt	13.8	3.9	28.4 b
NPA+Phyt	13.7	3.7	27.3 b

†Means with same letters not significantly different by Duncan’s Multiple Range Test, alpha = 0.05

2.3.4.c. Olsen Extraction of Feed Cu

The results of Olsen extraction of feed Cu are shown in Table 26. The alkaline Olsen solution was similar to the other two extractants in its ability to dissolve Cu in the rations. The pattern of Olsen solubility of Cu among the five diets was similar to that obtained with 0.01 M $CaCl_2$, although statistically significant differences were generally less pronounced. Again, feeds containing phytase tended to release the least Cu. Recall, however, that phytase enzyme in the feeds was probably not significantly activated by the alkaline Olsen extractant.

Table 26: Olsen Solubility of Cu in Feeds

Diet	Total Cu	Soluble Cu	
	mg kg^{-1}	mg kg^{-1}	% of total
NPA+P	13.7	4.9	35.9 a†
NPA	13.8	4.5	32.5 ab
HAP	13.8	4.5	32.4 ab
NPA+Phyt	13.7	3.7	26.8 bc
HAP+Phyt	13.8	3.4	24.4 c

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

2.3.4.d. Summary

Few meaningful conclusions can be drawn from the feed Cu extraction results. It appears that addition of defluorinated calcium phosphate to the baseline diet did not exert much influence on the availability of feed Cu. Dietary phytase enzyme and HAP corn, ingredients which presumably reduced the proportion of feed P in phytate form during the $CaCl_2$ and Mehlich III extractions, certainly did not improve the solubility of feed Cu. It is unclear how addition of these ingredients to the NPA rations caused the significant decreases in Cu solubility observed with all three extractants. As the following pages confirm, extraction of Cu from feeds did not reveal trends helpful for interpretation of the manure Cu extraction results.

2.3.5. MANURE CU EXTRACTION

2.3.5.a. $CaCl_2$ Extraction of Manure Cu

Individual Pen Manure Samples

Results of total Cu analysis conducted on $CaCl_2$ extracts of wet individual pen manures are summarized in Table 27. Extraction of the wet individual pen manures revealed no statistically significant differences in Cu solubility between the five manure types. Even when the results of statistical analysis are disregarded and solubility percentages are compared on a strictly numercial basis, no obvious relationships between diet formulation and water solubility of manure Cu can be discerned. The critical comparison between the NPA+P and NPA+Phyt manures reveals almost identical Cu water solubility percentages, with 33.5% and 33.4% of total manure Cu dissolved in the 0.01 M $CaCl_2$ extract.

Table 27: Water-soluble Cu in Manures from All Individual Pens

Manure Type	Total Cu	Soluble Cu	
	mg kg^{-1}	mg kg^{-1}	% of total
NPA+P	30.3	10.2	33.5 a†
NPA+Phyt	30.3	10.1	33.4 a
HAP	31.6	10.5	33.3 a
NPA	29.5	8.7	29.6 a
HAP+Phyt	29.4	7.6	26.0 a

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

Results from $CaCl_2$ extraction of Cu from manures from selected individual pens are summarized in Table 28. The only difference between this table and Table 27 is that Cu extraction data from the five pens that produced manures with unusually high Zn levels were not included in calculation of means in Table 28. Table 28 is presented in order to support the argument that potential Zn contamination in a few of the individual pen manure samples did not impact Cu solubility relationships between the five main manure types. Excluding data from the potentially contaminated pens increased the statistical significance of differences in Cu water-solubility between the manures. However, the relative solubility rankings among the five manure types were similar in Tables 27 and 28, with the NPA+P and NPA+Phyt manures showing the greatest Cu solubility in water and the HAP+Phyt manure showing the least. In addition, the key comparison between the NPA+P and NPA+Phyt manures again showed little difference in the percentages of total manure Cu soluble in the $CaCl_2$ extractant.

Table 28: Water-soluble Cu in Manures from Selected Individual Pens

Manure Type	Total Cu	Soluble Cu	
	mg kg^{-1}	mg kg^{-1}	% of total
NPA+Phyt	29.6	10.4	34.9 a†
NPA+P	30.3	10.2	33.5 ab
HAP	32.2	10.2	31.6 ab
NPA	30.0	7.9	26.2 bc
HAP+Phyt	29.8	7.1	23.8 c

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

<u>Composite Manure Samples</u>

The results of Cu analysis conducted on water extracts of wet composite manure samples are summarized in Table 29. As expected, these results are similar to those presented above for the wet individual pen extracts. Water extraction of wet composites did reveal more pronounced differences in solubility between the manures, with the NPA+Phyt, NPA+P, and HAP manures showing significantly greater Cu solubility percentages than the HAP+Phyt and NPA manures. Nevertheless, the relative solubility ranking among the five manure types remained consistent, as did the similarity in Cu extractability between the NPA+P (32.2%) and NPA+Phyt (29.6%) manures. Again, no obvious relationship between dietary formulation and manure Cu solubility could be identified.

The effects of drying on composite manure Cu solubility are shown in Table 30. Drying significantly enhanced the extractability of Cu in all five types of manure. However, the degree to which Cu solubility increased with drying varied across treatments. For example, Cu solubility doubled with drying in the HAP+Phyt manure, but only increased by a factor of 1.32 in the NPA+Phyt manure. The net result was a solubility pattern in which Cu in the two manures derived from phytase-amended diets was significantly less water soluble than Cu in the other three manures. No specific mechanism is proposed to explain this drying-induced shift in Cu solubility pattern, which produced a statistically significant difference in water solubility of Cu between the NPA+P and NPA+Phyt composite manures. While 48.5% of total Cu in the dried NPA+P manure dissolved in water, only 42.6% of Cu in the dry NPA+Phyt manure was water soluble.

Table 29: Water-soluble Cu in Wet Composite Manures

Manure Type	Total Cu	Soluble Cu	
	mg kg^{-1}	mg kg^{-1}	% of total
NPA+Phyt	30.7	9.9	32.2 a†
NPA+P	30.7	9.1	29.6 a
HAP	28.6	8.3	29.1 a
HAP+Phyt	29.6	6.8	23.0 b
NPA	30.5	6.6	21.6 b

†Means with same letters not significantly different by Duncan’s Multiple Range Test, alpha = 0.05

Table 30: Water-soluble Cu in Dry Composite Manures

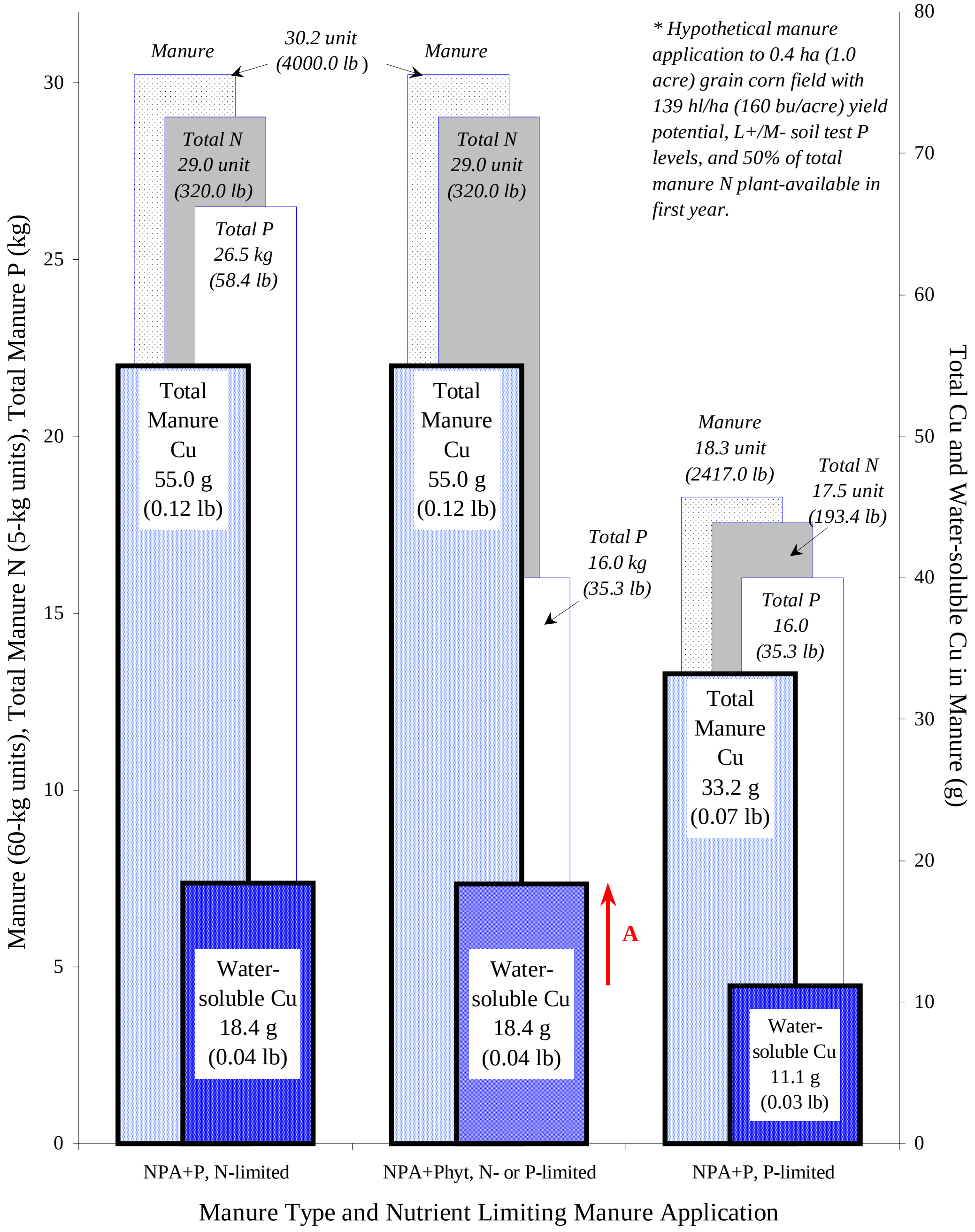
Manure Type	Total Cu	Soluble Cu	
	mg kg^{-1}	mg kg^{-1}	% of total
NPA+P	33.0	16.0	48.5 a†
NPA	32.4	15.6	48.1 a
HAP	34.9	16.5	47.4 a
HAP+Phyt	33.4	15.4	46.0 b
NPA+Phyt	34.0	14.5	42.6 c

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

Implications for Land Application

The implications of the Cu solubility results are illustrated with hypothetical land application examples similar to those used previously. The amounts of total Cu and water-soluble Cu that would be applied to soil if the NPA+P and NPA+Phyt individual pen manures were applied to a hypothetical corn field are shown in Figure 12. Again, both N- and P-based manure management scenarios are illustrated. Note that the quantities of manure, total N, and total P applied to soil (illustrated by the background columns) are identical to the examples used previously to illustrate the results of P extraction from wet manures. However, different units (left-hand axis) are used for these parameters in Figure 12. The foreground columns (values on the right-hand axis) compare the amounts of total manure Cu and water-soluble manure Cu applied to the land.

Figure 12: Water-soluble Cu Loadings from N- and P-limited Land Application of Wet NPA+P and NPA+Phyt Manures †



Under N-limited manure management, switching from the conventional to the phytase diet does not affect either total Cu or water-soluble Cu loadings to soil. This is because the wet conventional and alternative manures did not differ in total Cu content or in percentage of Cu soluble in $CaCl_2$. In the P-limited manure application scenario, conversion to phytase feeding produces a 65% increase in the total amount of manure Cu loaded on soils. This increase in total Cu applied in turn results in a 65% increase in water-soluble Cu loading, illustrated by line segment A in Figure 12. Although this increase in Cu loading is dramatic, it must be emphasized that even the highest Cu application in this particular example (0.13 kg ha^{-1} or 0.12 lb $acre^{-1}$ total Cu) is relatively low from an agronomic or environmental point of view. A harvest of 9.41 Mg (150 bu) of corn grain, as might be expected from the hypothetical 0.4 ha (1.0 acre) field in this example, removes approximately 0.07 kg (0.06 lb) of Cu from the field (Virginia Cooperative Extension, 1987).

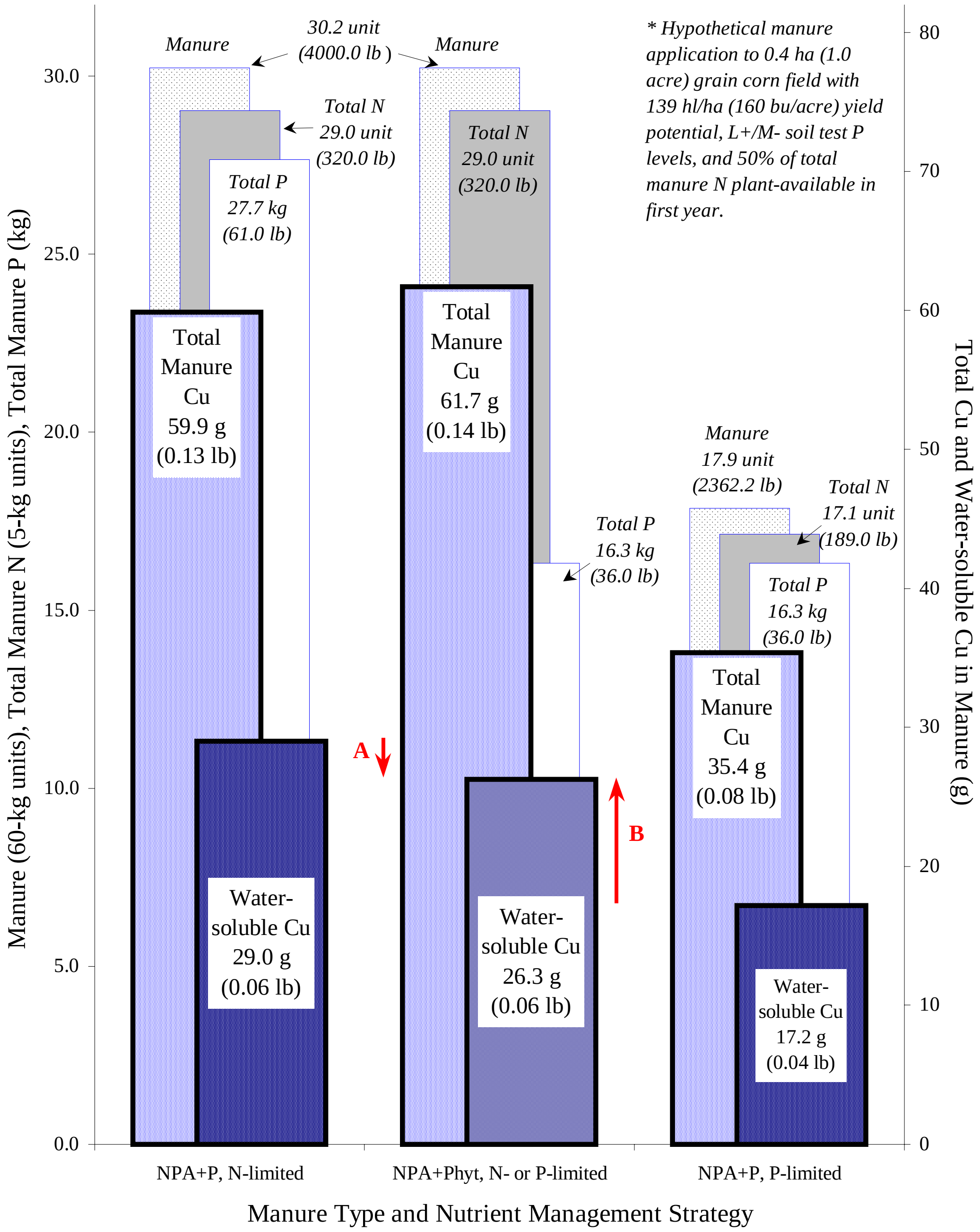
A similar hypothetical land application example is shown in Figure 13, but in this case, Cu loadings reflect the results of Cu extraction from dry composite manures from Table 30. When application of dry manure to soil is limited on the basis of N, converting from the NPA+P to the alternative NPA+Phyt feed results in a 3% increase in the total amount of manure Cu applied to the land, but a 10% decrease in the total amount of water-soluble Cu applied (from 29 g for NPA+P, N-limited to 26 g for NPA+Phyt). Under P-based manure management, switching from the conventional NPA+P feed to the NPA+Phyt feed results in a 74% increase in the total amount of Cu applied, but only a 53% increase in the total loading of water-soluble Cu (from 17 g for NPA+P, P-limited to 26 g for NPA+Phyt).

Summary

Extraction of wet manures with 0.01 M $CaCl_2$ showed that the Cu in conventional NPA+P and alternative NPA+Phyt manures did not differ in water solubility at the point of excretion. Overall, the five different dietary treatments produced no meaningful differences or trends in $CaCl_2$ extractability of Cu from wet manures. Drying the manures at moderate temperature for 4 d caused a minor shift in Cu solubility patterns. Water solubility of Cu was a few percentage points lower in dry manures derived from phytase-amended feeds than in other dry manures. These differences were statistically significant. Therefore, after drying, Cu solubility was significantly lower in the alternative NPA+Phyt manure than in the conventional NPA+P manure.

These results suggest that, when land application of manure is limited on an N basis, conversion to a phytase-amended diet may not affect the amount of total or water-soluble manure Cu applied to the land. This is especially true if manure is land-applied immediately after excretion. Transformation of manure compounds after excretion may significantly alter this outcome. For example, if the manures from this experiment had been land-applied on an equal-N basis after drying, the phytase-derived manure would have loaded 10% less water-soluble Cu on soils than the conventional manure. Although use of phytase may not significantly affect the percentage of manure Cu soluble in water, it produces major increases in both the total amount of manure and manure Cu that can be land applied when manure spreading is limited on a P basis. Therefore, under P-based nutrient management, conversion to phytase-amended diets is likely to cause a net increase in the total amount of readily dissolved manure Cu applied to the land.

Figure 13: Water-soluble Cu Loadings from N- and P-limited Land Application of Dry NPA+P and NPA+Phyt Manures †



2.3.5.b. Mehlich III Extraction of Manure Cu

Individual Pen Manure Samples

Results of total Cu analysis conducted on Mehlich III extracts of all wet individual pen manures are summarized in Table 31. The wet NPA+P manure contained the most acid-soluble forms of Cu, with 48.5% of manure Cu dissolved in the Mehlich III extractant. The manures derived from phytase-amended diets differed from each other numerically, but not statistically, in the percentage of total manure Cu soluble in Mehlich III, with 37.7% for HAP+Phyt and 32.8% for NPA+Phyt. The wet HAP and NPA manures contained the lowest percentage of Mehlich III-soluble Cu, with 28.6% and 26.8% of total Cu extracted, respectively. The NPA+Phyt, HAP, and NPA manures did not differ statistically from each other. When the means in Table 31 are adjusted by excluding data associated with the five pens suspected of Zn contamination, the values in Table 32 are obtained. The similarities between these two tables again suggests that any potential Zn contamination of manure samples did not affect overall Mehlich III Cu solubility relationships among the five manure types.

Table 31: Mehlich III-soluble Cu in Manures from Individual Pens, Means for Treatments

Manure Type	Total Cu	Soluble Cu	
	mg kg^{-1}	mg kg^{-1}	% of total
NPA+P	30.3	14.7	48.5 a†
HAP+Phyt	29.4	11.1	37.7 b
NPA+Phyt	30.3	9.9	32.8 bc
HAP	31.4	9.0	28.6 c
NPA	29.4	7.9	26.8 c

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

Table 32: Mehlich III-soluble Cu in Manures from Select Individual Pens, Means for Treatments

Manure Type	Total Cu	Soluble Cu	
	mg kg^{-1}	mg kg^{-1}	% of total
NPA+P	30.3	14.7	48.5 a†
HAP+Phyt	29.8	11.2	37.6 b
NPA+Phyt	29.6	10.8	36.4 bc
HAP	32.0	9.5	29.6 bc
NPA	29.9	8.4	28.0 c

†Means with same letters not significantly different by Duncan’s Multiple Range Test, alpha = 0.05

Composite Manure Samples

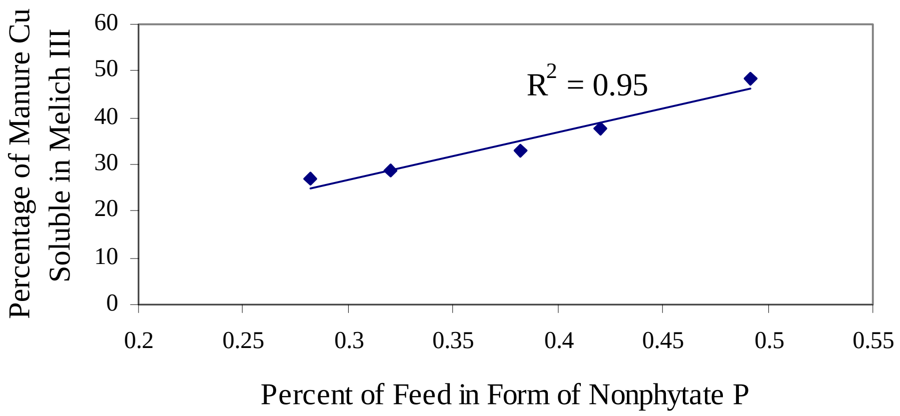
The results from Cu analysis of Mehlich III extracts of wet composite manures are shown in Table 33. This series of extractions revealed statistically significant differences in Cu solubility between all five manure types, with the greatest percentage of manure Cu solubilized from the NPA+P composite (44.2%) and the smallest from the NPA composite (20.6%). These Mehlich III extracts of wet composite manures therefore showed more pronounced Cu solubility differences among the five manure types than did the individual pen extracts. However, the three sets of means in Tables 31, 32, and 33 present an identical pattern of Cu solubility ranking among the five manure types. This solubility pattern can be directly related to the total nonphytate P contents of the feeds from which the manures were derived (recall that total nonphytate P includes both inorganic and plant-derived forms). The percentages of the different diets that consisted of nonphytate P, as predicted after activation of feed phytase enzyme, are shown in Table 2. These percentages of feed in nonphytate P form correlate very well ($r^2 = 0.95$) with wet manure Mehlich III Cu solubility percentages from Table 33, as shown in Figure 14. Correlations with select individual pen and wet composite solubility percentages from Tables 28 and 29 reveal r^2 values of 0.96 and 0.87, respectively (these two correlations not shown).

Table 33: Mehlich III-soluble Cu in Wet Composite Manures

Manure Type	Total Cu	Soluble Cu	
	mg kg^{-1}	mg kg^{-1}	% of total
NPA+P	30.7	13.6	44.2 a†
HAP+Phyt	29.6	8.6	29.2 b
NPA+Phyt	30.7	7.8	25.4 c
HAP	28.6	6.5	22.7 d
NPA	30.5	6.3	20.6 e

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

Figure 14: Relationship between Percentage of Manure Cu Soluble in Mehlich III Extractant and Nonphytate P Content of Feed



The results of total Cu analysis conducted on Mehlich III extracts of dry composite manures are summarized in Table 34. Drying at 40 °C for 4 d enhanced the Mehlich III extractability of manure Cu for all five manure types. However, the five manure types differed in the extent to which drying increased the solubility of the Cu they contained. The magnitudes of the drying-induced increases in manure Cu solubility were inversely proportional to the nonphytate P contents of feeds from which the manures were derived. The plot of this relationship (not shown) revealed an r^2 value of 0.98 for the correlation of nonphytate P feed percentage with percent increase (due to drying) in Mehlich III solubility of manure Cu. Since the effect of manure drying on Cu solubility varied in this manner, the net result was a final Mehlich III solubility pattern among the dry manures inconsistent with the pattern observed for the wet manures. The dry NPA+P manure still contained the most soluble form of Cu (53.8%), but the dry manures derived from phytase-amended feeds now contained the least soluble forms of Cu (46.8% for HAP+Phyt and 43.5% for NPA+Phyt).

Table 34: Mehlich III-soluble Cu in Dry Composite Manures

Manure Type	Total Cu	Soluble Cu	
	mg kg^{-1}	mg kg^{-1}	% of total
NPA+P	33.0	17.8	53.8 a†
NPA	32.4	16.0	49.3 b
HAP	34.9	16.5	47.2 c
HAP+Phyt	33.4	15.6	46.8 c
NPA+Phyt	34.0	14.8	43.5 d

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

Although strong correlations between Mehlich III solubility of manure Cu and feed formulation can be readily drawn, few specific suggestions are offered regarding the underlying causal mechanism. The phytate molecule readily chelates metals such as Cu to form highly insoluble complexes. It is tempting to suggest that, as nonphytate P feed content increased, the solubility of Cu in the manures increased due to a reduction in manure phytate content and phytate chelation of Cu. This does not appear to be correct, however, because the NPA+P diet was the feed containing the highest level of phytate as well as total nonphytate P (Table 2). What can be stated with confidence is that Mehlich III solubility of Cu in wet manures increased as the total amount of nonphytate P (i.e., plant-derived nonphytate P and inorganic P) in feeds increased. The variable influence of drying on manure Cu solubility is also difficult to explain. The principal observation to be made is that drying at moderate temperature appears to have increased the Mehlich III solubility of Cu that was associated in some way with feed components other than nonphytate P.

Implications for Land Application

Despite the complexity of the preceeding discussion, the implications of the Mehlich III Cu solubility results are relatively straightforward. The Cu in the conventional NPA+P manures was significantly more Mehlich III-soluble than the Cu in the alternative NPA+Phyt manures. This was true when both wet manures and dry manures were extracted. The results of Mehlich III extraction of Cu from wet individual pen manures are shown in Figure 15 in the context of the familiar hypothetical land application example. Under N-based nutrient management, switching from the conventional NPA+P diet to the alternative NPA+Phyt diet does not affect total Cu applied to soil, but reduces loading of Mehlich III-soluble Cu by 32% (from 26.7 g for NPA+P to 18.0 g for NPA+Phyt). Under P-based nutrient management, the conversion to phytase-amended feed boosts total Cu loadings to soil by 65%, but only causes a 12% increase in the amount of land-applied Mehlich III-soluble Cu (from 16.1 g for NPA+P to 18.0 g for NPA+Phyt).

Drying reduced the numerical differences in Mehlich III Cu solubility between the NPA+P and NPA+Phyt manures. Figure 16 shows how manure drying affects Cu loadings to a hypothetical corn field receiving manure. Under N-based nutrient management, switching from the conventional NPA+P diet to the alternative NPA+Phyt diet causes a slight increase in total Cu applied to soil, but reduces loading of Mehlich III-soluble Cu by 17% (from 32.2 g for NPA+P to 26.8 g for NPA+Phyt). When manure application is limited on a P basis, the conversion to phytase-amended feed boosts total Cu loadings to soil by 74% and causes a 41% increase in land application of Mehlich III-soluble Cu (from 19.0 g for NPA+P to 26.8 g for NPA+Phyt).

Figure 15: Mehlich III-soluble Cu Loadings from N- and P-limited Land Application of Wet NPA+P and NPA+Phyt Manures†

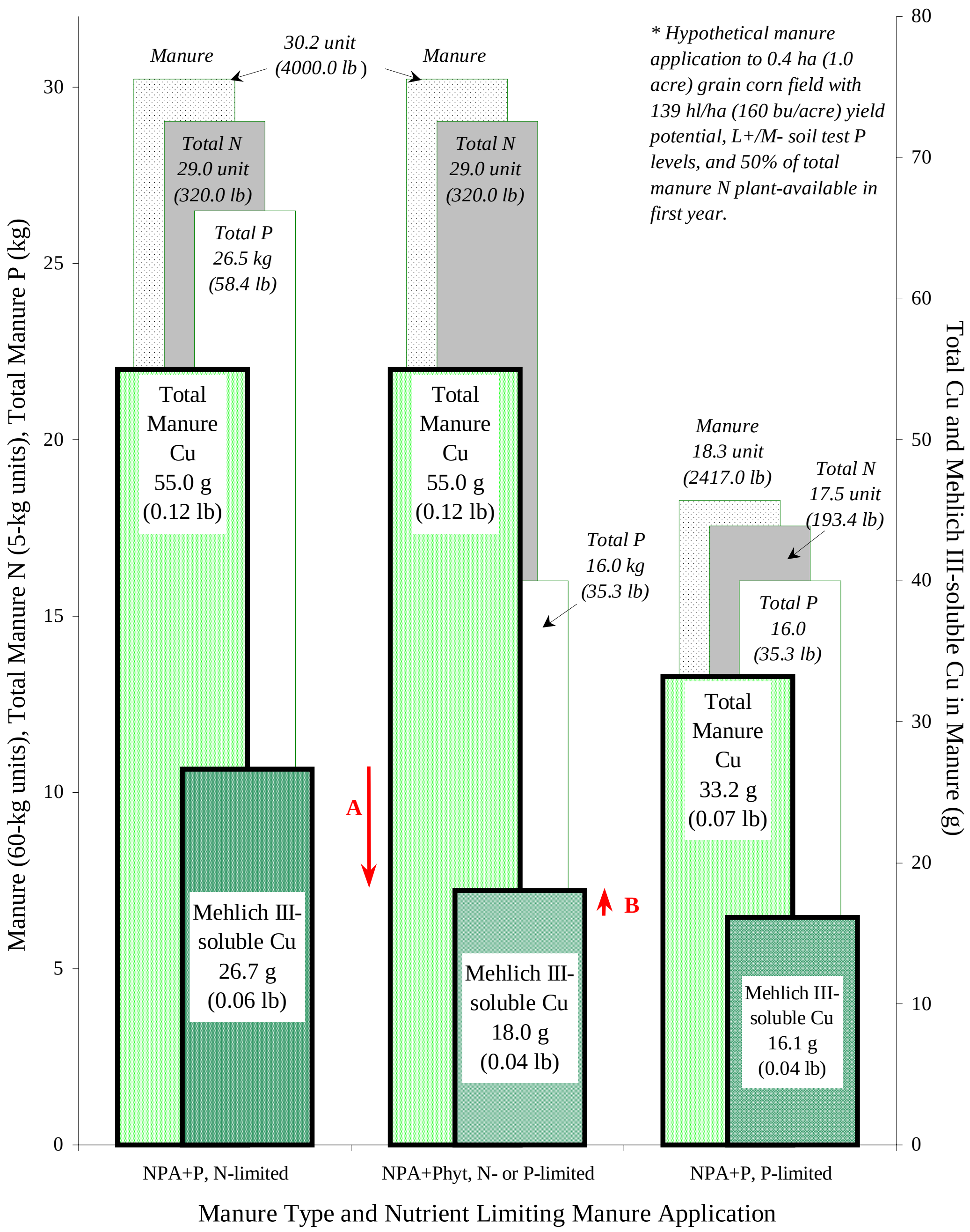
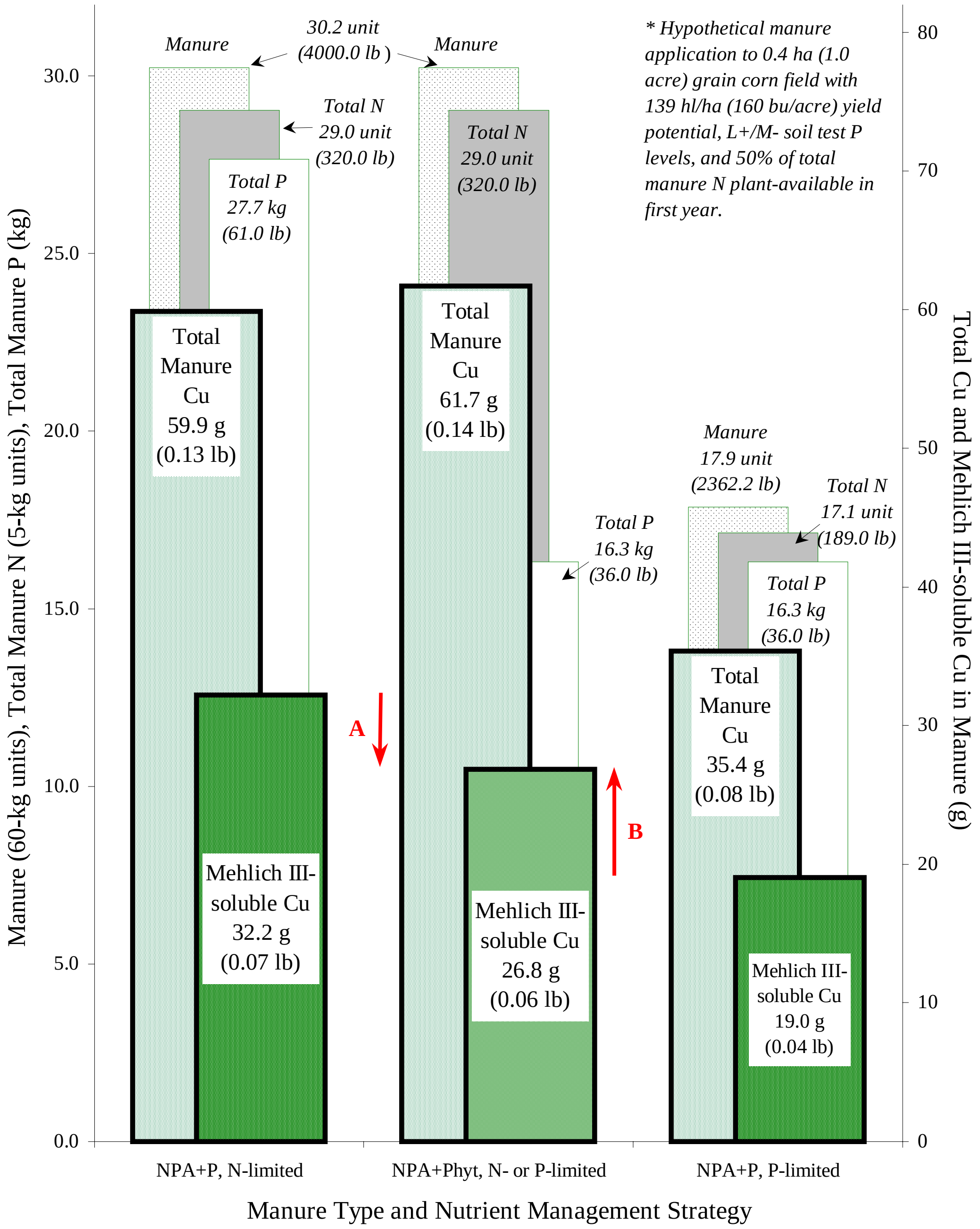


Figure 16: Mehlich III-soluble Cu Loadings from N- and P-limited Land Application of Dry NPA+P and NPA+Phyt Manures †



As a final note, it should be emphasized that differences in Mehlich III solubility of Cu between the NPA+P and NPA+Phyt manures would probably be minimized under commercial conditions. This is because the experimental NPA+Phyt feed contained less than NRC-recommended levels of total nonphytate P (as predicted after phytase activation). As explained earlier, a commercial producer would likely boost total nonphytate P levels in the NPA+Phyt manure to approach those in a traditional ration. It has been shown that Mehlich III manure Cu solubility was directly related to total nonphytate P in feeds. This suggests that equalization of total nonphytate P levels in the two types of feeds would lead to similar acid solubility of Cu between manures derived from the conventional and alternative feeds.

Summary

Mehlich III extraction of Cu from wet manures showed that Cu solubility was directly proportional to the percentage of feed P in nonphytate form. The net result was that all modifications to the baseline NPA diet, including addition of phytase enzyme and addition of defluorinated phosphate, led to numerical increases in Mehlich III solubility of manure Cu at the point of excretion. Another key result was that Cu in the conventional NPA+P manure was significantly more Mehlich III-soluble than Cu in the alternative NPA+Phyt manure. It should be noted that, in a commercial situation, total nonphytate P levels in alternative and phytase feeds would likely be equalized. The linear relationship between feed nonphytate P levels and Cu solubility observed may indicate that Mehlich III Cu solubility differences would disappear under these circumstances.

Drying manures prior to Mehlich III extraction shifted the overall Cu solubility pattern. The Cu in the conventional NPA+P manure remained significantly more Mehlich III-soluble than Cu in the alternative NPA+Phyt manure, although drying produced a numerical decrease in the solubility difference. After drying, manures derived from phytase-containing feeds contained the least soluble forms of Cu.

Implications for land application are as follows. If the wet experimental manures were land applied under N-based nutrient management, switching from the conventional NPA+P diet to the alternative NPA+Phyt diet would not change total Cu loading, but would reduce soil loadings of acid-soluble manure Cu by 32%. Drying reduced Mehlich III Cu solubility differences between the manures. Therefore, N-limited application of the alternative NPA+Phyt manure would load soils with 17% less acid-soluble Cu than the conventional NPA+P manure. If P were the nutrient limiting manure applications, conversion to the alternative NPA+Phyt diet would increase total Cu loading by 65% to 74% for wet and dry manures, respectively. Soil loadings of acid-soluble manure Cu would increase by 12% and 41% under the same circumstances.

2.3.5.c. Olsen Extractability of Manure Cu

Composite Manure Samples

Results of Cu analysis conducted on Olsen extracts of wet composite manures are summarized in Table 35. These results reveal a Cu solubility pattern among the five manures similar to that obtained by water extraction of the composites (Table 29). The NPA+Phyt and NPA+P manures showed numerically and statistically similar Olsen Cu solubility percentages. These manures contained the most Olsen-soluble forms of Cu, while the Cu in the NPA and HAP+Phyt manures was the least soluble. Statistical differences among the manures were somewhat less pronounced with Olsen than with water extraction of Cu. Overall, the $NaHCO_3$ solution was slightly more effective in solubilizing Cu from wet manures than was the $CaCl_2$

extractant. No clear relationship between manure Cu Olsen solubility percentage and feed formulation could be identified.

Table 35: Olsen-soluble Cu in Wet Composite Manures

Manure Type	Total Cu	Soluble Cu	
	mg kg^{-1}	mg kg^{-1}	% of total
NPA+Phyt	30.7	11.1	36.1 a†
NPA+P	30.7	11.0	35.7 a
HAP	28.6	9.3	32.5 ab
NPA	30.5	9.6	31.6 ab
HAP+Phyt	29.6	8.1	27.5 b

†Means with same letters not significantly different by Duncan’s Multiple Range Test, alpha = 0.05

The effects of drying manure on Olsen solubility of Cu are shown in Table 36. Drying increased the alkaline solubility of Cu for all five manure types, although not as greatly as it increased water extractability. The five manures differed in the degree to which drying enhanced Olsen solubility. This variability produced the familiar pattern in which, after drying, the two manures derived from feeds containing phytase contained the least soluble forms of Cu. Therefore, Cu in the dry NPA+P manure was significantly more Olsen-extractable than Cu in the dry NPA+Phyt manure. No particular mechanism is proposed to explain why this pattern was observed with all three extractants.

Table 36: Olsen-soluble Cu in Dry Composite Manures

Manure Type	Total Cu	Soluble Cu	
	mg kg^{-1}	mg kg^{-1}	% of total
HAP	34.9	15.6	44.8 a†
NPA	32.4	14.4	44.4 a
NPA+P	33.0	14.6	44.1 a
HAP+Phyt	33.4	14.6	43.8 a
NPA+Phyt	34.0	13.1	38.6 b

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

Implications for Land Application

The Olsen extractant revealed relative differences in Cu solubility between the NPA+P and NPA+Phyt manures much like those observed upon extraction with $CaCl_2$. This was true of wet as well as dry composite manures. Therefore, incorporating the Olsen Cu extraction results into the familiar land application example would produce charts very similar to Figures 12 and 13.

Summary

Olsen extraction of wet excreta revealed no statistically significant differences in Cu solubility between the NPA+P and NPA+Phyt manures. Overall, there were few statistically significant differences between treatments in alkaline solubility of manure Cu. No obvious relationships between Olsen solubility of manure Cu and dietary formulation were discerned. Drying manures increased Olsen extractability of manure Cu and caused a shift in solubility patterns. After drying, the NPA+P manure contained Cu that was significantly more base-soluble than Cu in the NPA+Phyt manure.

2.3.6. FEED ZN EXTRACTION

2.3.6.a. $CaCl_2$ Extraction of Feed Zn

Results of extracting Zn from feeds with 0.01 M $CaCl_2$ are shown in Table 37. Overall, water solubility of Zn in the feeds was low. Since phytate is a strong chelator of Zn, alternative feeds containing relatively less phytate P and more plant-derived nonphytate P were expected to release more Zn during extraction. This trend was generally observed, as significantly more Zn was dissolved from the NPA+Phyt and HAP+Phyt feeds than from the baseline NPA feed. The NPA+P and NPA feeds did not differ in the amount of phytate versus plant-derived nonphytate P they contained. Therefore, it is unclear why Zn in the NPA+P feed was so much more water-soluble than Zn in the baseline NPA feed.

Table 37: $CaCl_2$ Solubility of Zn in Feeds

Diet	Total Zn	Soluble Zn	
	mg kg^{-1}	mg kg^{-1}	% of total
NPA+Phyt	104.4	10.4	10.0 a†
NPA+P	104.3	8.1	7.8 b
HAP+Phyt	104.9	7.9	7.6 b
HAP	104.6	6.4	6.2 c
NPA	105.2	5.1	4.8 c

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

2.3.6.b. Mehlich III Extraction of Feed Zn

Results of Zn analysis of the Mehlich III feed extracts are presented in Table 38. The acidic, chelate-containing Mehlich III extractant solubilized more Zn from feed samples than did the dilute $CaCl_2$ solution. While water solubility of feed Zn was increased by the supplemental calcium phosphate in the NPA+P diet, the NPA+P and baseline NPA feeds did not differ in Mehlich III extractability of Zn. Furthermore, no clear pattern of phytase enzyme or HAP corn influence on acid solubility of feed Zn could be discerned, since significant differences in acid solubility of Zn were observed between the HAP+Phyt and the NPA+Phyt and HAP feeds.

Table 38: Mehlich III Solubility of Zn in Feeds

Diet	Total Zn	Soluble Zn	
	mg kg^{-1}	mg kg^{-1}	% of total
NPA+Phyt	104.4	56.9	54.5 a†
HAP	104.6	55.5	53.0 a
NPA+P	104.3	48.5	46.5 b
NPA	105.2	48.8	46.4 b
HAP+Phyt	104.9	39.3	37.5 c

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

2.3.6.c. Olsen Extraction of Feed Zn

Results of Olsen extraction of Zn from feeds are shown in Table 39. In this case, phytase enzyme addition and calcium phosphate supplementation to the baseline NPA diet appeared to depress Olsen solubility of Zn. It is important to recall that phytase enzyme activation was probably minimal during the alkaline Olsen extraction process. This may help explain the contrast between the Olsen and $CaCl_2$ Zn solubility patterns. The conventional NPA+P feed was numerically lower, but not statistically different in Zn solubility from the alternative NPA+Phyt feed.

2.3.6.d. Summary

As might be expected, Zn in feeds containing phytase enzyme and/or HAP corn was generally more water-soluble than Zn in the baseline NPA feed. This pattern was not observed with the Mehlich III and Olsen extractants. Addition of supplemental defluorinated calcium phosphate to the baseline NPA diet increased $CaCl_2$ solubility of feed Zn, decreased Olsen extractability of feed Zn, and did not modify Mehlich III solubility of feed Zn. Overall, few clear and convincing trends regarding the effects of dietary formulation on solubility of feed Zn were revealed.

Table 39: Olsen Solubility of Zn in Feeds

Diet	Total Zn	Soluble Zn	
	mg kg^{-1}	mg kg^{-1}	% of total
HAP	104.6	32.8	31.4 a†
NPA	105.2	31.6	30.1 a
NPA+Phyt	104.4	30.2	28.9 ab
NPA+P	104.3	26.0	24.9 bc
HAP+Phyt	104.9	24.0	22.9 c

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

2.3.7. MANURE ZN EXTRACTION

As indicated previously, five pens in the original feeding trial produced manures with unusually high Zn contents. The most likely explanation is contamination by metal trays used in excreta collection. Since $CaCl_2$ and Mehlich III extractions were conducted on subsamples of manure from each of the 34 individual pens manures used in this study, it was possible to exclude data associated with the five suspect pens from calculation of means and other analyses. The preceding sections on manure P and Cu solubility compared means for individual pen manures calculated both with and without data from the five suspect pens. The comparisons suggested that potential Zn contamination did not affect the P and Cu solubility trends among the study's five manure types. This supports the argument that results from P and Cu extraction of composite manures are valid, even though manures from the five suspect pens were included when composites were prepared. The following paragraphs present evidence that potential Zn contamination likewise did not affect the Zn solubility relationships between the study's five manure types. Nevertheless, conclusions about Zn solubility will be drawn primarily from individual pen extraction results after exclusion of data from the five suspect pens. Since the composite manures probably contained Zn from a source other than the birds, results from extraction of composites must be viewed with caution.

2.3.7.a. $CaCl_2$ Extraction of Manure Zn

Individual Pen Manure Samples

Results of $CaCl_2$ extraction of Zn from all individual pen manures are shown in Table 40. Results of $CaCl_2$ extraction of Zn from select individual pens (i.e., data from the five pens suspected of contamination are excluded) are summarized in Table 41. Note the dramatic differences between the two tables with regard to the "Total Zn" column. Despite these differences, the tables present extremely similar Zn solubility trends, with identical statistical groupings and comparable solubility percentages for each manure type. Table 41 shows that $CaCl_2$ solubility of Zn for the NPA+P manure (11%) was significantly lower than for the other four manure types (51.0% to 61.5%). The NPA, HAP, NPA+Phyt, and HAP+Phyt manures differed from each other in Zn water solubility percentage numerically, but not statistically. Note how little Zn (28.9 mg) was extracted per kg of NPA+P manure in comparison with the other manure types.

Table 40: Water Solubility of Zn in Manures from All Individual Pens

Diet	Total Zn	Soluble Zn	
	$mg\ kg^{-1}$	$mg\ kg^{-1}$	% of total
NPA+Phyt	444.6	277.7	62.5 a†
HAP	343.5	185.2	53.9 a
HAP+Phyt	342.3	180.9	52.9 a
NPA	364.9	174.2	47.7 a
NPA+P	263.7	28.9	11.0 b

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

Table 41: Water Solubility of Zn in Manures from Selected Individual Pens

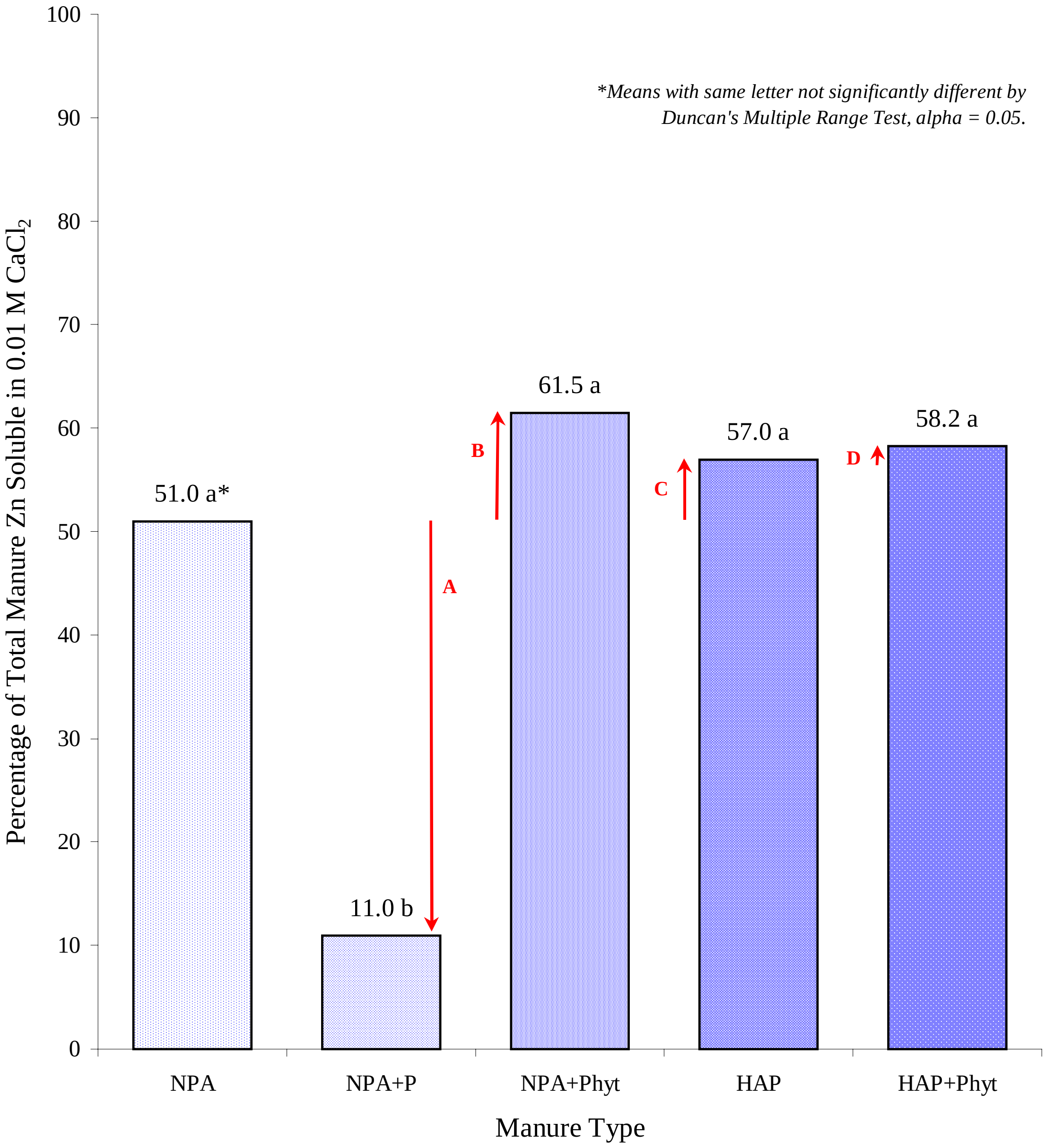
Diet	Total Zn	Soluble Zn	
	mg kg^{-1}	mg kg^{-1}	% of total
NPA+Phyt	246.6	151.7	61.5 a†
HAP+Phyt	223.5	130.2	58.2 a
HAP	258.6	147.2	57.0 a
NPA	284.2	145.0	51.0 a
NPA+P	263.7	28.9	11.0 b

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

The data in Table 41 are presented in graphic form in Figure 17. This figure illustrates the dramatic difference in water solubility of manure Zn between the conventional NPA+P (11.0%) and alternative NPA+Phyt (61.5%) manures, represented by the combined length of line segments A and B. It appears that the supplemental defluorinated phosphate in the baseline NPA diet, represented by line segment A, accounted for the majority of this difference. This is not surprising when one considers that Zn tends to bond with phosphates to form insoluble compounds. As a result of dietary inorganic P supplementation, the NPA+P manure contained by far the largest amounts of P of all the excreta types. It is hypothesized that water-extractable Zn was lowest in the NPA+P manure at least in part because more P was available in this material for the formation of water-insoluble Zn compounds.

The addition of phytase enzyme to poultry rations is known to improve the nutritional availability of Zn by reducing the number of phytate chelates available for metal complexation. One question this study sought to answer was whether the same enhancement of Zn availability would carry through to manure. Figure 17 shows that a reduction in phytate P levels in feeds (through use of phytase enzyme, HAP corn, or both) increased $CaCl_2$ solubility of manure Zn numerically, but not statistically. For example, the addition of phytase to the baseline NPA diet caused water solubility of manure Zn to rise from 51.0% to 61.5%, as represented by line segment B. However, this solubility difference was not statistically significant. Given that the NPA and NPA+Phyt manures did not differ statistically in Zn solubility percentage, differences in Zn extractability between the conventional NPA+P and alternative NPA+Phyt manures can be attributed primarily to the solubility-depressing effect of dietary inorganic P supplementation. Substitution of HAP to the baseline NPA diet as well as addition of phytase to the HAP diet also increased water solubility of manure Zn numerically, but not statistically, as represented by line segments C and D.

Figure 17: Water Solubility of Zn from Manures from Select Individual Pens



Composite Manure Samples

Results of water extraction of Zn from wet and dry composite manures are presented in Tables 42 and 43. As indicated previously, the composites included quantities of the five individual pen manures potentially contaminated with Zn. Therefore, important conclusions regarding Zn solubility should be drawn from the results of extraction of select individual pen manures in Table 41. The composite $CaCl_2$ extraction results are presented here mainly to point out the effect of drying on manures derived from phytase-amended feeds. Drying enhanced or did not modify the percentage of total Zn extracted with $CaCl_2$ from the NPA, HAP, and NPA+P composites. However, drying dramatically decreased the water solubility of manure Zn in both the NPA+Phyt and HAP+Phyt composites. This may be a more dramatic example of the same phenomenon seen with Cu, where manure drying caused the NPA+Phyt and HAP+Phyt manures to exhibit the lowest metal solubility. Regardless of the cause, the net result was that differences in $CaCl_2$ solubility of Zn between the conventional NPA+P and the alternative NPA+Phyt manures were dramatically reduced (but not erased) by drying. No particular mechanism is proposed here to explain this intriguing effect of drying on water solubility of manure Zn.

Table 42: Water Solubility of Zn in Wet Composite Manures

Manure Type	Total Zn	Soluble Zn	
	mg kg^{-1}	mg kg^{-1}	% of total
NPA+Phyt	483.0	315.9	65.4 a†
HAP	393.8	253.6	64.4 a
NPA	422.7	210.9	49.9 b
HAP+Phyt	413.0	193.7	46.9 b
NPA+P	293.1	21.7	7.4 c

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

Table 43: Water Solubility of Zn in Dry Composite Manures

Manure Type	Total Zn	Soluble Zn	
	mg kg^{-1}	mg kg^{-1}	% of total
HAP	403.3	255.3	63.3 a†
NPA	410.0	255.0	62.2 a
NPA+Phyt	506.1	176.1	34.8 b
HAP+Phyt	492.9	143.4	29.1 c
NPA+P	304.0	32.5	10.7 d

†Means with same letters not significantly different by Duncan’s Multiple Range Test, alpha = 0.05

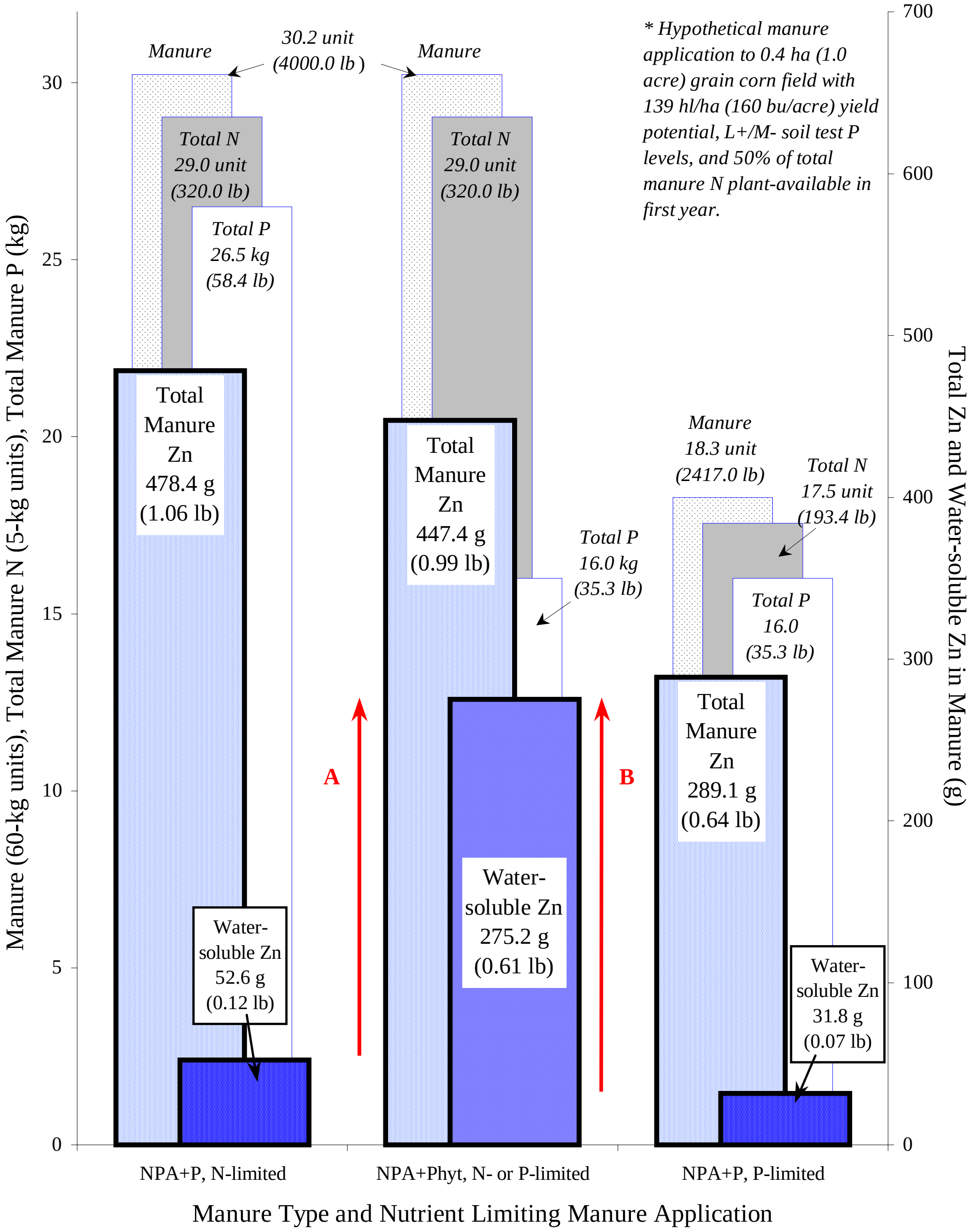
Implications for Land Application

The implications of the manure Zn water solubility results are illustrated in Figure 18. In this case, the results of $CaCl_2$ extraction of Zn from selected wet individual pen manure samples (Table 41) are presented in the context of the same land application example used for Cu and P. Figure 18 compares the amounts of total and water-soluble manure Zn that would be applied to soil if the NPA+P and NPA+Phyt wet individual pen manures were applied to a hypothetical corn field under both N- and P-limited nutrient management. The quantities of manure, total N, and total P applied to soil (illustrated by the background columns) are identical to those used in the previous examples involving wet manure. The foreground columns (units on the right-hand axis) compare the amounts of total manure Zn and water-soluble manure Zn applied to soil.

Under N-limited manure application, switching from the conventional to the phytase-amended diet results in a 6% decrease in the total amount of Zn applied to the land. This is because the alternative NPA+Phyt manure contained slightly less Zn than the conventional NPA+P manure. However, the Zn in the NPA+Phyt excreta was the more water soluble by far. Therefore, converting to the phytase diet results in a 423% increase in the amount of water-soluble Zn applied to the land under N-based manure management (represented by line segment A in Figure 18). In the P-limited manure application scenario, switching to the phytase diet produces a 55% increase in the total amount of Zn applied to the hypothetical field and a 765% increase (line segment B) in the amount of water-soluble Zn applied to the land.

The results of Zn extraction from dry composite manures are not presented in the context of the land application example, due to the contamination concerns described previously. It is worth noting, however, that Figure 18 would be dramatically different if it showed the results of $CaCl_2$ extraction of Zn from dry manures. For the N-based manure management scenario, the increase in water-soluble manure Zn loading would change from 423% to 204%. For the P-based nutrient management scenario, the increase in water-soluble manure Zn loading would change from 765% to 415%.

Figure 18: Water-soluble Zn Loadings from N- and P-limited Land Application of Wet NPA+P and NPA+Phyt Manures†



Summary

Water solubility of Zn in the conventional NPA+P manure was dramatically lower than in the other four manures. This statistically significant difference apparently occurred because the NPA+P manure contained more Ca and P than the other manures. It is hypothesized that phosphates readily bound Zn, reducing the metal's solubility in the dilute $CaCl_2$. Feeding phytase enzyme, HAP corn, or HAP plus phytase all enhanced water solubility of Zn in wet manures numerically, but not statistically. The solubility-depressing calcium phosphate effect was therefore the main reason why water solubility of Zn in the alternative NPA+Phyt manure (61.5%) was 5.5 times greater than in the conventional NPA+P manure (11.0%).

Based on the results of $CaCl_2$ extraction of wet individual pen manures, it is estimated that converting from the conventional NPA+P feed to the alternative NPA+Phyt feed in an N-based manure management situation would result in a 423% increase in the amount of water-soluble Zn applied to soil. Under P-limited manure spreading, the same dietary change would increase total manure Zn loadings by 55% and water-soluble manure Zn loadings by 765%.

Drying of composite manures prior to water extraction dramatically decreased the solubility of Zn in the NPA+Phyt and HAP+Phyt manures. In contrast, drying slightly increased water extractability of Zn in the NPA+P manure. As a result, the solubility of Zn in the dry NPA+Phyt manure (34.8%) was only 3.3 times greater than the solubility of Zn in the dry NPA+P manure (10.7%).

2.3.7.b. Mehlich III Extraction of Manure Zn

Individual Pen Manure Samples

Results of Mehlich III extraction of Zn from all wet individual pen manures are presented in Table 44. Results of Mehlich III extraction of Zn from select wet individual pen manures (i.e., data from the five pens suspected of contamination are excluded) are presented in Table 45. Note again the dramatic differences between the two tables with regard to the "Total Zn" column and the general similarity between the tables in terms of Zn solubility percentages. Table 45 shows that Mehlich III was a more effective extractant of Zn from wet manures than was 0.01 M $CaCl_2$, with more than 90% of Zn solubilized for all five manure types. In addition, with Mehlich III extraction, the dramatic difference in Zn solubility between the NPA+P manure and the other four manure types disappeared. This supports the idea that acid-soluble, water-insoluble calcium phosphate compounds played a major role in controlling the $CaCl_2$ solubility of Zn in the NPA+P manure. Overall, there were few statistically significant differences in Mehlich III extractability of Zn among the five manures. For example, the NPA+P and NPA+Phyt manures did not differ significantly in solubility percentage. When the results are considered on a strictly numerical basis, however, the Mehlich III solubility of Zn in the wet individual pen manures can be related to feed formulation. Correlation of percentage of total feed P in nonphytate form (as predicted after phytase activity, see Table 2) and percentage of manure Zn soluble in Melich III revealed an r^2 value of 0.92 (plot not shown). Therefore, increasing the ratio of nonphytate P to phytate P in feed increased Mehlich III solubility of manure Zn.

Table 44: Mehlich III Solubility Zn in Manures from All Individual Pens

Manure Type	Total Zn	Soluble Zn	
	mg kg^{-1}	mg kg^{-1}	% of total
HAP+Phyt	379.6	379.6	100.0 a†
NPA+P	267.1	263.0	98.5 ab
NPA+Phyt	461.5	441.6	95.7 abc
HAP	359.5	338.8	94.2 bc
NPA	362.4	327.7	90.4 b

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

Table 45: Mehlich III Solubility of Zn in Manures from Select Individual Pens

Manure Type	Total Zn	Soluble Zn	
	mg kg^{-1}	mg kg^{-1}	% of total
HAP+Phyt	232.3	232.3	100.0 a†
NPA+Phyt	267.1	263.0	98.5 ab
NPA+P	250.2	246.2	98.4 ab
HAP	263.1	249.8	95.0 b
NPA	281.3	256.1	91.0 b

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

Composite Manure Samples

The results of Mehlich III extraction of Zn from wet and dry composite manures are presented in Tables 46 and 47. Again, results from extraction of composite manures must be considered with caution, since the composites included portions of the five individual pen manures suspected of Zn contamination. The composite extraction results are presented here primarily in order to point out that drying of composite manures prior to Mehlich III extraction did not produce the pattern seen with $CaCl_2$ solubility, in which drying caused a disproportionate drop in extractability of Zn in manures derived from phytase-amended feeds.

Table 46: Mehlich III Solubility of Zn in Wet Composite Manures

Manure Type	Total Zn	Soluble Zn	
	mg kg^{-1}	mg kg^{-1}	% of total
NPA+Phyt	483.0	468.5	97.0 a†
HAP+Phyt	413.0	393.2	95.2 a
NPA+P	293.1	266.1	90.8 ab
HAP	393.8	335.1	85.1 bc
NPA	422.7	340.7	80.6 c

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

Table 47: Mehlich III Solubility of Zn in Dry Composite Manures

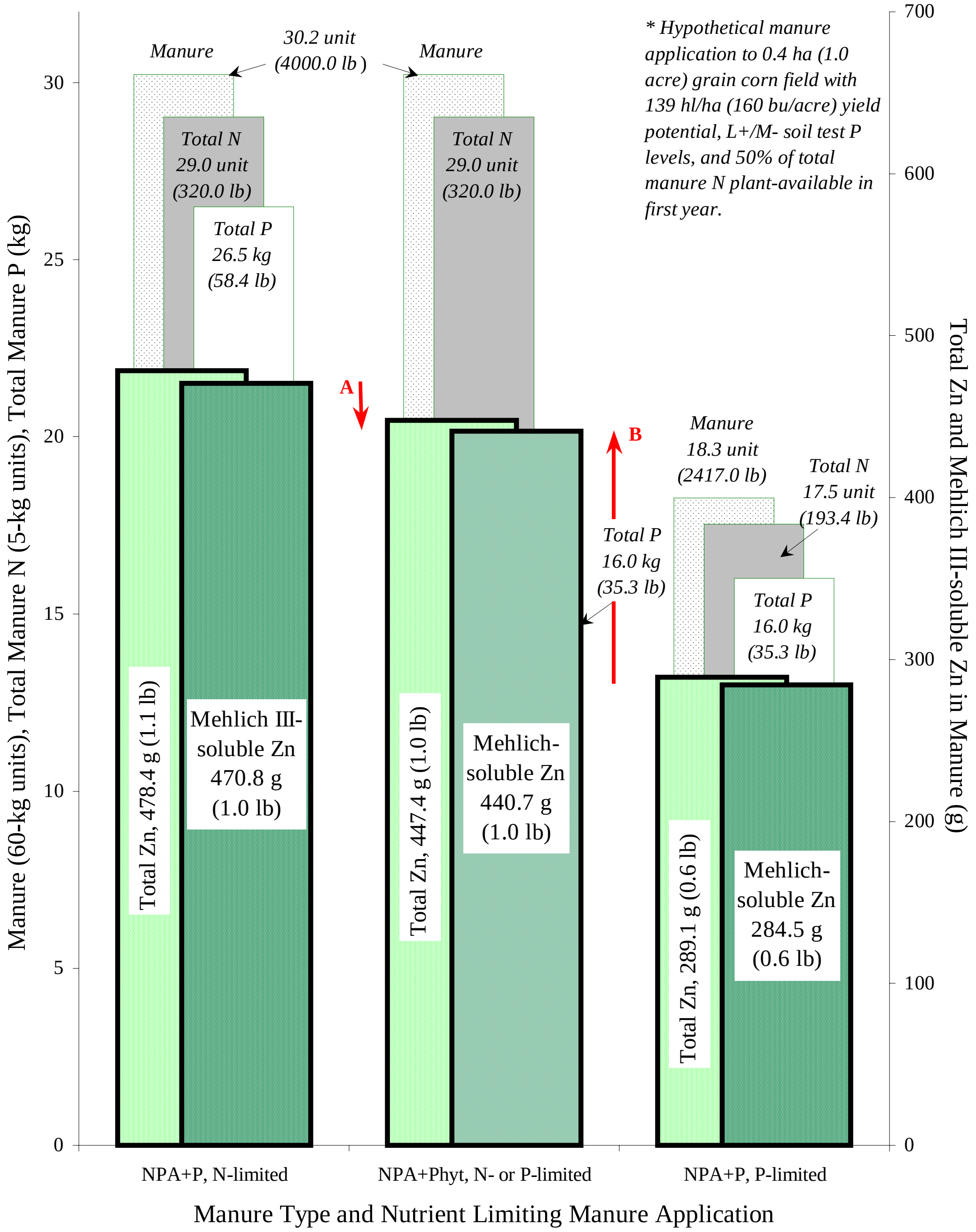
Manure Type	Total Zn	Soluble Zn	
	mg kg^{-1}	mg kg^{-1}	% of total
NPA+Phyt	506.1	506.1	100.0 a†
NPA	410.0	410.0	100.0 a
HAP	403.3	402.1	99.7 a
NPA+P	304.0	283.3	93.2 b
HAP+Phyt	492.9	453.5	92.0 b

†Means with same letters not significantly different by Duncan’s Multiple Range Test, alpha = 0.05

Implications for Land Application

Figure 19 illustrates the potential implications of the manure Zn Mehlich III solubility results. The results of Mehlich III extraction of Zn from selected wet individual pen manure samples are presented in the context of the familiar land application example. Under N-based manure management, switching from the conventional to the phytase-amended diet produces a 6% decrease in the total amount of Zn applied to the land. Since the NPA+P and NPA+Phyt manures did not differ in Mehlich III Zn solubility percentage, this dietary change results in a proportional 6% decrease in the amount of acid-soluble Zn applied to the soil, represented by line segment A. Under P-based manure management, converting to the phytase diet increased both total Zn and Mehlich III-soluble Zn loadings to soil by 55% (the latter increase is represented in Figure 19 by line segment B).

Figure 19: Mehlich III-soluble Zn Loadings from N- and P-limited Land Application of Wet NPA+P and NPA+Phyt Manures†



Summary

The acidic, chelate-containing Mehlich III extractant solubilized over 90% of total Zn in each of the five different manures. It is hypothesized that the Mehlich III solution dissolved the phosphate compounds which disproportionately limited $CaCl_2$ solubility of Zn in the NPA+P manure. This theory helps explain why the wet individual pen NPA+P manures did not differ significantly from any of the other individual pen manures in Mehlich III extractability of Zn. The wet NPA+P and NPA+Phyt manures showed almost identical Mehlich III Zn solubility percentages (98.4% and 98.5%, respectively). It appears that phytate P levels in the manures controlled Mehlich III solubility of Zn to some degree. A direct linear relationship was observed between percentage of total manure Zn soluble in Mehlich III and percentage of feed P in total (inorganic plus plant-derived) nonphytate form. However, this influence translated into few statistically meaningful differences in Mehlich III solubility of Zn between different manure types.

The results of Mehlich III extraction of wet individual pen manures suggest that, in an N-based manure management scenario, conversion from feeding conventional NPA+P rations to alternative NPA+Phyt rations would not change the amount of water-soluble manure Zn applied to soils. Under P-based manure management, the dietary shift would result in an increase in the amount of total manure Zn applied to soil and a proportional increase in the amount of water-soluble manure Zn applied to soil.

2.3.7.c. Olsen Extraction of Manure Zn

Composite Manure Samples

The results of Olsen extractions conducted on wet and dry composite manures are presented in Tables 48 and 49, respectively. These results show that, of the three extracting solutions, the alkaline $NaHCO_3$ was the least effective at solubilizing Zn from wet as well as dry manures. Olsen extraction of the wet composite manures produced a Zn solubility pattern not related in any obvious way to correponding dietary formulations. Manure drying shifted Olsen solubility trends, resulting in a pattern in which the HAP and NPA manures contained the most soluble forms of Zn, the HAP+Phyt and NPA+Phyt manures contained Zn of intermediate solubility, and the NPA+P manure contained the least soluble forms of Zn.

Table 48: Olsen-soluble Zn in Wet Composite Manures

Manure Type	Total Zn	Soluble Zn	
	mg kg^{-1}	mg kg^{-1}	% of total
HAP+Phyt	413.0	114.4	27.7 a†
HAP	393.8	105.9	26.9 ab
NPA+P	293.1	70.1	23.9 bc
NPA	422.7	98.5	23.3 c
NPA+Phyt	483.0	107.2	22.2 c

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

Table 49: Olsen-soluble Zn in Dry Composite Manures

Manure Type	Total Zn	Soluble Zn	
	$mg\ kg^{-1}$	$mg\ kg^{-1}$	% of total
HAP	403.3	115.3	28.6 a†
NPA	410.0	111.1	27.1 ab
HAP+Phyt	492.2	129.4	26.3 b
NPA+Phyt	506.1	113.9	22.5 c
NPA+P	304.0	56.5	18.6 d

†Means with same letters not significantly different by Duncan's Multiple Range Test, alpha = 0.05

2.4. CONCLUSIONS

2.4.1. PRIMARY CONCLUSIONS

Extracting manures at the point of excretion (i.e., wet manures) with 0.01 M $CaCl_2$ showed that converting from the conventional NPA+P diet to the alternative, phytase-containing NPA+Phyt diet produced a statistically significant increase in water solubility of manure P. For example, only 51.8% of total P in the wet individual pen NPA+P manure was water soluble, while 78.5% of total P in the NPA+Phyt manure was water soluble. Note that, although the P in the conventional NPA+P manure was significantly less water soluble on a percentage basis, a greater total amount of P was extracted from the wet NPA+P manure than from an equal quantity of wet NPA+Phyt manure (7568 mg soluble P per kg NPA+P manure versus 6929 mg soluble P per kg NPA+Phyt manure). This was because the conventional manure contained higher levels of total P than the alternative NPA+Phyt manure.

The statistically significant difference in water solubility of P between the wet NPA+P and NPA+Phyt manures was caused in large part by the greater quantity of defluorinated calcium phosphate in the conventional feed. For example, simple addition of defluorinated calcium phosphate to the baseline NPA diet caused a statistically significant drop in water solubility of manure P, from 69.1% for wet individual pen NPA manures to 51.8% for NPA+P manures. It is hypothesized that, because of the higher levels of calcium phosphate in the NPA+P feed, a greater proportion of P in the NPA+P manure was in water-insoluble calcium phosphate form. Phytase enzyme in the alternative feed also contributed to the differences in water solubility of P between wet NPA+P and NPA+Phyt manures. Addition of phytase enzyme to the baseline NPA diet without defluorinated phosphate modification caused a statistically significant increase in the $CaCl_2$ extractability of P in wet manures (for example, from 69.1% for the wet individual pen NPA manure to 78.5% for the NPA+Phyt manure).

The solubility-depressing effect of supplemental dietary defluorinated phosphate was strongly enhanced by drying manure at moderate temperature prior to $CaCl_2$ extraction. Therefore, drying prior to extraction increased differences in water solubility of manure P between the conventional NPA+P and alternative NPA+Phyt manures. For example, only 34.1% of total P in the dried composite NPA+P manure was water soluble, while 75.8% of total P in the dried composite NPA+Phyt manure was water soluble. The phytase-related enhancement of manure P solubility observed in wet manures did not persist after manures were dried.

2.4.2. SECONDARY CONCLUSIONS

Extraction of wet manures with 0.01 M $CaCl_2$ showed that water solubility of Cu in the conventional NPA+P and alternative NPA+Phyt manures did not differ significantly at the point of excretion. For example, 33.5% of total Cu in the wet individual pen NPA+P manure samples was soluble in $CaCl_2$, compared to 33.4 % of total Cu in the NPA+Phyt manure. However, converting to the phytase-amended diet produced a dramatic, 5.6-fold increase in $CaCl_2$ solubility of manure Zn at the point of excretion. For example, while only 11.0% of total Zn in the wet individual pen NPA+P manures was water extractable, 61.5% of total Zn in the alternative NPA+Phyt manures was soluble in $CaCl_2$. Feeding phytase enzyme alone enhanced $CaCl_2$ solubility of Zn in wet manures numerically, but not statistically. Therefore, this dramatic effect of diet on Zn extractability was caused primarily by depression of Zn solubility associated with supplemental calcium phosphate in the NPA+P feed. It is hypothesized that the relatively large amount of P in the NPA+P manure bound Zn and limited the metal's water solubility.

Drying of composite manures at moderate temperature for 4 d caused a minor shift in manure Cu water solubility patterns. Water extractability of Cu was a few percentage points lower for dry manure derived from phytase-amended feeds than for other dry manures. These differences were statistically significant. Therefore, after drying, water solubility of Cu was significantly lower in the alternative NPA+Phyt manure (42.6%) than in the conventional NPA+P manure (48.5%). Drying also affected water solubility of manure Zn. Drying manures prior to $CaCl_2$ extraction dramatically decreased the solubility of Zn in the NPA+Phyt manures. In contrast, drying slightly increased water extractability of Zn in the NPA+P manure. As a result, the solubility of Zn in the dried alternative NPA+Phyt manure was only 3.3 times greater than the solubility of Zn in the dried conventional NPA+P manure (34.8% versus 10.7%).

The P in manures derived from either alternative diet containing HAP corn was significantly more water soluble than was P in the conventional NPA+P manure, both at the point of excretion and after drying. For example, 51.8% of total P in the wet individual pen NPA+P manure was $CaCl_2$ extractable, while 79.3% and 84.3% of total P in the HAP and HAP+Phyt manures, respectively, was water soluble. Switching from the conventional NPA+P diet to either of the alternative diets containing HAP corn involved reductions in feed defluorinated calcium phosphate content. The calcium phosphate effect described previously therefore played an important role in these P solubility differences between the conventional NPA+P and alternative HAP and HAP+Phyt manures. Comparisons involving the baseline NPA manure showed that feeding HAP corn or HAP corn plus phytase enzyme alone (i.e., without dietary defluorinated phosphate modification) also contributed to these P solubility differences among the wet manures. Note that drying manures eliminated the HAP-plus-phytase effect, but not the solubility-enhancing effect of feeding HAP corn alone.

Extraction of wet individual pen manures with 0.01 M $CaCl_2$ revealed no statistically significant differences in Cu solubility at the point of excretion between the conventional NPA+P manure (33.5% of total Cu soluble) and the alternative HAP and HAP+Phyt manures (33.3% and 26.0% of total Cu soluble, respectively). After individual pen manures were combined to form wet composites, the Cu solubility differences between the NPA+P and HAP+Phyt manures became statistically significant (29.6% for NPA+P versus 23.0% for HAP+Phyt). Note that neither feeding HAP corn nor HAP corn plus phytase individually (i.e., without modification of feed calcium phosphate levels) significantly affected water solubility of manure Cu at the point of excretion. Drying composite manures at moderate temperature enhanced the overall water extractability of manure Cu, but did not notably affect the Cu solubility relationships observed at the point of excretion between the NPA+P, HAP, and HAP+Phyt composite manures.

Switching from the conventional NPA+P diet to the alternative HAP and HAP+Phyt diets significantly increased $CaCl_2$ extractability of manure Zn at the point of excretion. While only 11.0% of total Zn in the wet individual pen NPA+P manures was water extractable, 57.0% of total Zn in the HAP manures and 58.2% of total Zn in the HAP+Phyt manures was soluble in $CaCl_2$. These solubility differences at the point of excretion were very similar to those observed upon conversion to the NPA+Phyt diet and described above. Feeding HAP corn and HAP corn plus phytase individually (without modification in feed calcium phosphate) enhanced $CaCl_2$ solubility of Zn in wet manures numerically, but not statistically. Therefore, these dramatic effects of diet on Zn extractability can again be attributed to a depression in Zn solubility associated with the supplemental calcium phosphate in the conventional NPA+P feed and manure. The impact of manure drying on water solubility of manure Zn differed between the HAP and HAP+Phyt treatments. The percentage of total manure Zn extractable from the HAP composite manure was not notably affected by drying, but was dramatically reduced for the HAP+Phyt manure. Water solubility of Zn in the dried HAP and HAP+Phyt manures remained significantly greater than in the dried NPA+P manures.

CHAPTER 3: SOIL EXTRACTION STUDY

3.1. OBJECTIVES

3.1.1. PRIMARY OBJECTIVES

(a) Investigate how converting from a conventional diet to a phytase diet changes the water solubility of P in soils amended on an equal-N or equal-P basis with turkey manure.
(b) Investigate how converting from a conventional diet to a phytase diet changes the plant availability of P in soils amended on an equal-N or equal-P basis with turkey manure.
(c) Investigate the effect of increasing manure loading, increasing incubation time, and different soil types on any observed changes in P solubility or availability.
(d) Explain how differences in diet formulation and/or manure composition cause any observed changes in P solubility or availability.

3.1.2. SECONDARY OBJECTIVES

(a) Investigate how converting from a conventional diet to a phytase diet changes the water solubility and plant availability of Cu in soils amended on an equal-N and or equal-P basis with turkey manure.
(b) Investigate how converting from a conventional diet to a diet formulated with HAP corn or HAP corn plus phytase enzyme changes the water solubility and plant availability of P and Cu in soils amended on an equal-N or equal-P basis with turkey manure.
(c) Investigate the effect of increasing manure loading, increasing incubation time, and different soil types on any observed changes in Cu or P solubility or availability.
(d) Explain how differences in diet formulation and/or manure composition cause any observed changes in P or Cu solubility or availability.

3.2. MATERIALS AND METHODS

3.2.1. MANURE PRODUCTION AND CHARACTERIZATION

This study involved extracting soils after those soils were treated with five different types of turkey manure. The turkey manures used to amend soils were the wet composite manure samples described in the Materials and Methods part of Chapter 2. The reader is referred to that portion of Chapter 2 for a detailed discussion of the five dietary treatments consumed by the birds and the manure handling and characterization procedures used. Results of characterization of the five wet composite manures are described in the Results and Discussion part of Chapter 2.

3.2.2. SOIL COLLECTION, HANDLING, AND CHARACTERIZATION

Approximately 100 kg of soil were collected from the plow layer (0 to 15 cm) from three different agricultural fields. The objective was to obtain samples representative of productive agricultural soils from the Appalachian Ridge and Valley, Piedmont, and Atlantic Coastal Plain regions of Virginia. The Ridge and Valley soil was a Groseclose loam, classified as a clayey, mixed, mesic Typic Hapludult. The Groseclose sample was taken from a tilled garden site formerly in grass hay in Montgomery County. The Piedmont soil was a Cecil loam, classified as a fine, kaolinitic, thermic Typic Kanhapludult. The Cecil sample was taken from a field of soybeans located in Amelia County. The Coastal Plain soil was a Mahan sandy loam, classified as a fine, kaolinitic, thermic Typic Hapludult. The Mahan sample was collected from a cotton field in Isle of Wight County.

All soils were spread on benches to dry (air-dried) and clods were broken up by hand using a mortar and pestle. Each sample was then sifted through a 2-mm sieve to remove coarse fragments and thoroughly homogenized by mixing in a portable electric cement mixer. Subsamples were submitted to the Virginia Cooperative Extension Soil Testing Laboratory at Virginia Tech. The lab estimated plant-available P, K, Ca, Mg, Zn, and Cu content of the soils by extraction with Mehlich I solution followed by ICP analysis of the extract. The lab also determined pH (1:1 ratio of soil to water) and percent organic matter. Exchangeable bases and cation exchange capacity were determined by the Soil Survey Laboratory at Virginia Tech. Exchangeable bases were determined by extraction with ammonimum acetate (NH_4OAc) and exchangeable acidity was determined by extraction with potassium chloride (KCl) (National Soil Survey Center, 1996). The moisture content of the air-dried soils was determined by weighing before and after 24 h of incubation in an oven maintained at 110 °C. The water-holding capacity of the three soils was estimated using the technique of (Kuo, 1996). This estimate of the amount of water held by the soils after being allowed to drain freely will be referred to herein as “container capacity.”

3.2.3. MANURE TREATMENT AND INCUBATION OF SOILS

Soils were treated using the five wet composite manures described in Chapter 2. This means that manures collected at the point of excretion were applied to soils with no intervening drying or other modification of manure. As explained previously, most poultry manure is litter containing bedding. Poultry litter produced in commercial facilities also undergoes a wide variety of handling and storage processes prior to land application. Manures used in this study were produced without bedding. Furthermore, it was not possible to identify or replicate in the lab the handling and storage processes to which “typical” poultry manure is subjected between the moment of excretion and the point of land application. Therefore, it was decided that soils would simply be treated with wet manures collected at the point of excretion.

Manure treatments that were applied to the three soils are shown in Table 50. No manure was applied to soils for the control treatment. Six basic manure treatments were mixed with soils under two application regimes. The NPA, HAP, NPA+Phyt, HAP+Phyt, and NPA+P Pbasis treatments involved mixing the five different manure types with soil on an equal-P basis (i.e., so that a similar amount of total manure P was added to soil for each of the five manure types). Note that applying the NPA, HAP, NPA+Phyt, and HAP+Phyt manures on an equal-P basis also meant that these four manure types were applied on a similar total-weight and total-manure-N basis. The sixth treatment, the NPA+P Nbasis treatment, was formulated so as to load a similar amount of total manure and manure N on soils as was achieved with the NPA, HAP, NPA+Phyt, and HAP+Phyt treatments. Therefore, the conventional NPA+P manure was the only type of manure that was used in two different treatments. This choice of treatments allowed both equal-P basis and equal-N basis comparisons to be made among soils amended with the five different types of manures. Note that the manuring rates for the low application regime were simply doubled to obtain the high regime manuring rates.

As indicated previously (Table 8), all five types of composite manures contained similar concentrations of Cu. Therefore, application of manures to soils on an equal-manure-weight basis resulted in very similar loadings of manure Cu as shown in Table 50. Note however, that no combination of treatments resulted in equal loadings of manure Zn on soils. This is because the wet composite manures used in the study contained widely varying concentrations of Zn, as explained previously (Table 8). Therefore, it was possible to compare the impacts of the dietary treatments on solubility and availability of Cu in manured soils, but not on solubility and availability of Zn in manured soils.

Table 50: Manure and Manure Elements Applied to Soils†

Manure Treatment	Manure g kg^{-1} or ton $acre^{-1}$	P		N		Cu		Zn	
		mg kg^{-1} soil	lb P_2O_5 $acre^{-1}$	mg kg^{-1} soil	lb $acre^{-1}$	mg kg^{-1} soil	lb $acre^{-1}$	mg kg^{-1} soil	lb $acre^{-1}$
Control	0.0	0.0	0.0	0.0	0.0	0.00	0.00	0.00	0.00
Low Manure Application Regime									
NPA	22.8	190.4	871.9	1904.2	3808.4	0.70	1.39	9.64	19.28
HAP	24.4	190.8	873.9	2082.6	4165.2	0.70	1.40	9.61	19.22
NPA+Phyt	23.8	188.7	864.4	1866.7	3733.3	0.73	1.46	11.50	22.99
HAP+Phyt	24.9	188.5	863.3	2050.7	4101.5	0.74	1.47	10.28	20.57
NPA+P Pbasis	14.4	191.2	875.8	1114.0	2228.0	0.44	0.88	4.22	8.44
NPA+P Nbasis	24.0	318.7	1459.7	1856.7	3713.3	0.74	1.47	7.03	14.07
High Manure Application Regime									
NPA	45.6	380.8	1743.9	3808.4	7616.8	1.39	2.78	19.28	38.55
HAP	48.8	381.6	1747.8	4165.2	8330.4	1.40	2.79	19.22	38.43
NPA+Phyt	47.6	377.5	1728.8	3733.3	7466.7	1.46	2.92	22.99	45.98
HAP+Phyt	49.8	377.0	1726.6	4101.5	8202.9	1.47	2.95	20.57	41.13
NPA+P Pbasis	28.8	382.5	1751.7	2228.0	4456.0	0.88	1.77	8.44	16.88
NPA+P Nbasis	48.0	637.4	2919.5	3713.3	7426.7	1.47	2.95	14.07	28.14

†All concentrations in table expressed on oven-dry basis

Relatively large amounts of manure were applied to soils, even under the low application regime, as shown in Table 50. Given the study's goals, it was deemed essential that soils be mixed with sufficient manure and P in manure form so that any subtle treatment-related differences in P chemistry not be masked by the soil's capacity to adsorb added P or release native P. Preliminary trials involving mixing of manure and soil under study conditions were conducted to evaluate different application rates. These trials suggested that the use of agronomic manuring rates currently recommended for Virginia farmers (up to 11.2 Mg dry manure ha^{-1} or 10,000 lb acre^{-1})might lead to situations where treated soils mixed with manure and control soils not mixed with manure would not differ significantly in extractable P content. Given these considerations, approximate targets for the low and high manuring rates were set at 56 Mg ha^{-1} (50,000 lb acre^{-1}) and 112 Mg ha^{-1} (100,000 lb acre^{-1}), respectively. More specific P-based manure application rates in the target range were then developed.

All manures were applied to soil in wet, "as excreted" form. Therefore, since varying amounts of water were entrained in the different manures, it was necessary to equalize the amount of moisture added to soils across treatments. Initial trials showed that the desired heavy loadings of wet manure added more moisture to all three soils than their calculated "container capacity." Visual observation during initial trials suggested that wetting soils to 150% of calculated container capacity produced an acceptable soil moisture content for purposes of the incubation study. Therefore, all soils amended with all treatments were moistened to 150% of calculated container capacity. For control soils which recieved no manure applications, all of this moisture was added in the form of water. For other soils, the amount of moisture entrained in soil-applied manures was determined. Then water was added to bring total moisture in the soil to 150% of container capacity. The coarse-textured Mahan soil had such little water-holding capacity that high application regime manure treatments caused soil moisture to exceed 150% of container capacity. Therefore, only low application regime treatments were applied to the Mahan soil. Low and high regime treatments were applied to both the Groseclose and Cecil soils.

Soils and manures were mixed and incubated in round 0.36-L (12-ounce) plastic containers ("cups") fitted with air-tight lids. Wet manure was combined with soil in these cups in the following manner. First, 100 g (oven-dry basis) quantities of air-dried soil were weighed out and set aside. Next, calculated quantities of wet manures were weighed into labeled cups, which were sealed with lids until soil was added. The full 100 g of soil were added to each cup in incremental amounts. Slow addition of dry soil to wet manure along with vigorous mixing by hand was needed to ensure thorough mixing of manures with soil. The stiff plastic handle of a small paint brush proved to be the best strirring rod available for this mixing. After soils and manures were mixed, any additional water needed to bring the soil-manure mixture to 150% of container capacity was added to the soil surface using a micropipette. The cups were then sealed and weighed. The weight of each cup was recorded on the container itself, then four small air holes were punched in the lid. The cups were then ready for incubation. Manure-amended soils were incubated for 4 or 28 d prior to extraction. The incubation chamber was completely dark and maintained at 20 °C.

A total of seven different treatments (control plus six different manure types or rates) were applied to soils under a total of 10 different combinations of soil type, application regime, and incubation time factors (keep in mind that there were no Mahan soil x high application regime combinations). This brought the total number of different treatment x factor combinations to 70. The initial objective was to replicate each of these 70 treatment x factor combinations three times, requiring the preparation of a total of 210 individual soil cups. However, certain treatment x factor combinations were replicated only twice. Therefore, the total number of individual soil cups prepared and incubated in the soil extraction study was 189.

3.2.4. EXTRACTION OF MANURED SOILS

At the end of the incubation period, subsamples of soil were removed from cups for extraction. Prior to withdrawal of samples, soil cups were weighed in order to estimate any loss of moisture during incubation. Any difference between the weight of a cup at the start of the incubation period (recorded on the cup) and the weight at the end of the incubation period was attributed to evaporation of water through the holes in the cup's lid. Since moisture content of the soil was known to be 150% of container capacity at the start of incubation, the water loss information was used to calculate the moisture content of the soil at the time of extraction.

The same three extraction procedures used to extract P, Cu, and Zn from manures were used for extraction of soils. The reader is referred to the Materials and Methods portion of Chapter 2 for a detailed description of the $CaCl_2$, Mehlich III, and Olsen extracting solutions and procedures. Subsamples of soil were weighed directly from the soil cups into centrifuge tubes for extraction. Since the soils contained a known quantity of entrained water, this moisture was counted as part of the volume of extractant. Therefore, the strategy of using "double-strength" extractants to make up 50% of extractant volume and using water (including water entrained in soil) to make up the remaining 50% of extractant volume was again adopted. A key difference between the soil and water extracts was that the soil extracts contained much less suspended material after centrifugation. Therefore, soil extracts were forced through the syringe filtration systems by hand. The SampleTek Vacuum Extractor was not used for filtration of soil extracts.

Subsamples of soil drawn from all 189 soil cups were subjected to the three different extraction procedures, with one exception. Olsen extraction was not conducted on Groseclose soils amended with high application regime treatments and incubated for 28 d. On average, two subsamples from each soil cup were subjected to 0.01 M $CaCl_2$ extraction. The same was true of the Mehlich III extraction. A grand total of 363 0.01 M $CaCl_2$ extractions and 365 Mehlich III extractions were conducted on the soil samples. Since the Olsen extraction was less critical to the goals of this study, a total of only 262 extractions of this type were carried out on the soil samples. All extracts were analyzed for total P and Cu by ICP spectrometry. The only extracts diluted prior to analysis were the alkaline Olsen extracts, which required acidification to ensure compatibility with the ICP instrument. Acidification by addition of 1 M HNO_3 resulted in a 2x dilution of the $NaHCO_3$ Olsen extracts.

The results of ICP analysis were used to calculate the amount of P or Cu extracted per unit of soil. The following equation was used in this calculation:

$$(A \times B \times C) = D$$

where

A = mg kg^{-1} element in extract or mg element per L of analyzed extract
B = dilution factor or mL analyzed extract per mL of extractant used
C = extractant:dry matter ratio or mL extractant used per g dry material extracted
D = mg kg^{-1} element in dry matter or mg element soluble per kg of dry soil extracted

Note that the dilution factor (B) was equal to “1” for all $CaCl_2$ and Mehlich III extracts and equal to “2” for all Olsen extracts. The above calculations were preformed using Excel spreadsheets. All data and calculation results were then imported into SAS for statistical analysis. The data were subjected to analysis of variance (ANOVA) using the general linear model procedure (proc GLM) of SAS. The principal tool used for comparing means was Duncan’s Multiple Range Test using the default significance level of alpha = 0.05.

3.3. RESULTS AND DISCUSSION

3.3.1. CHARACTERIZATION OF SOILS

The results of characterization of the three untreated soils by the Virginia Cooperative Extension Soil Testing Lab are shown in Table 51. Soil pH and extractable nutrient levels show that the soils collected for use in the study were representative of highly productive, heavily fertilized agricultural land from each of Virginia's three physiographic provinces. Exchangeable bases, cation exchange capacity, and percent base saturation for the three soils are presented in Table 51. These values are also indicative of agricultural soils under a high level of management.

Table 51: Native Soil Characterization I: pH, Organic Matter, and Plant-Available Nutrient Levels

Soil Type	pH	Organic Matter (%)	mg kg^{-1} in soil, Mehlich I extractable				
			P	K	Ca	Mg	Cu
Groseclose	6.7	3.8	152 (VH)†	155 (H+)	1086 (VH)	91 (H)	0.9
Cecil	6.6	3.1	57 (VH)	176 (VH)	753 (H-)	83 (M-)	0.9
Mahan	6.1	2.3	34 (H)	112 (H)	456 (M-)	84 (M-)	1.7

†Virginia Cooperative Extension nutrient availability rankings: VH = very high, H = high, M = medium

Table 52: Native Soil Characterization II: Exchangeable Bases and Acidity, Cation Exchange Capacity, Base Saturation

Soil Type	cmol$_c$ kg^{-1} soil							Base Saturation (%)
	Ca	Mg	K	H	Al	ECEC	CEC	
Groseclose	3.81	0.75	0.69	0.20	0.15	5.40	5.45	96.33
Cecil	3.08	0.63	0.70	0.20	0.15	4.56	4.61	95.66
Mahan	1.86	0.62	0.48	0.80	0.25	3.21	3.76	78.72

3.3.2. EXTRACTION OF SOIL P

3.3.2.a. $CaCl_2$ Extraction of Soil P

Effect of Manure Treatments

The results of $CaCl_2$ extraction of soil P are summarized in Table 53. This table incorporates data from a total of 363 $CaCl_2$ extractions, including results from all 10 combinations of application regime, incubation time, and soil type factors used in the study. Therefore, Table 53 offers a reliable overall picture of how the different manure treatments affected water solubility of soil P under a variety of conditions. When analyzing soil extraction results such as those in Table 53, it is important to keep in mind that only five of the seven treatments resulted in equal applications of manure P to soil. These were the NPA+P Pbasis, NPA, HAP, NPA+Phyt, and HAP+Phyt treatments. Therefore, the reader should focus on these five treatments when comparing how the five manure types affected soil P extractability. The NPA+P Nbasis results should be considered when the reader is interested in assessing how dietary changes affected soil P solubility under N-based manure management (this treatment is also important for Cu solubility comparisons, as explained later in this chapter). Note that under the control treatment, no manure was applied to soil.

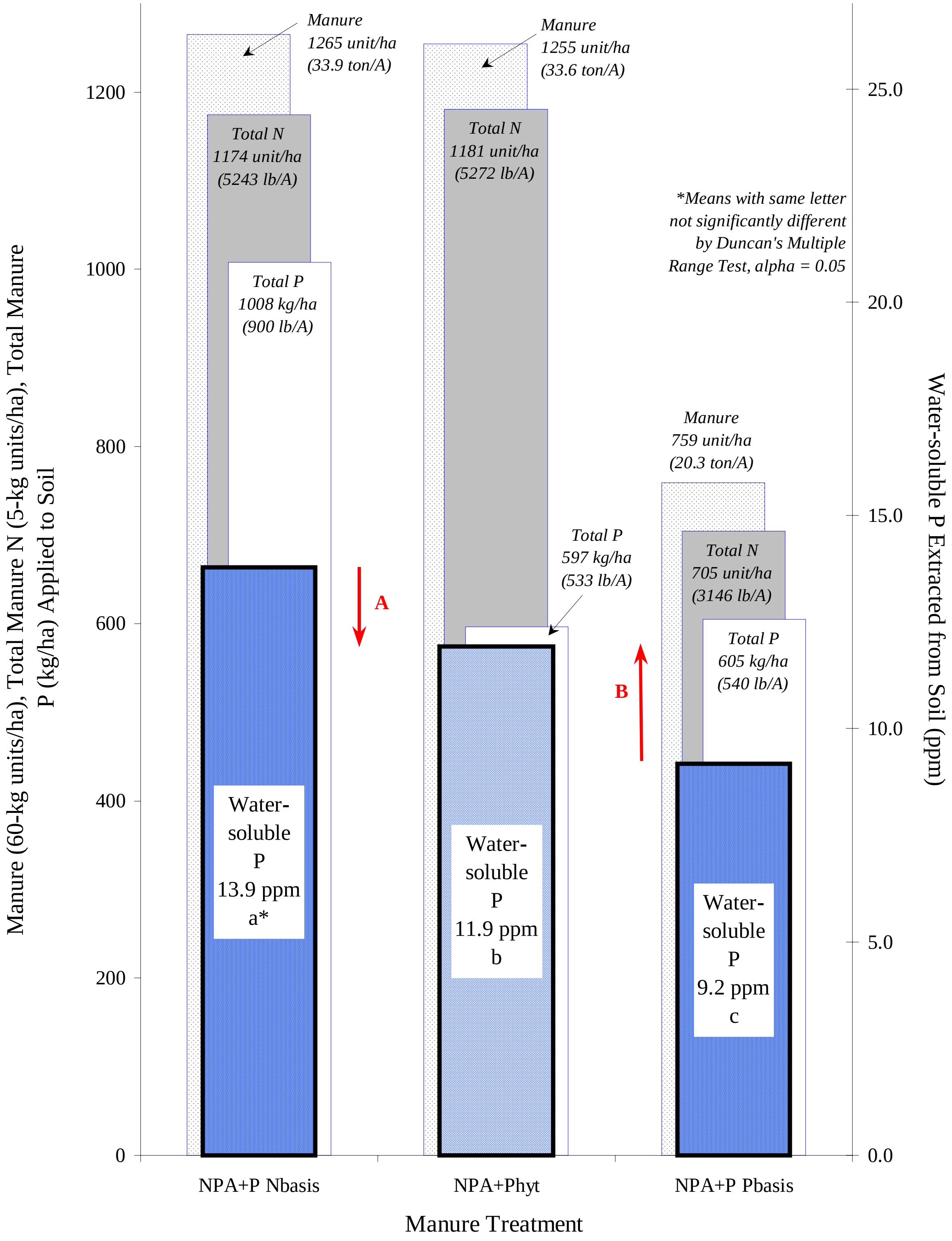
Table 53: Water-soluble P in Manured Soils, Overall Comparison
(All Application Regimes, Incubation Times, Soil Types)

Treatment	mg P kg^{-1}
NPA+P Nbasis	13.8 a†
HAP+Phyt	13.7 a
HAP	12.5 b
NPA	12.2 bc
NPA+Phyt	11.9 c
NPA+P Pbasis	9.2 d
Control	3.7 e

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05

The most critical issue to consider is how switching from the conventional NPA+P diet to the alternative NPA+Phyt diet affected $CaCl_2$ solubility of soil P. In order to more clearly illustrate the effect of this dietary change under both N- and P-based manure applications, the NPA+P Nbasis, NPA+Phyt, and NPA+P Pbasis results from Table 53 are presented in Figure 20. The background columns in this figure compare the total amounts of manure, manure N, and manure P applied to soils as a result of the NPA+P Nbasis, NPA+Phyt, and NPA+Pbasis treatments. The foreground columns show the amount of water-soluble P the soils contained as a result of the three treatments. The values in these foreground columns are drawn directly from Table 53.

Figure 20: Water-soluble P Comparison, Soils Treated with NPA+P and NPA+Phyt Manures



Soils amended with the NPA+P Nbasis treatment contained significantly more $CaCl_2$-extractable P than soils amended with the NPA+Phyt treatment (13.9 mg kg^{-1} versus 11.9 mg kg^{-}

^{1}). This means that, under N-based manure management, feeding phytase enzyme produced a significant 14% decrease in water-extractable soil P, represented in Figure 20 by line segment A. Note how modest this decrease in water-soluble soil P was relative to the reduction in total P loading achieved by feeding phytase. Under N-based manure management, switching from the conventional to the alternative diet produced a 41% drop in total manure P loading to soils, from 1008 kg/ha to 597 kg/ha. Consider next the P-based manure management scenario, illustrated by the NPA+P Pbasis and NPA+Phyt treatments. Under P-based manure allocation, switching from the conventional to the alternative phytase diet did not affect the total amount of manure P applied to soils. However, conversion to phytase feeding resulted in a statistically significant 29% increase in water-soluble soil P levels, from 9.2 mg kg^{-1} for NPA+P Pbasis to 11.9 mg kg^{-1} for NPA+Phyt. This increase is represented in Figure 20 by line segment B.

Why did soils amended with NPA+Phyt manure contain more water-soluble P than soils amended with NPA+P manure on an equal-P basis? It is proposed that this difference occurred because a greater proportion of P in the NPA+P manure was in calcium phosphate form. This hypothesis is supported by the following observations. The NPA and NPA+P diets differed only in that the latter was formulated to contain an extra 0.21% defluorinated calcium phosphate, as fed. It was shown in Chapter 2 that this additional dietary defluorinated phosphate depressed water extractability of manure P, most likely by increasing the proportion of manure P in calcium phosphate form. Table 53 suggests that water solubility of soil P was subject to the same calcium phosphate effect. Increasing the level of defluorinated phosphate in the baseline NPA feed reduced water solubility of P in manure-amended soils by 25%, from 12.2 mg kg^{-1} to 9.2 mg kg^{-1}. Note that the same dietary change reduced water solubility of P in wet composite manures by a statistically significant 23% (Table 17), which implies that soil application and incubation did not diminish the solubility-depressing effect of supplemental calcium phosphate. In contrast, feeding phytase had no significant effect on water solubility of soil P. As shown in Table 53, very similar water-soluble P levels were observed for the NPA and NPA+Phyt soils. Recall that addition of phytase to the baseline NPA diet produced a significant increase in water extractability of manure P (Tables 13 and 17), suggesting that soil application and incubation diminished phytase-induced differences in water solubility of manure P. Regardless of the mechanisms involved, these results indicate that a dietary phytase effect was not responsible for the differences in water-soluble P levels between between the NPA+P Pbasis and NPA+Phyt soils.

The next question to be addressed is whether, under P-based manure management, switching from the conventional NPA+P diet to either the alternative HAP diet or the alternative HAP+Phyt diet increased water-soluble P levels in soils. As shown in Table 53, soils amended with either alternative manure contained significantly more water-extractable P than the NPA+P Pbasis soil. In the case of the HAP versus NPA+P comparison, it is again proposed that soil P solubility differences were primarily the result of a calcium phosphate effect. Soils amended with the HAP treatment were statistically similar in water-extractable P content to soils treated with NPA manure (12.5 mg kg^{-1} versus 12.2 mg kg^{-1}). This indicates that there was no HAP effect on water solubility of P in manured soils. In the case of the HAP+Phyt versus NPA+P comparison, it appears that soil P solubility differences were the result of both an HAP-plus-phytase enhancement of P solubility as well as a calcium phosphate solubility-depressing effect. As shown in Table 53, HAP+Phyt-amended soils contained significantly more water-soluble P than the NPA-amended soils (13.7 mg kg^{-1} versus 12.2 mg kg^{-1}). Note that water extraction of raw manures revealed more pronounced HAP and HAP-plus-phytase effects than were observed with soils. This suggests that mixing and incubating manures with soils diminished the impact that these dietary alternatives had on P water solubility.

Effect of Application Regime, Incubation Time, and Soil Type Factors.

The data in Table 54 are presented in order to explore the effect of changing manure application regime on the treatment comparisons discussed above. Note that high application regime treatments were not used on any Mahan soils. Therefore, no data from extraction of Mahan soils are included in Table 54. As expected, doubling the amount of manure applied to soils produced an increase in water-extractable P levels across all treatments. However, changes in manure application regime did not notably influence the P water solubility pattern observed among soils amended with different treatments. For example, under the low application regime, soils amended with the alternative NPA+Phyt manure contained 27% more water-soluble P than soils mixed on an equal-P basis with conventional NPA+P manure (7.4 mg kg^{-1} for NPA+P Pbasis versus 9.4 mg kg^{-1} for NPA+Phyt). Under the high application regime, NPA+Phyt-treated soils also contained about 27% more water-soluble P than soils subjected to the NPA+P Pbasis treatment (12.8 mg kg^{-1} for NPA+P Pbasis versus 16.3 mg kg^{-1} for NPA+Phyt).

Table 54: Water-Soluble P in Manured Soils, Application Regime Comparison

Groseclose and Cecil Soils, Both Incubation Times			
Low Application Regime		High Application Regime	
Treatment	mg P kg^{-1}	Treatment	mg P kg^{-1}
NPA+P Nbasis	10.9 a	HAP+Phyt	19.0 a
HAP+Phyt	10.6 a	NPA+P Nbasis	18.9 a
HAP	9.6 b	HAP	17.3 b
NPA+Phyt	9.4 b	NPA	16.8 bc
NPA	9.4 b	NPA+Phyt	16.3 c
NPA+P Pbasis	7.4 c	NPA+P Pbasis	12.8 d
Control	4.2 d	Control	4.2 e

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05. Statistical analyses were conducted separately for each of the two application regimes.

Water-soluble P contents of manure-amended soils incubated for 4 d and 28 d prior to extraction are compared in Table 55. Across all treatments (including the control), lengthening incubation time prior to extraction increased water solubility of soil P. Changing incubation time also modified the extent of differences between certain treatments. For example, as incubation period increased, differences in water-soluble P content between soils mixed with alternative NPA+Phyt manure and soils mixed with conventional NPA+P manure on an equal-P basis also increased. This apparently occurred because longer incubation enhanced the solubility-reducing effect of the supplemental defluorinated calcium phosphate in the NPA+P diet. This conclusion follows from the fact that (1) differences in water-soluble P content between NPA and NPA+P Pbasis soils increased with longer incubation times and (2) water-soluble P levels were almost identical in the NPA-treated and NPA+Phyt-treated soils at both incubation times. The overall implication is that increasing opportunties for chemical reactions between soil and manure components may do little to diminish increases in water solubility of soil P caused by conversion to alternative diets.

Table 55: Water-soluble P in Manured Soils, Incubation Time Comparison

All Soils, Both Application Regimes			
4-Day Incubation		28-Day Incubation	
Treatment	mg P kg^{-1}	Treatment	mg P kg^{-1}
NPA+P Nbasis	11.9 a†	HAP+Phyt	15.9 a
HAP+Phyt	11.4 a	NPA+P Nbasis	15.6 a
HAP	10.0 b	HAP	14.9 b
NPA+Phyt	9.8 b	NPA	14.3 c
NPA	9.5 b	NPA+Phyt	14.3 c
NPA+P Pbasis	7.8 c	NPA+P Pbasis	10.5 d
Control	3.4 d	Control	4.2 e

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05. Statistical analyses were conducted separately for each of the two incubation times.

Increasing incubation period decreased numerical differences in water-soluble P content between the NPA-treated soil and soils treated with either the HAP or HAP+Phyt manures. For example, after 4 d of incubation, the HAP+Phyt soils contained 20% more water-extractable P than soils mixed with NPA manure. After 28 d of incubation, the HAP+Phyt soils contained 11% more water-extractable P than soils mixed with NPA manure (differences were statistically significant for both incubation times). Note that, from a percentage standpoint, increasing incubation time also reduced water-soluble P differences between the HAP-amended soils and the NPA-amended soils. However, P solubility differences between the HAP and NPA soils actually became more statistically significant with increasing incubation time.

The influence of soil type on water-soluble P differences between soils amended with different manure treatments is explored in Table 56. Note that only low application regime data are included in the comparison, since the Mahan soil did not receive high regime treatments.

The data in Table 56 show that the three soil types differed in native water-soluble P content (i.e., prior to manure addition). These differences in native or background P help explain why the manure-amended Mahan soils contained the least water-soluble P across all treatments. Soil type also affected comparisons between treatments. For all three soil types, soils amended with NPA+Phyt manures contained statistically higher levels of water-soluble P than soils treated with NPA+P manure on an equal-P basis. However, from a numerical standpoint, the degree to which conversion to the alternative diet increased solubility of soil P varied by soil type. Groseclose soils amended with NPA+Phyt manure contained 14% more water-soluble P than Groseclose soils amended with the NPA+P Pbasis treatment. (7.9 mg kg^{-1} for NPA+P Pbasis versus 9.0 mg kg^{-1} for NPA+Phyt). For manure-amended Cecil and Mahan soils, the shift from NPA+P to NPA+Phyt manure resulted in 40% and 56% increases in water-solubility of soil P, respectively (Cecil, 7.0 mg kg^{-1} for NPA+P Pbasis versus 9.8 mg kg^{-1} for NPA+Phyt; Mahan, 5.0 mg kg^{-1} for NPA+P Pbasis versus 7.8 mg kg^{-1} for NPA+Phyt). This variation in NPA+P versus NPA+Phyt comparison can be attributed to a variation in calcium phosphate effect. That is because, across all soil types, soils treated with NPA and NPA+Phyt manures contained nearly identical levels of water-soluble P. It is intriguing to note that conversion to a phytase diet produced the least P solubility enhancement on the Groseclose, which contained the highest levels of native water-extractable P.

Comparisons of water-soluble P data for all 10 combinations of application regime, incubation time, and soil type factors are presented in Table 57. Note that three individual combinations of factors (e.g., high application regime x 4-d incubation time x Groseclose soil) did not produce statistically significant differences in P water solubility between soils amended with NPA+P Pbasis and NPA+Phytase treatments. Nevertheless, from a numerical point of view, the NPA+P Pbasis soil contained less water-soluble P than NPA+Phyt-amended soils under all conditions.

Table 56: Water-soluble P in Manured Soils, Soil Type Comparison

Low Application Regime, Both Incubation Times					
Groseclose Soil		Cecil Soil		Mahan Soil	
Treatment	mg P kg^{-1}	Treatment	mg P kg^{-1}	Treatment	mg P kg^{-1}
NPA+P Nbasis	10.5 a†	NPA+P Nbasis	11.4 a	NPA+P Nbasis	8.7 a
HAP+Phyt	10.0 a	HAP+Phyt	11.4 a	HAP+Phyt	8.5 ab
NPA	9.2 b	HAP	10.3 ab	HAP	8.1 bc
NPA+Phyt	9.0 b	NPA+Phyt	9.8 b	NPA+Phyt	7.8 c
HAP	8.9 b	NPA	9.5 b	NPA	7.6 c
NPA+P Pbasis	7.9 c	NPA+P Pbasis	7.0 c	NPA+P Pbasis	5.0 d
Control	5.6 d	Control	2.7 d	Control	0.9 e

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05. Statistical analyses were conducted separately for each of the three soil types

Table 57: Water-soluble P in Manured Soils, Comparison of All Factor Combinations

Low Manure Application Regime				High Manure Application Regime			
4-Day Incubation		28-Day Incubation		4-Day Incubation		28-Day Incubation	
Groseclose Soil							
Treatment	mg kg^{-1}	Treatment	mg kg^{-1}	Treatment	mg kg^{-1}	Treatment	mg kg^{-1}
NPA+P Nbasis	9.1 a†	NPA+P Nbasis	11.9 a	NPA+P Nbasis	15.7 a	HAP+Phyt	24.0 a
HAP+Phyt	8.2 b	HAP+Phyt	11.7 a	HAP+Phyt	14.9 a	NPA+P Nbasis	22.7 ab
NPA+Phyt	7.2 c	NPA	11.0 ab	NPA	12.2 b	HAP	21.4 bc
HAP	7.2 c	NPA+Phyt	10.8 abc	HAP	12.1 b	NPA+Phyt	20.6 c
NPA	6.9 c	HAP	10.6 bc	NPA+Phyt	11.3 b	NPA	19.7 c
NPA+P Pbasis	5.9 d	NPA+P Pbasis	9.8 c	NPA+P Pbasis	10.6 b	NPA+P Pbasis	15.6 d
control	5.2 e	control	6.1 d	control	5.2 c	control	6.1 e
Cecil Soil							
Treatment	mg kg^{-1}	Treatment	mg kg^{-1}	Treatment	mg kg^{-1}	Treatment	mg kg^{-1}
NPA+P Nbasis	11.6 a	HAP	12.5 a	HAP+Phyt	16.5 a	NPA+P Nbasis	20.4 a
HAP+Phyt	10.0 ab	HAP+Phyt	12.5 a	HAP	16.3 a	HAP+Phyt	19.7 ab
NPA+Phyt	8.3 bc	NPA+Phyt	11.4 b	NPA+P Nbasis	15.9 ab	NPA	19.5 ab
HAP	8.2 bc	NPA+P Nbasis	11.2 b	NPA	15.4 ab	HAP	18.7 bc
NPA	8.0 bc	NPA	11.0 b	NPA+Phyt	14.5 b	NPA+Phyt	17.9 c
NPA+P Pbasis	6.6 c	NPA+P Pbasis	7.5 c	NPA+P Pbasis	11.6 c	NPA+P Pbasis	12.9 d
control	2.5 d	control	3.1 d	control	2.5 d	control	3.1 e
Mahan Soil							
Treatment	mg kg^{-1}	Treatment	mg kg^{-1}				
NPA+P Nbasis	7.4 a	NPA+P Nbasis	10.1 a				
HAP+Phyt	7.0 ab	HAP+Phyt	10.0 a				
HAP	6.3 bc	HAP	9.8 a				
NPA+Phyt	6.2 bc	NPA+Phyt	9.3 a				
NPA	6.1 c	NPA	9.2 a				
NPA+P Pbasis	4.4 d	NPA+P Pbasis	5.7 b				
control	0.9 e	control	0.8 c				

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05.
Statistical analyses were conducted separately for each of the 10 combinations of factors.

Summary

Extracting manure-amended soils with 0.01 M $CaCl_2$ showed that, under a P-limited manure application scenario, switching from the conventional NPA+P diet to the alternative NPA+Phyt diet significantly increased the amount of water-soluble P in those soils. When data from all 10 combinations of application regime, incubation time, and soil type factors used in the study are considered together, the amount of water-soluble P extracted from soils increased by 29% as a result of converting to the phytase-amended diet. It should be noted that, when wet manures were applied to soil on an equal-N and equal-manure-weight basis, the conversion from the NPA+P diet to the alternative NPA+Phyt diet produced a modest, yet statistically significant decrease in the the amount of $CaCl_2$-extractable P in those soils.

The $CaCl_2$ extraction data strongly suggest that differences in water-soluble P content between soils amended with conventional and alternative manures were directly related to the fact that the NPA+Phyt feed contained less defluorinated calcium phosphate than the conventional NPA+P feed. When phytase enzyme was added to the baseline diet without any modification in feed defluorinated phosphate levels, $CaCl_2$-extractable P levels in manured soils were not affected. Meanwhile, simple addition of defluorinated phosphate to the baseline diet signficantly reduced water-soluble P levels in manured soils. These observations provide convincing evidence of the critical role that dietary defluorinated calcium phosphate levels played in controlling $CaCl_2$ extractability of P in manured soils.

It should be noted that the same calcium phosphate effect described above was observed during extraction of raw manures. Apparently, mixing wet manures with soil did not diminish this calcium phosphate effect. In fact, increasing manure-soil incubation time appeared to enhance the way in which increasing dietary calcium phosphate levels depressed P solubility. This suggests that P water solubility differences between conventional and alternative poultry manures may persist in the soil environment. The idea that calcium phosphate compounds derived from conventional poultry manures may persist in soils for many years after land application is not new. For example, Sharpley and Smith (1995) found that application of poulty litter and other manures over 8 to 35 yr to 20 Southern Plains soils dramatically increased the proportion of soil P in acid-soluble, Ca-bound form. It is also interesting to note that drying raw manures heightened differences in P water solubility between the NPA+P and NPA+Phyt manures. A drying-induced enhancement of the calcium phosphate effect appeared to be involved (Figure 4). Since application of wet manures to soil in no way mitigated the solubility-reducing calcium phosphate effect, it is intriguing to consider that use of dried manures in the soil incubation experiment might have produced even more dramatic water-soluble P differences between NPA+P Pbasis- and NPA+Phyt-treated soils.

Finally, it should be emphasized that water solubility of manure P at the point of excretion was significantly increased by adding phytase enzyme, HAP corn, and HAP corn plus phytase enzyme to the baseline NPA diet (Tables 13 and 17). After wet manures were mixed and incubated with soils, the phytase-induced enhancement of P water solubility disappeared. No HAP-induced enhancement of water-soluble P in manured soils was observed either. Water solubility of P in manured soils was enhanced by HAP-plus-phytase modification of the baseline diet. However, this HAP-plus-phytase effect was less pronounced than in the raw manures. These results may indicate that P water solubility differences between conventional and alternative poultry manures that are produced strictly by feeding phytase or HAP corn (i.e., without modification of feed calcium phosphate levels) may be short-lived in the soil environment.

3.3.2.b. Mehlich III Extraction of Soil P

Effect of Manure Treatments

Results of Mehlich III extraction of soil P are summarized in Table 58. This table shows means for data from a total of 365 Mehlich III extractions, including results from all 10 combinations of application regime, incubation time, and soil type factors used in the study. Therefore, Table 58 offers a reliable overall picture of how the different manure treatments affected plant availability of soil P under a range of conditions. Note that the acidic Mehlich III solution was a far more effective extractant of soil P than was the dilute $CaCl_2$ solution, solubilizing over 20 times more P than the water extractant.

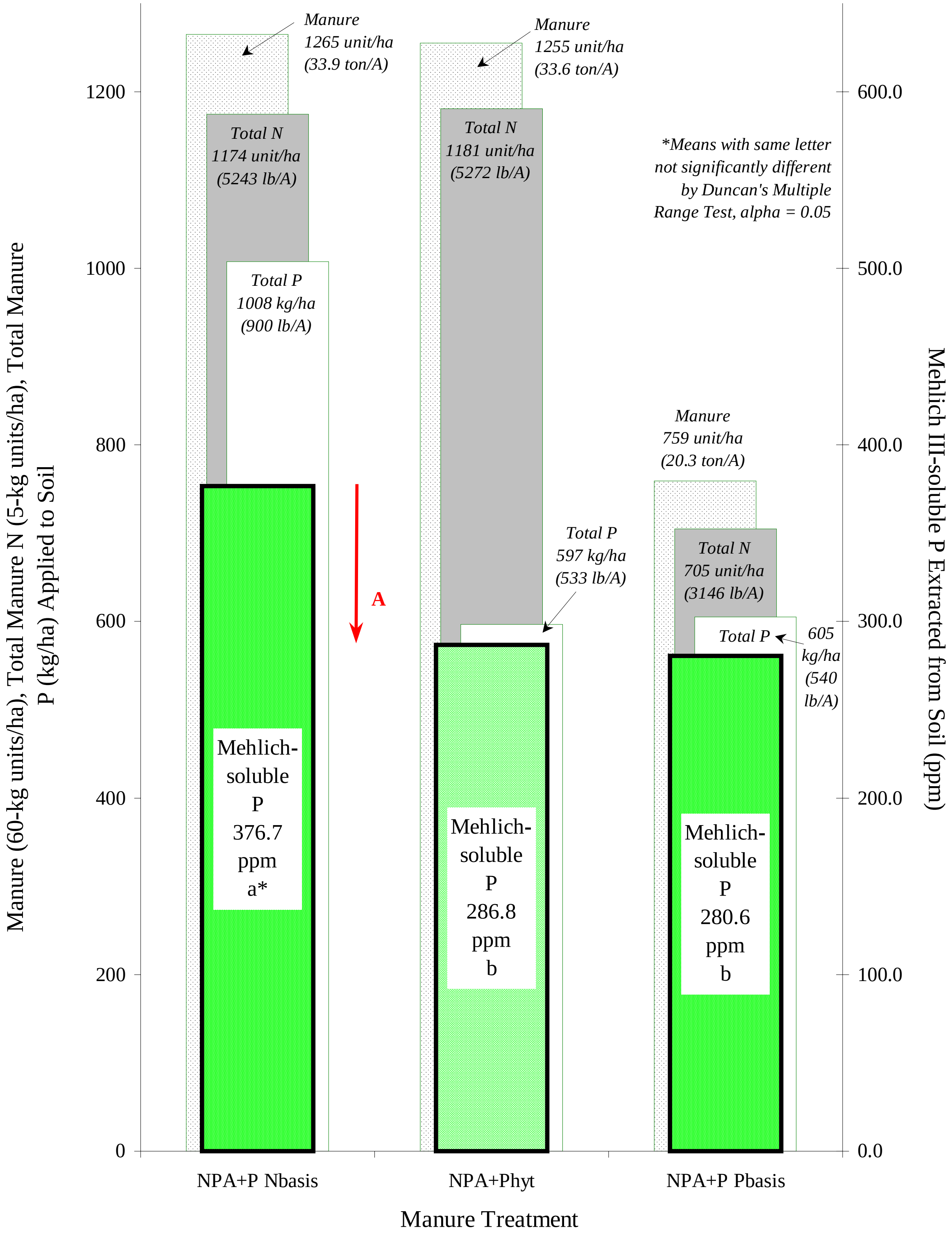
Table 58: Mehlich III-Soluble P in Manured Soils, Overall Comparison (All Application Regimes, Incubation Times, Soil Types)

Treatment	mg P kg^{-1}
NPA+P Nbasis	376.7 a†
HAP+Phyt	303.2 b
NPA+Phyt	286.8 c
HAP	286.6 c
NPA+P Pbasis	280.6 c
NPA	280.0 c
Control	145.9 d

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05

The first issue to consider is how conversion from the conventional NPA+P diet to the alternative NPA+Phyt diet affected plant availability of soil P. In order to more clearly illustrate the impact of the dietary change under both N- and P-based nutrient management, the NPA+P Nbasis, NPA+Phyt, and NPA+P Pbasis results from Table 58 are presented in Figure 21. The background columns in this figure again compare the total amounts of manure, manure N, and manure P applied to soils as part of the NPA+P Nbasis, NPA+Phyt, and NPA+P Pbasis treatments. The foreground columns show the amount of Mehlich III-soluble P that the soils contained as a result of the three treatments. The values in these foreground columns are taken directly from Table 58.

Figure 21: Mehlich III-Soluble P Comparison, Soils Treated with NPA+P and NPA+Phyt Manures



Soils amended with the NPA+P Nbasis treatment contained significantly more plant-available P than soils amended with the NPA+Phyt treatment (376.7 mg kg^{-1} for NPA+P Nbasis versus 286.8 mg kg^{-1} for NPA+Phyt). This means that, when manure was applied to soils on an equal-N and equal-manure-weight basis, feeding phytase enzyme resulted in a significant decrease in Mehlich III-extractable P levels in those soils. This 24% decrease is represented by line segment A in Figure 21. This reduction in Mehlich-soluble P levels was still modest relative to the 41% decrease in total P loading achieved by feeding the NPA+Phyt diet. Recall, however, that water-soluble P content of manured soils decreased by only 14% under the same circumstances (Figure 20). Consider next the P-based manure management scenario, illustrated by the NPA+P Pbasis and NPA+Phyt treatments. When manure applications were limited on a P basis, switching from the conventional to the alternative phytase diet did not affect the total amount of manure P loaded on soils. Phytase feeding also did not significantly change the amount of plant-available P in the manured soils. Amending soils with NPA+Phyt manure instead of NPA+P manure resulted in a statistically insignificant 2% increase in Mehlich III-extractable P in those soils (from 280.6 mg kg^{-1} for NPA+P Pbasis to 286.8 mg kg^{-1} for NPA+Phyt). Under the same circumstances, water solubility of P in manured soils increased by 29% (Figure 20).

Why did soils amended with NPA+P and NPA+Phyt manures on an equal-P basis differ in water-soluble P content, but not in Mehlich III-soluble P content? The answer lies in the hypothesis stated previously that the NPA+P manure contained a greater proportion of P in calcium phosphate form than did the NPA+Phyt manure. It is proposed that these calcium phosphate compounds persisted after application to soils and were readily solubilized by the acidic Mehlich III solution, but not by the $CaCl_2$ extractant. The data in Table 58 indicate that the Mehlich III extractant solubilized nearly identical amounts of P from the NPA- and NPA+P Pbasis-amended soils. Since the NPA and NPA+P feeds differed dramatically in defluorinated calcium phosphate content, this supports the theory that calcium phosphate compounds did not limit Mehlich III solubility of P in manured soils. Note that Mehlich III extraction of wet manures did not reveal statistically significant differences in P solubility between the NPA and NPA+P manures either (Tables 19 and 21).

The Mehlich III solution dissolved statistically similar amounts of P from soils amended with the NPA, NPA+Phyt, and HAP treatments, as shown in Table 58. This suggests that plant availability of P in manured soils was not significantly affected by adding phytase enzyme or HAP corn to the baseline NPA diet without modification of calcium phosphate levels. The absence of a statistically significant phytase or HAP effect on Mehlich III P solubility is consistent with results from extraction of wet manures (Tables 19 and 21). The data in Table 58 does indicate that a statistically significant difference in Mehlich III-soluble P levels was observed between soils amended with the NPA and HAP+Phyt treatments (280.0 mg kg^{-1} for NPA versus 303.2 mg kg^{-1} for HAP+Phyt). Note that this same significant HAP-plus-phytase effect on Mehlich III solubility of P was observed during extraction of wet individual pen manures (Table 19).

Influence of Application Regime, Incubation Time, and Soil Type Factors

Data in Table 59 allow one to explore the influence of changing manure application regime on the treatment comparisons discussed above. Again, no data from extraction of Mahan soils is included in Table 59, because high application regime treatments were not used on any soils of this type. As anticipated, doubling the amount of manure applied to soils produced a dramatic increase in plant-available soil P across all treatments. Changing application regime caused only one notable shift in the effects of dietary treatment on plant availability of P in manured soils. Under the low application regime, there was no significant difference in Mehlich III P solubility between soils treated with HAP+Phyt manure and soils treated with NPA manure. Under the high application regime, soils treated with HAP+Phyt manure contained significantly more Mehlich III-soluble P than did soils amended with NPA manure (398.0 mg kg^{-1} for HAP+Phyt versus 362.8 mg kg^{-1} for NPA).

Table 59: Mehlich III-Soluble P in Manured Soils, Application Regime Comparison

Groseclose and Cecil Soils, Both Incubation Times			
Low Application Regime		High Application Regime	
Treatment	mg P kg^{-1}	Treatment	mg P kg^{-1}
NPA+P Nbasis	312.7 a†	NPA+P Nbasis	493.0 a
HAP+Phyt	256.3 b	HAP+Phyt	398.0 b
NPA+Phyt	246.1 b	HAP	374.1 c
NPA+P Pbasis	244.0 b	NPA+Phyt	369.8 c
HAP	242.1 b	NPA	362.8 c
NPA	242.0 b	NPA+P Pbasis	359.5 c
Control	158.4 c	Control	158.4 d

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05. Statistical analyses were conducted separately for each of the two incubation times.

The amounts of Mehlich III-soluble P in manure-amended soils incubated for 4 d and 28 d prior to extraction are compared in Table 60. Across all treatments except the control, increasing incubation time resulted in increases in Mehlich III extractability of soil P. More importantly, the additional 24 d of incubation brought out a number of statistically significant differences between treatments that were not observed after 4 d of incubation. After 28 d of incubation, soils amended with NPA+Phyt manures contained significantly higher levels of plant-available P than soils treated with NPA+P on a P basis. It should be emphasized that this consisted of a mere 4% increase in Mehlich III extractability of soil P. Nevertheless, comparisons among the NPA, NPA+P Pbasis, and NPA+Phyt treatments show that increasing incubation time heightened (numerically, not statistically) the solubility-enhancing effect of feeding phytase enzyme and the solubility-reducing effect of supplemental dietary calcium phosphate. Longer incubation time produced a statistically significant difference in plant-available P content between soils amended with NPA and HAP manures, as well as a significant difference between soils amended with

NPA and HAP+Phyt manures. These results suggest that increasing the amount of time available for reactions between soil and manure components may actually enhance differences in plant-available P content between soils treated with different types of manure.

Table 60: Mehlich III-Soluble P in Manured Soils, Incubation Time Comparison

All Soils, Both Application Regimes			
4-Day Incubation		28-Day Incubation	
Treatment	mg P kg^{-1}	Treatment	mg P kg^{-1}
NPA+P Nbasis	361.0 a†	NPA+P Nbasis	391.9 a
HAP+Phyt	270.7 b	HAP+Phyt	334.4 b
NPA+P Pbasis	262.1 b	HAP	319.3 c
NPA+Phyt	261.2 b	NPA+Phyt	311.5 cd
HAP	254.0 b	NPA	307.4 de
NPA	251.6 b	NPA+P Pbasis	298.3 e
Control	147.0 c	Control	144.1 f

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05. Statistical analyses were conducted separately for each of the two incubation times.

The influence of soil type on plant availability of P in manured soils is explored in Table 61. Note that the native soils (prior to manure addition) differed in Mehlich III-soluble P content. The Groseclose control contained 215.1 mg kg^{-1} Mehlich III-soluble P, while the Cecil and Mahan controls contained 91.9 mg kg^{-1} and 83.2 mg kg^{-1} acid-soluble P, respectively. These differences in native or background P help explain why the manure-amended Groseclose soils contained the most Mehlich III-soluble P across all treatments, while the Mahan soils contained the least Mehlich III-soluble P across all treatments. Only one significant instance of soil type influence on treatment comparisons was observed. Groseclose soils amended with HAP+Phyt manure contained significantly more Mehlich III-soluble P than Groseclose soils amended with NPA manure (302.3 mg kg^{-1} for HAP+Phyt versus 278.4 mg kg^{-1} for NPA). Such differences were not observed with the Cecil and Mahan soils.

Table 61: Mehlich III-Soluble P in Manured Soils, Soil Type Comparison

Low Application Regime, Both Incubation Times					
Groseclose Soil		Cecil Soil		Mahan Soil	
Treatment	mg P kg^{-1}	Treatment	mg P kg^{-1}	Treatment	mg P kg^{-1}
NPA+P Nbasis	345.7 a†	NPA+P Nbasis	279.7 a	NPA+P Nbasis	260.6 a
HAP+Phyt	302.3 b	HAP+Phyt	210.3 b	HAP+Phyt	197.8 b
NPA+Phyt	289.0 bc	NPA	205.6 b	NPA+Phyt	194.1 b
NPA+P Pbasis	284.8 bc	HAP	203.9 b	NPA+P Pbasis	188.0 b
HAP	284.5 bc	NPA+Phyt	203.2 b	HAP	187.8 b
NPA	278.4 c	NPA+P Pbasis	203.2 b	NPA	182.4 b
Control	215.1 d	Control	91.9 c	Control	83.2 c

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05. Statistical analyses were conducted separately for each of the.

Mehlich III extraction data from all 10 combinations of application regime, incubation time, and soil type factors are compared in Table 62. This table shows that, for almost every individual combination of factors, Mehlich III solubility of soil P was not significantly affected by any of the study's four major dietary modifications (defluorinated phosphate addition, phytase addition, HAP corn substitution, and phytase addition plus HAP corn substitution) to the baseline NPA diet. For three combinations of factors involving long incubation times, soils amended with the HAP+Phyt treatment contained significantly more Mehlich III-soluble P than soils amended with the NPA treatment.

Summary

Extracting manure-amended soils with Mehlich III solution showed that, under P-based manure management, switching from the conventional NPA+P diet to the alternative NPA+Phyt diet did not significantly affect the amount of plant-available P in those soils. It is hypothesized that the acidic Mehlich III extractant more completely solubilized the manure-derived calcium phosphate compounds that caused differences in P water solubility between soils mixed with NPA+P and NPA+Phyt manures on an equal-P basis. When wet manures were applied to soils on an equal-N basis, the conversion from the NPA+P diet to the alternative NPA+Phyt diet produced a statistically significant 24% decrease in plant-available soil P levels.

It is assumed that the forms of P soluble in the acidic, chelate-containing Mehlich III extractant would be available over time to plants or microbes in the soil environment. The Mehlich III extraction results therefore suggest that, although dietary changes may result in land application of different forms of manure P, most of those different forms of P are equally available for uptake by plants or other soil organisms. This implies that, given sufficient time, differences among these P compounds have the potential to be erased as a result of P uptake, biological transformation, and cycling back into the soil environment. Nevertheless, it is important to note that not all forms of manure P were equally soluble in the Mehlich III extractant. Overall, soils amended with HAP+Phyt manures contained statistically higher levels of plant-available P than NPA-treated soils. This effect was more pronounced as incubation time increased, suggesting its potential for persistence in the soil environment. It should also be noted that only fresh or wet manures were applied to soils in this study. Drying composite manures prior to extraction produced significant differences in Mehlich III P solubility between the raw NPA+P and NPA+Phyt manures. This was apparently the result of a drying-induced decrease in the acid solubility of manure calcium phosphate compounds. It is intriguing to consider the possibility that, if dried instead of wet manures had been used in the soil incubation experiment, significant differences in plant-available P levels might have been observed between soils amended with the NPA+Phyt and NPA+P Pbasis treatments.

Table 62: Mehlich III-Soluble P in Manured Soils, Comparison of All Factor Combinations

Low Manure Application Rate				High Manure Application Rate			
4-Day Incubation		28-Day Incubation		4-Day Incubation		28-Day Incubation	
Groseclose Soil							
Treatment	mg kg^{-1}	Treatment	mg kg^{-1}	Treatment	mg kg^{-1}	Treatment	mg kg^{-1}
NPA+P Nbasis	331.7 a†	NPA+P Nbasis	359.6 a	NPA+P Nbasis	490.4 a	NPA+P Nbasis	521.2 a
NPA+P Pbasis	278.2 b	HAP+Phyt	327.2 b	HAP+Phyt	394.8 b	HAP+Phyt	480.3 b
HAP+Phyt	277.3 b	HAP	315.4 bc	NPA+Phyt	380.4 b	HAP	446.7 c
NPA+Phyt	265.6 b	NPA+Phyt	312.3 bc	HAP	368.8 b	NPA+Phyt	436.6 c
HAP	259.8 b	NPA	302.1 cd	NPA+P Pbasis	361.9 b	NPA+P Pbasis	429.8 c
NPA	254.6 b	NPA+P Pbasis	291.3 d	NPA	357.8 b	NPA	422.6 c
control	220.2 c	control	207.8 e	control	220.2 c	control	207.8 d
Cecil Soil							
Treatment	mg kg^{-1}	Treatment	mg kg^{-1}	Treatment	mg kg^{-1}	Treatment	mg kg^{-1}
NPA+P Nbasis	259.3 a	NPA+P Nbasis	300.1 a	NPA+P Nbasis	467.5 a	NPA+P Nbasis	487.5 a
NPA+P Pbasis	189.7 b	HAP+Phyt	242.7 b	HAP+Phyt	310.2 b	HAP+Phyt	390.3 b
NPA	181.4 b	HAP	238.1 b	NPA+Phyt	304.3 b	HAP	371.9 bc
HAP+Phyt	177.8 b	NPA+Phyt	237.2 b	NPA+P Pbasis	299.1 b	NPA	368.5 c
HAP	169.6 b	NPA	229.7 b	HAP	294.6 b	NPA+Phyt	344.6 d
NPA+Phyt	169.2 b	NPA+P Pbasis	216.6 b	NPA	290.4 b	NPA+P Pbasis	333.1 d
control	93.1 c	control	89.1 c	control	93.1 c	control	89.1 e
Mahan Soil							
Treatment	mg kg^{-1}	Treatment	mg kg^{-1}				
NPA+P Nbasis	256.0 a	NPA+P Nbasis	265.2 a				
HAP+Phyt	193.1 b	HAP+Phyt	202.4 b				
NPA+Phyt	186.3 b	NPA+Phyt	201.8 b				
NPA+P Pbasis	181.5 b	HAP	198.1 b				
HAP	177.4 b	NPA+P Pbasis	194.6 b				
NPA	174.0 b	NPA	190.7 b				
control	85.1 c	control	81.2 c				

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05. Statistical analyses were conducted separately for each of the 10 combinations of factors.

3.3.2.c. Olsen Extraction of Soil P

Effect of Manure Treatments

The results of Olsen extraction of P from manured soils are presented in Table 63. The Olsen-soluble P means in Table 63 reflect data from 262 individual extractions. All nine combinations of application regime, incubation time, and soil type factors that were subjected to Olsen extraction are represented. Therefore, Table 63 provides an overall picture of how the different manure treatments affected Olsen solubility of soil P under a variety of conditions. These results show that the Olsen solution was generally a more effective extractant of soil P than 0.01 M $CaCl_2$, but a weaker P extractant than the Mehlich III solution. Table 63 also reveals a number of similarities between the Olsen-soluble P data and both the water-soluble and Mehlich III-soluble P results with regard to the relative rank and statistical grouping of treatment means.

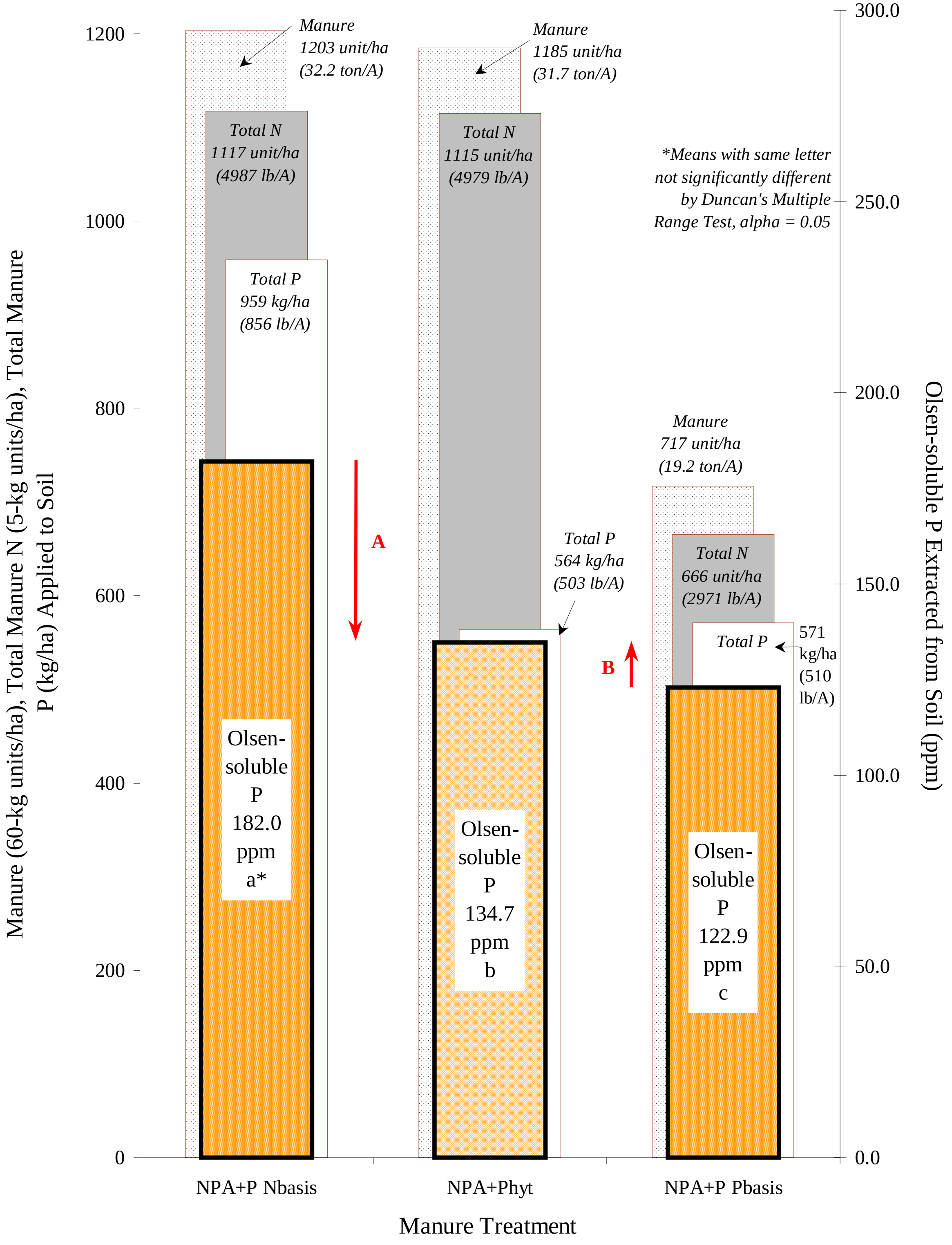
Table 63: Olsen-Soluble P in Manured Soils, Overall Comparison

Treatment	mg P kg^{-1}
NPA+P Nbasis	182.0 a†
HAP+Phyt	161.4 b
NPA	142.8 c
HAP	141.5 c
NPA+Phyt	134.7 c
NPA+P Pbasis	122.9 d
Control	61.9 e

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05

The first issue to consider is how switching from the conventional NPA+P diet to the alternative NPA+Phyt diet affected Olsen solubility of soil P. In order to graphically illustrate the effect of this dietary shift under both N- and P-based manure management, the NPA+N Pbasis, NPA+Phyt, and NPA+P Pbasis results from Table 63 are presented in Figure 22. The background columns in this figure compare the total amounts of manure, manure N, and manure P applied to soils as a result of the three treatments, while the foreground columns show the amount of water-soluble P the soils contained following the manure treatments. The values in the foreground columns are drawn directly from Table 63.

Figure 22: Olsen-Soluble P Comparison, Soils Treated with NPA+P and NPA+Phyt Manures



Soils amended with the NPA+P Nbasis treatment contained significantly more Olsen-soluble P than soils amended with the NPA+Phyt treatment (182.0 mg kg^{-1} versus 134.7 mg kg^{-1}). This means that, when manure was applied to soils on an equal-N and equal-manure-weight basis, conversion to the phytase diet resulted in a significant decrease in Olsen-extractable P levels in those soils (represented by line segment A in Figure 22). This 26% drop in Olsen-soluble P was similar to the decrease in Mehlich III-extractable P observed under the same circumstances (Figure 21). Consider next the P-based manure management scenario, illustrated by the NPA+P Pbasis and NPA+Phyt treatments. When manure was applied to soils on an equal-P basis, switching from the conventional to the alternative phytase diet did not change the total amount of manure P applied to soils. However, feeding the alternative diet resulted in a significant increase in Olsen-soluble P levels in soils, from 122.9 mg kg^{-1} to 134.7 mg kg^{-1} (represented by line segment B in Figure 22). Although statistically significant, this shift represented only a 10% increase in Olsen availability of P from manured soils. This increase was intermediate between the statistically significant 29% rise in water solubility and the insignificant 2% rise in Mehlich III solubility of soil P observed under the same circumstances.

Why did soils amended with NPA+Phyt manure contain more Olsen-soluble P than soils amended with NPA+P manure on an equal-P basis? It is again proposed that the greater proportion of P in calcium phosphate form in the NPA+P manure caused this difference. As shown in Table 63, soils amended with the NPA+P Pbasis treatment contained a statistically significant 14% less Olsen soluble P than soils treated with NPA manure. It is logical that the pH 8.5 Olsen extractant was a relatively inefficient extractant of calcium phosphate P. Meanwhile, the NPA+Phyt- and NPA-treated soils did not differ in Olsen extractability of P, indicating that a phytase enzyme effect was not involved. Olsen extraction also revealed no significant difference in P solubility between soils mixed with HAP manure and soils mixed with NPA manure. In contrast, soils amended with HAP+Phyt manures contained significantly more Olsen-soluble P than did NPA soils (161.4 mg kg^{-1} versus 142.8 mg kg^{-1}). It is interesting to note that the phytase, HAP, and HAP-plus-phytase effects shown for manured soils in Table 63 were not consistent with the effects that these three dietary modifications had on Olsen solubility of P in wet composite manures. For example, Olsen extraction of wet composite manures showed that the NPA+Phyt manure contained a significantly higher P solubility percentage than the baseline NPA manure, but that the HAP+Phyt manure did not (Table 29).

Influence of Application Regime, Incubation Time, and Soil Type Factors

The data in Table 64 allows investigation of the influence of changing manure application regime on Olsen extractability of soil P. Note that high application regime treatments were not used on any Mahan soils. In addition, Groseclose soils treated with high regime manure applications and incubated for 28 d were not extracted with Olsen solution. Therefore, Table 64 only shows the results of Olsen extraction of Groseclose and Cecil soils after short incubation time. As the amount of manure applied to these soils doubled, Olsen-soluble P levels rose across all treatments. Note that in these particular soils, no statistically significant differences in Olsen-extractable P levels were observed between NPA+P Pbasis and NPA+Phyt soils. However, higher manure loadings enhanced the solubility-reducing calcium phosphate effect (Olsen P solubility in NPA+P Pbasis soils was 5% lower than in NPA soils under the low application regime, 11% lower under the high application regime). The shift from low to high application regime also enhanced the solubility-increasing HAP-plus-phytase effect. Differences in Olsen-soluble P levels between soils amended with HAP+Phyt manures and soils mixed with NPA manure became statistically significant under the high application regime.

Table 64: Olsen-Soluble P in Manured Soils, Application Regime Comparison

Groseclose and Cecil Soils, 4-Day Incubation			
Low Application Regime		High Application Regime	
Treatment	mg P kg^{-1}	Treatment	mg P kg^{-1}
NPA+P Nbasis	144.9 a†	NPA+P Nbasis	243.8 a
HAP+Phyt	121.7 b	HAP+Phyt	219.1 b
HAP	111.3 bc	NPA	190.6 c
NPA+Phyt	110.4 bc	HAP	178.5 cd
NPA	109.2 bc	NPA+P Pbasis	169.3 cd
NPA+P Pbasis	103.6 c	NPA+Phyt	166.7 d
Control	66.0 d	Control	66.0 e

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05. Statistical analyses were conducted separately for each of the two incubation times.

Olsen-soluble P means for manure-amended soils incubated for 4 d and 28 d prior to extraction are compared in Table 65. Note that only low application regime results are included. Increasing incubation time produced 14% to 20% increases in Olsen-extractability of soil P for soils treated with NPA, HAP, NPA+Phyt, and HAP+Phyt manures. However, the NPA+P Nbasis soil showed only a 3% increase in Olsen P solubility, while the NPA+P Pbasis soil decreased slightly in Olsen-soluble P content with lengthening incubation. The net result was that increasing incubation time enhanced the calcium phosphate effect and led to a statistically significant difference in Olsen-soluble P content between soils treated with NPA+Phyt and NPA+P manures on an equal-P basis. Greater incubation time also enhanced the HAP-plus-phytase effect and caused Olsen-soluble P levels in HAP+Phyt-treated soils to be significantly higher than in NPA-amended soils.

Table 65: Olsen-Soluble P in Manured Soils, Incubation Time Comparison

All Soils, Low Application Regime			
4-Day Incubation		28-Day Incubation	
Treatment	mg P kg^{-1}	Treatment	mg P kg^{-1}
NPA+P Nbasis	141.6 a†	NPA+P Nbasis	146.5 a
HAP+Phyt	115.2 b	HAP+Phyt	138.7 b
HAP	106.4 bc	HAP	124.9 c
NPA+Phyt	105.8 bc	NPA	123.0 c
NPA	105.8 bc	NPA+Phyt	120.6 c
NPA+P Pbasis	98.8 c	NPA+P Pbasis	96.2 d
Control	64.3 d	Control	56.0 e

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05. Statistical analyses were conducted separately for each of the two incubation times.

The influence of soil type on differences in Olsen-soluble P content between soils amended with different manure treatments is explored in Table 66. Note that only low application regime data are included in the comparison. The three native soils (prior to manure addition) differed in Olsen-soluble P content. These differences in native or background P help explain why the manure-amended Groseclose soils contained the most base-soluble P across all treatments, while the Mahan soils contained the least base-soluble P across all treatments. A statistically significant calcium phosphate effect was observed for all three soil types. However, this effect was most pronounced in the Groseclose and Mahan soils. As a result, soils of these types mixed with NPA+Phyt manure contained significantly more Olsen-soluble P than soils of these types amended with the NPA+P Pbasis treatment. For Cecil soils, the NPA+Phyt versus NPA+P Pbasis comparison did not reveal statistically significant differences in Olsen solubility of P. A different trend was observed with regard to the HAP-plus-phytase effect, which was significant for Groseclose and Cecil soils, but not for soils of the Mahan type. It should be emphasized that similar trends among the treatment means were observed for all three soil types. Only the magnitude of the differences between treatments changed as soil type changed.

Table 66: Olsen-Soluble P in Manured Soils, Soil Type Comparison

Low Application Regime, Both Incubation Times					
Groseclose Soil		Cecil Soil		Mahan Soil	
Treatment	mg P kg^{-1}	Treatment	mg P kg^{-1}	Treatment	mg P kg^{-1}
HAP+Phyt	141.3 a†	NPA+P Nbasis	159.0 a	NPA+P Nbasis	134.7 a
NPA+P Nbasis	139.6 ab	HAP+Phyt	129.1 b	HAP+Phyt	104.4 b
NPA+Phyt	123.3 b	HAP	118.9 c	HAP	102.7 b
NPA	122.8 b	NPA	117.4 c	NPA	99.3 b
HAP	122.2 b	NPA+Phyt	114.6 cd	NPA+Phyt	97.5 b
NPA+P Pbasis	102.0 c	NPA+P Pbasis	104.9 d	NPA+P Pbasis	83.8 c
Control	82.5 d	Control	48.2 e	Control	39.2 d

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05. Statistical analyses were conducted separately for each of the three soil types.

Base-soluble P results from all nine combinations of application regime, incubation time, and soil type factors subjected to Olsen extraction are compared in Table 67. These results confirm that a significant calcium phosphate effect (i.e., difference between NPA and NPA+P Pbasis soils) was observed after 28 d of incubation, but not after 4 d. The data in Table 67 also suggest that increasing application regime as well as incubation time heightened the solubility-enhancing effect of amending the baseline diet with HAP corn plus phytase enzyme.

Summary

Extraction of manured soils with the 0.5 M $NaHCO_3$ Olsen solution showed that, under P-based manure management, switching from the conventional NPA+P diet to the alternative NPA+Phyt diet produced a modest, yet statistically significant increase in the amount of alkaline-soluble P in those soils. When all available Olsen extraction data are considerd together, water solubility of soil P increased by 10% as a result of conversion to the phytase feed. Note that, when wet manures were applied to soil on an equal-N or equal-manure-weight basis, the switch from NPA+P diet to alternative NPA+Phyt diet produced a statistically significant decrease in the amount of Olsen extractable P in those soils.

The base extraction data suggest that differences in Olsen-soluble P content between soils amended with conventional and alternative manures were again caused by differences in defluorinated calcium phosphate content between the NPA+Phyt and NPA+P feeds. Addition of phytase enzyme to the baseline NPA diet in the absence of defluorinated phosphate modification did not affect the Olsen solubility of P in manured soils. Note that the calcium phosphate effect (i.e., difference between NPA and NPA+P treatments) became statistically significant only after the long incubation period. Increasing manure loadings also enhanced this effect. One can again speculate about how drying of manure prior to mixing with soils might have influenced Olsen extractability of P from those soils. Olsen extraction of composite manures showed that drying dramatically heightened the solubility-reducing calcium phosphate effect (Tables 22 and 23).

Olsen extraction revealed significant differences in P solubility between soils amended with HAP+Phyt and NPA manures. This HAP-plus-phytase effect was stronger as manure loadings and incubation time increased. Note that no solubility-enhancing HAP-plus-phytase effect was observed during Olsen extraction of raw manures.

Table 67: Olsen-soluble P in Manured Soils, Comparison of All Factor Combinations

Low Manure Application Rate				High Manure Application Rate			
4-Day Incubation		28-Day Incubation		4-Day Incubation		28-Day Incubation	
Groseclose Soil (Ridge and Valley)							
Treatment	mg kg^{-1}	Treatment	mg kg^{-1}	Treatment	mg kg^{-1}		
NPA+P Nbasis	136.9 a†	HAP+Phyt	153.3 a	NPA+P Nbasis	229.4 a		
HAP+Phyt	129.2 a	NPA+P Nbasis	141.8 ab	HAP+Phyt	206.1 ab		
NPA+Phyt	117.4 a	NPA	132.5 b	NPA	179.1 bc		
HAP	115.4 a	HAP	130.8 b	HAP	168.8 bc		
NPA	113.1 ab	NPA+Phyt	129.1 b	NPA+P Pbasis	163.7 c		
NPA+P Pbasis	106.0 ab	NPA+P Pbasis	97.9 c	NPA+Phyt	153.9 c		
control	83.4 b	control	79.6 d	control	83.4 d		
Cecil Soil (Piedmont)							
Treatment	mg kg^{-1}	Treatment	mg kg^{-1}	Treatment	mg kg^{-1}	Treatment	mg kg^{-1}
NPA+P Nbasis	151.3 a	NPA+P Nbasis	179.7 a	NPA+P Nbasis	258.2 a	NPA+P Nbasis	308.0 a
HAP+Phyt	114.3 b	HAP+Phyt	166.2 b	HAP+Phyt	232.0 b	HAP+Phyt	285.8 b
HAP	107.1 b	HAP	148.3 c	NPA	202.1 c	HAP	258.6 c
NPA	105.4 b	NPA	147.6 c	HAP	188.2 cd	NPA	245.5 cd
NPA+Phyt	103.3 b	NPA+Phyt	142.8 c	NPA+Phyt	179.6 cd	NPA+Phyt	233.0 d
NPA+P Pbasis	101.1 b	NPA+P Pbasis	114.3 d	NPA+P Pbasis	174.9 d	NPA+P Pbasis	195.6 e
control	48.6 c	control	46.6 e	control	48.6 e	control	46.6 f
Mahan Soil (Coastal Plain)							
Treatment	mg kg^{-1}	Treatment	mg kg^{-1}				
NPA+P Nbasis	126.8 a	NPA+P Nbasis	137.8 a				
NPA	88.3 b	HAP+Phyt	113.1 b				
NPA+Phyt	83.2 b	HAP	110.8 b				
HAP	82.4 b	NPA	103.7 b				
HAP+Phyt	82.4 b	NPA+Phyt	103.2 b				
NPA+P Pbasis	75.3 b	NPA+P Pbasis	87.2 c				
control	37.4 c	control	39.9 d				

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05. Analyses conducted separately for each of the nine combinations of factors.

3.3.3. EXTRACTION OF SOIL CU

3.3.3.a. $CaCl_2$ Extraction of Soil Cu

Effect of Manure Treatments

The results of water extraction of soil Cu are summarized in Table 68. This table reflects data from a total of 363 $CaCl_2$ extractions, including results from all 10 combinations of application regime, incubation time, and soil type factors used in the study. Table 68 therefore provides an overall picture of how the different manure treatments affected water solubility of soil Cu under a variety of conditions. When reviewing soil Cu extraction results such as those in Table 68, the reader should keep in mind that, for these particular manures, equal-N basis application also resulted in equal-Cu basis application. Therefore, the NPA+P Nbasis, NPA, HAP, NPA+Phyt, and HAP+Phyt treatments all resulted in equal loadings of Cu to soils. The reader should focus on these five treatments when comparing how the five manure types affected soil Cu solubility. The NPA+P Pbasis results should only be considered when the reader is interested in assessing how dietary changes affected soil Cu solubility under P-based manure management.

Table 68: Water-Soluble Cu in Manured Soils, Overall Comparison (All Application Regimes, Incubation Times, Soils).

Treatment	mg Cu kg^{-1}
HAP+Phyt	0.75 a†
HAP	0.73 a
NPA	0.70 b
NPA+Phyt	0.69 b
NPA+P Nbasis	0.65 c
NPA+P Pbasis	0.36 d
Control	0.04 e

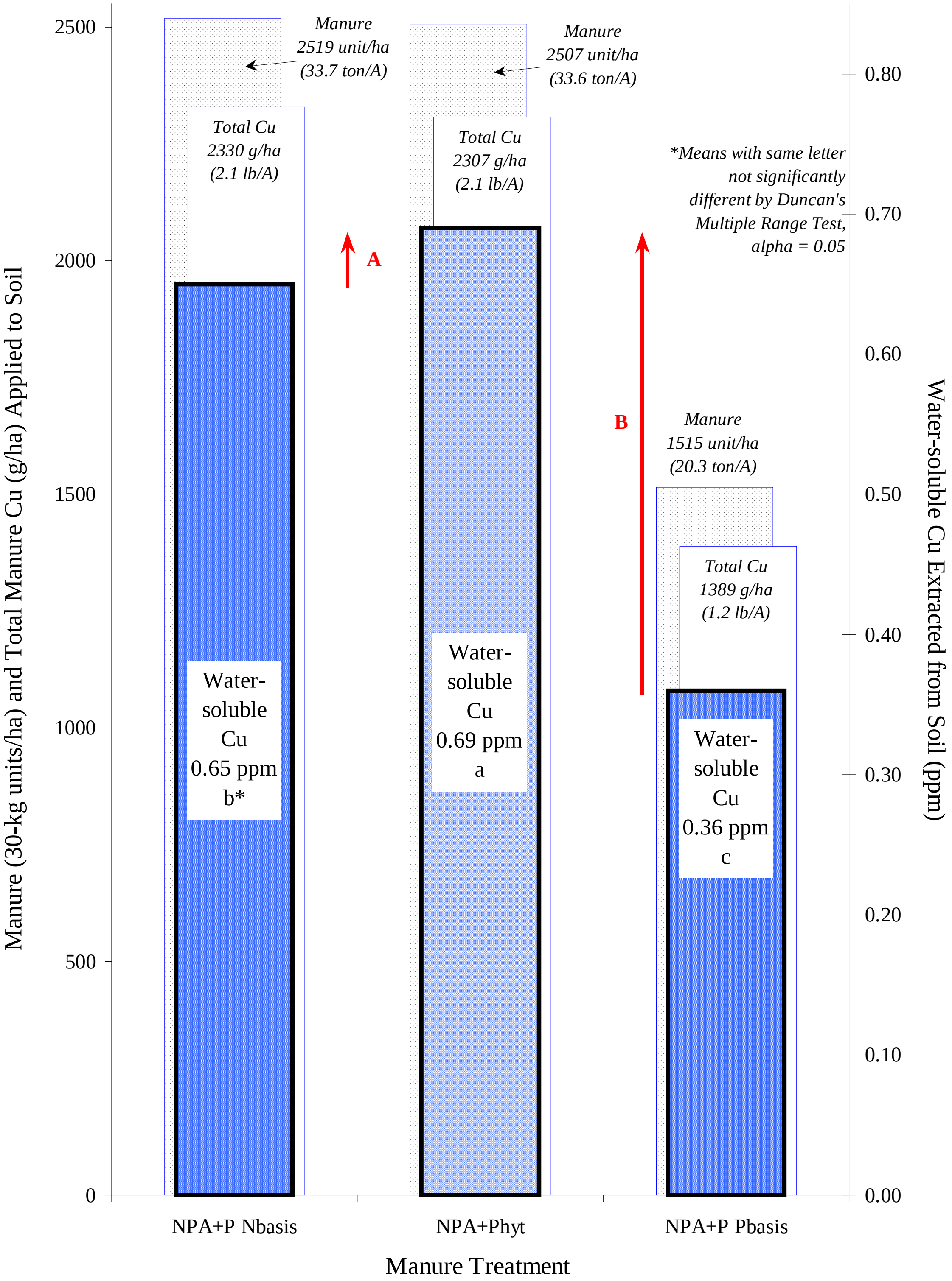
† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05

The first issue to consider is how switching from the conventional NPA+P diet to the alternative NPA+Phyt diet affected water solubility of Cu in the manure-amended soils. In order to more clearly illustrate the effect of this dietary change under both N- and P-based manure management, the NPA+P Nbasis, NPA+Phyt, and NPA+P Pbasis results from Table 69 are presented in Figure 23. The background columns in this figure compare the total amounts of manure and manure Cu applied to soils as a result of the NPA+P Nbasis, NPA+Phyt, and NPA+P Pbasis treatments. The foreground columns show the amount of water-soluble Cu that the soils contained after application of the manure treatments. The values in these foreground columns are drawn directly from Table 68.

Soils amended with the NPA+Phyt treatment yielded significantly more water-soluble Cu than soils amended with the NPA+P Nbasis treatment (0.69 mg kg^{-1} versus 0.65 mg kg^{-1}). Similar amounts of manure Cu were applied to both soils. This means that water extractability of soil Cu increased by 6% (line segment A) as a result of applying the alternative NPA+Phyt manure instead of the NPA+P manure to soil. Consider next the P-based manure management scenario, illustrated by the NPA+P Pbasis and NPA+Phyt treatments. In this situation, converting to a phytase diet caused a 67% increase in the total quantity of manure Cu applied to soils. This dietary change also caused the amount of water-soluble Cu extracted from soils to increase by a statistically significant 92%, from 0.36 mg kg^{-1} to 0.69 mg kg^{-1} (line segment B).

Why did soils amended with the NPA+Phyt manure contain 6% more water-soluble Cu than soils amended with NPA+P manure on an equal-Cu basis? Data in Table 68 suggest that the difference can again be attributed to the supplemental defluorinated calcium phosphate in the NPA+P feed, since NPA+P and NPA-amended soils differed significantly in water-soluble Cu content, while the NPA+Phyt and NPA soils did not. It should be noted that this is a reversal of the calcium phosphate effect observed in water extracts of composite manures. Extraction of wet and dry manures with $CaCl_2$ indicated that Cu in the NPA+P manure was generally more soluble than Cu in the NPA manures. This indicates that reactions between soil and manure led to important changes in the water solubility of manure-derived Cu. Indeed, extraction of manured soils produced a very different Cu solubility pattern than was obtained by water extraction of wet or dry manures (compare Table 68 with Tables 29 and 30). Table 68 shows a significant HAP-corn effect on Cu solubility, since both HAP+Phyt- and HAP-amended soils contained significantly more water-extractable Cu than soils mixed with NPA manure. Note that no phytase effect on water solubility of soil Cu was observed.

Figure 23: Water-Soluble Cu Comparison, Soils Treated with NPA+P and NPA+Phyt Manures



Influence of Application Regime, Incubation Time, and Soil Type Factors

The data in Table 69 allows one to investigate the effect of changing manure application regime on the treatment comparisons discussed above. No data from extraction of Mahan soils are included in Table 69, since high application regime treatments were not used on any Mahan soils. As expected, doubling the amount of manure applied to soils produced a dramatic increase in water-extractable Cu levels across all treatments. Increasing manure loading rates also enhanced statistical differences between soils amended with different treatments. For example, no calcium phosphate effect was observed on soils amended with low regime treatments. However, under the high application regime, soils amended with the NPA+P Nbasis treatment contained significantly less water-soluble Cu than soils amended with NPA or NPA+Phyt manures (0.83 mg kg^{-1} versus 0.89 mg kg^{-1}). Increasing manure loadings also enhanced the statistical significance of the HAP effect.

Table 69: Water-Soluble Cu in Manured Soils, Application Regime Comparison

Groseclose and Cecil Soils, Both Incubation Times			
Low Application Regime		High Application Regime	
Treatment	mg Cu kg^{-1}	Treatment	mg Cu kg^{-1}
HAP+Phyt	0.48 a†	HAP	0.93 a
HAP	0.46 ab	HAP+Phyt	0.93 a
NPA+Phyt	0.42 b	NPA	0.89 b
NPA	0.42 b	NPA+Phyt	0.89 b
NPA+P Nbasis	0.41 b	NPA+P Nbasis	0.83 c
NPA+P Pbasis	0.16 c	NPA+P Pbasis	0.50 d
Control	0.04 d	Control	0.04 e

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05. Statistical analyses were conducted separately for each of the two application regimes.

Water-soluble Cu contents of manure-amended soils incubated for 4 d and 28 d prior to extraction are compared in Table 70. Across all treatments other than the control, increasing incubation time prior to extraction decreased extractability of Cu in the manured soils. This probably occurred because longer incubation time permitted the formation of stronger bonds of adsorption between soil particles and Cu derived from manures. The overall pattern of Cu solubility rankings and relationships among the different treatments was not notably affected by increasing incubation time.

Table 70: Water-Soluble Cu in Manured Soils, Incubation Time Comparison

All Soils, Both Application Regimes			
4-Day Incubation		28-Day Incubation	
Treatment	mg Cu kg^{-1}	Treatment	mg Cu kg^{-1}
HAP+Phyt	0.86 a†	HAP+Phyt	0.64 a
HAP	0.84 ab	HAP	0.63 ab
NPA	0.82 ab	NPA	0.59 bc
NPA+Phyt	0.81 b	NPA+Phyt	0.57 cd
NPA+P Nbasis	0.75 c	NPA+P Nbasis	0.54 d
NPA+P Pbasis	0.45 d	NPA+P Pbasis	0.27 e
Control	0.04 e	Control	0.04 f

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05. Statistical analyses conducted separately for each of the two incubation times.

The data presented in Table 71 explores the influence of soil type on differences in water solubility of Cu between soils amended with different manure treatments. Note that only low application regime data are included, since the Mahan soil did not receive high regime treatments. Although the three soil types did not differ in native water-soluble Cu content (i.e., prior to manure addition), manured Mahan soils released dramatically larger quantities of Cu than did other soils during the water extraction. It is suggested that since the sandy Mahan soil contained a lower proportion of reactive, clay-sized particles than the Groseclose or Cecil soils, the Mahan adsorbed less manure Cu than the other soils. Differences in soil type did not notably change Cu solubility rankings and relationships among the treatments. All three soil types showed the established pattern in which, at least numerically, soils amended with HAP+Phyt and HAP manure contained the most water-extractable Cu, while soils amended with the NPA+P Nbasis treatment contained the least. Across all three soil types, the NPA-treated soils did not differ statistically from soils amended with the four other types of manure on a equal Cu and equal weight basis. Only on the Mahan soils was a statistically meaningful difference observed between the NPA- and NPA+P Nbasis-amended soils.

Table 71: Water-Soluble Cu in Manured Soils, Soil Type Comparison

Low Application Regime, Both Incubation Times					
Groseclose Soil		Cecil Soil		Mahan Soil	
Treatment	mg Cu kg^{-1}	Treatment	mg Cu kg^{-1}	Treatment	mg Cu kg^{-1}
HAP+Phyt	0.49 a†	HAP+Phyt	0.48 a	HAP+Phyt	0.86 a
HAP	0.49 a	HAP	0.43 ab	HAP	0.85 a
NPA+Phyt	0.46 ab	NPA	0.41 ab	NPA	0.83 a
NPA+P Nbasis	0.46 ab	NPA+Phyt	0.38 b	NPA+Phyt	0.78 ab
NPA	0.42 b	NPA+P Nbasis	0.37 b	NPA+P Nbasis	0.72 b
NPA+P Pbasis	0.15 c	NPA+P Pbasis	0.17 c	NPA+P Pbasis	0.45 c
Control	0.04 d	Control	0.05 d	Control	0.04 d

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05. Statistical analyses were conducted separately for each of the three soil types.

Water-soluble Cu data for all 10 combinations of application regime, incubation time, and soil type factors are presented in Table 72. For most combinations, this table shows the established pattern of Cu solubility rankings among soils manured on an equal-Cu basis, with the HAP+Phyt- and HAP-treated soils containing the greatest amount of water-soluble Cu and the NPA+P Nbasis soils containing the least. However, relatively few statistically significant differences in Cu solubility existed between soils amended with different treatments within each of the 10 groups of means.

Summary

Soils amended with NPA+Phyt manure released 6% more Cu to the $CaCl_2$ extractant than did soils amended with NPA+P manure on an equal-Cu (as well as equal-N and equal-weight) basis. Although modest, this increase in Cu solubility was statistically significant and was apparently the result of a solubility-depressing calcium phosphate effect. This effect became more pronounced with increasing soil-manure incubation time. Note that this calcium phosphate effect was not observed in water extracts of raw manures. These two observations suggest that soil-manure reactions played an important role in promoting this calcium phosphate effect. Under P-based manure management, converting from NPA+P feed to NPA+Phyt feed caused a 92% increase in the amount of water-soluble Cu extracted from manured soils. The dramatic nature of this increase was directly linked to the fact that feeding the alternative diet produced a 67% rise in the amount of total manure Cu applied to soils.

Simply feeding phytase without modifying dietary defluorinated calcium phosphate levels did not significantly affect water solubility of Cu in manured soils. However, a statistically significant HAP-induced enhancement in solubility of soil Cu was observed. Water extraction of raw manures did not reveal such a prominent solubility-enhancing HAP effect. Meanwhile, increasing incubation time prior to extraction promoted the HAP effect. These observations suggest that soil-manure reactions also promoted the HAP effect on water solubility of soil Cu.

Table 72: Water-Soluble Cu in Manured Soils, Comparison of All Factor Combinations

Low Manure Application Rate				High Manure Application Rate			
4-Day Incubation		28-Day Incubation		4-Day Incubation		28-Day Incubation	
Groseclose Soil							
Treatment	mg kg^{-1}	Treatment	mg kg^{-1}	Treatment	mg kg^{-1}	Treatment	mg kg^{-1}
HAP	0.67 a†	HAP+Phyt	0.32 a	NPA+Phyt	1.15 a	HAP+Phyt	0.90 a
HAP+Phyt	0.66 ab	HAP	0.31 a	HAP	1.13 a	HAP	0.88 a
NPA+Phyt	0.62 ab	NPA+Phyt	0.30 a	NPA	1.12 a	NPA	0.83 ab
NPA+P Nbasis	0.62 ab	NPA+P Nbasis	0.30 a	HAP+Phyt	1.11 a	NPA+Phyt	0.80 ab
NPA	0.59 b	NPA	0.29 a	NPA+P Nbasis	1.04 b	NPA+P Nbasis	0.75 b
NPA+P Pbasis	0.21 c	NPA+P Pbasis	0.09 b	NPA+P Pbasis	0.58 c	NPA+P Pbasis	0.34 c
control	0.04 d	control	0.03 b	control	0.04 d	control	0.03 d
Cecil Soil							
Treatment	mg kg^{-1}	Treatment	mg kg^{-1}	Treatment	mg kg^{-1}	Treatment	mg kg^{-1}
HAP+Phyt	0.54 a	HAP+Phyt	0.43 a	HAP	1.00 a	HAP	0.74 a
HAP	0.48 a	HAP	0.38 b	HAP+Phyt	0.99 ab	HAP+Phyt	0.74 a
NPA	0.45 a	NPA	0.37 b	NPA+Phyt	0.95 abc	NPA	0.71 a
NPA+Phyt	0.44 a	NPA+P Nbasis	0.35 bc	NPA	0.93 bc	NPA+Phyt	0.68 a
NPA+P Nbasis	0.39 a	NPA+Phyt	0.33 c	NPA+P Nbasis	0.90 c	NPA+P Nbasis	0.66 a
NPA+P Pbasis	0.21 b	NPA+P Pbasis	0.14 d	NPA+P Pbasis	0.61 d	NPA+P Pbasis	0.48 b
control	0.05 c	control	0.05 e	control	0.05 e	control	0.05 c
Mahan Soil							
Treatment	mg kg^{-1}	Treatment	mg kg^{-1}				
HAP+Phyt	0.96 a	HAP	0.77 a				
NPA	0.94 a	HAP+Phyt	0.75 a				
HAP	0.94 a	NPA	0.72 ab				
NPA+Phyt	0.89 a	NPA+Phyt	0.68 ab				
NPA+P Nbasis	0.82 a	NPA+P Nbasis	0.62 b				
NPA+P Pbasis	0.65 b	NPA+P Pbasis	0.26 c				
control	0.05 c	control	0.04 d				

†Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05. Statistical analyses conducted separately for each of the 10 combinations of factors.

3.3.3.b. Mehlich III Extraction of Soil Cu

Effect of Manure Treatments

Results of Mehlich III extraction of soil Cu are summarized in Table 73. This table shows means for data from a total of 365 Mehlich III extractions, including results from all 10 combinations of application regime, incubation time, and soil type factors used in the study. The acidic Mehlich III extractant containing EDTA was generally a more effective extractant of soil Cu than was the dilute $CaCl_2$ solution. It is essential to note, however, that the Mehlich III extractant did not solubilize much more Cu from soils amended with large quantities of manure than from untreated control soils. Apparently, combining organic manure material with soil produced a medium that strongly adsorbed Cu originating in the manure, forming acid-insoluble compounds. It is also possible that the solubility of the Cu originating in the soil was affected by manure applications. Regardless of the exact cause, soils loaded with relatively large amounts of Cu in manure form released statistically similar amounts of Cu during the Mehlich III extraction as did untreated control soils.

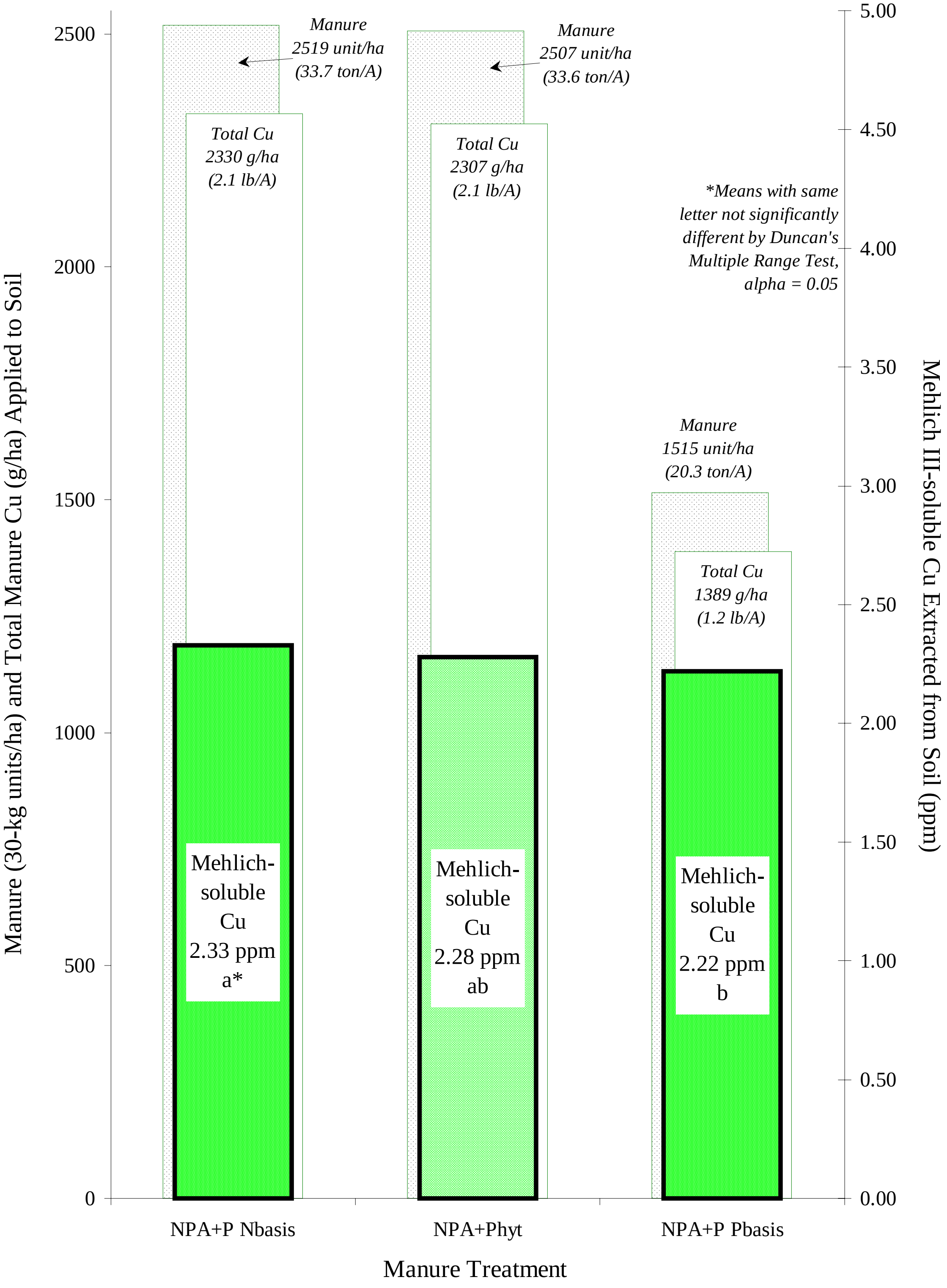
Table 73: Mehlich III-Soluble Cu in Manured Soils, Overall Comparison (All Application Regimes, Incubation Times, Soil Types)

Treatment	mg Cu kg^{-1}
NPA+P Nbasis	2.33 a
HAP+Phyt	2.32 ab
NPA+Phyt	2.28 ab
NPA	2.27 ab
HAP	2.26 ab
NPA+P Pbasis	2.23 bc
Control	2.16 c

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05

As always, the principal issue to consider is how converting from the conventional NPA+P diet to the alternative NPA+Phyt diet affected plant availability of soil Cu. In order to more clearly illustrate the impact of the dietary change under both N- and P-based manure management, the NPA+P Nbasis, NPA+Phyt, and NPA+P Pbasis results from Table 74 are presented in Figure 24. The background columns in this figure compare the total amounts of manure and manure Cu applied to soils as a result of the NPA+P Nbasis, NPA+Phyt, and NPA+P Pbasis treatments. The foreground columns show the amounts of Mehlich III-soluble Cu that the soils contained after application of the manure treatments. The values in these foreground columns are drawn directly from Table 73.

Figure 24: Mehlich III-Soluble Cu Comparison, Soils Treated with NPA+P and NPA+Phyt Manures



Soils amended with the NPA+P Nbasis and NPA+Phyt treatments contained statistically similar amounts of Mehlich III-soluble Cu (2.33 mg kg^{-1} versus 2.28 mg kg^{-1}). Equal amounts of manure Cu were applied to both soils. This suggests that the plant availability of soil Cu was not affected by applying the alternative NPA+Phyt rather than the NPA+P manure to soil. In the P-based manure management scenario, illustrated by the NPA+P Pbasis and NPA+Phyt treatments, converting to the phytase diet caused a 67% increase in the total amount of manure Cu applied to soils. Yet, surprisingly, the amount of Mehlich III-soluble Cu in these soils did not change significantly. The overall implication of these results is that manure treatments did not exert much influence on the amount of Cu extracted from soils with Mehlich III, regardless of the quantities or types of manure applied. This is confirmed by comparing the NPA+P Pbasis and NPA+P Nbasis soils, which received the same type of manure. Soils amended with the NPA+P Nbasis treatment were loaded with approximately 67% more manure and manure Cu than soils treated on a P basis. However, plant availability of Cu from those soils increased by only 5% (note that this increase was statistically significant). This disproportionately small increase in the amount of Cu dissolved strongly suggests that most Cu extracted by the Mehlich III solution originated from native soils, not from manure.

The net result is that the data from Mehlich III extraction of soil Cu allow for few meaningful comparisons between treatments. Table 73 shows that no statistically significant differences existed between soils amended with any of the five manure types on an equal-Cu basis. It is interesting to note that, from a strictly numerical standpoint, soils amended on an equal-Cu basis with NPA+P and phytase-derived manures tended to release the most plant-available P, while soils treated with HAP and NPA manure released the least. This pattern is consistent with the results obtained by Mehlich III extraction of wet composite manures. Nevertheless, it is clear that reactions between soils and manures dramatically affected the form and acid solubility of manure-derived Cu.

Influence of Application Regime, Incubation Time, and Soil Type Factors

The data in Table 74 shows the influence of changing manure application regime on Mehlich III solubility of Cu from manured soils. As might be expected, doubling the amount of manure Cu applied to soils increased Mehlich III extractability of Cu across all treatments. However, these increases were relatively modest, which lends further support to the theory that much of the Cu extracted from manured soils with Mehlich III originated in the native soils. Increasing application regime also increased statistical differences between soils amended with different manure treatments. Under the low application regime, none of the soils, including the untreated control, differed in Mehlich III-extractable Cu content. High regime applications of manure produced some statistically significant differences in plant availability of Cu between soils, particularly between the control and other treatments.

Table 74: Mehlich III-Soluble Cu in Manured Soils, Application Regime Comparison

Groseclose and Cecil Soils, Both Incubation Times			
Low Application Regime		High Application Regime	
Treatment	mg Cu kg^{-1}	Treatment	mg Cu kg^{-1}
NPA+P Nbasis	2.29 a†	HAP+Phyt	2.44 a
NPA+Phyt	2.22 a	NPA+P Nbasis	2.41 a
HAP+Phyt	2.21 a	NPA	2.38 ab
NPA+P Pbasis	2.19 a	HAP	2.38 ab
NPA	2.18 a	NPA+Phyt	2.37 ab
Control	2.15 a	NPA+P Pbasis	2.24 bc
HAP	2.15 a	Control	2.15 c

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05. Statistical analyses were conducted separately for each of the two application regimes.

Mehlich III-soluble Cu contents of manure-amended soils incubated for 4 d and 28 d prior to extraction are compared in Table 75. Across all treatments including the control, increasing incubation time reduced Mehlich III solubility of Cu from the manured soils. In addition, increasing incubation time eliminated statistical differences in plant-available Cu levels between control soils and manured soils. This suggests that longer incubation time promoted adsorption and/or chelation of Cu to reactive soil and organic matter surfaces.

Table 75: Mehlich III-Soluble Cu in Manured Soils, Incubation Time Comparison

All Soils, Both Application Regimes			
4-Day Incubation		28-Day Incubation	
Treatment	mg Cu kg^{-1}	Treatment	mg Cu kg^{-1}
NPA+P Nbasis	2.48 a†	NPA+P Nbasis	2.18 a
HAP+Phyt	2.48 a	HAP+Phyt	2.17 a
NPA+Phyt	2.42 a	NPA+Phyt	2.15 a
NPA	2.41 a	HAP	2.14 a
HAP	2.39 a	NPA+P Pbasis	2.14 a
NPA+P Pbasis	2.30 ab	NPA	2.13 a
Control	2.21 b	Control	2.09 a

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05. Statistical analyses were conducted separately for each of the two incubation times.

The influence of soil type on Mehlich III solubility of Cu from manured soils is explored in Table 76. Note that only low application regime data are included, since the Mahan soil did not receive high regime treatments. For all three soil types, no statisically significant differences in plant-available Cu content were observed between soils amended with any treatments, including the control. Note that differences in Mehlich III-extractable Cu content among the untreated soils explain why Groseclose soils contained the most plant-available Cu across all treatments, while Cecil soils contained the least.

Mehlich III-soluble Cu data for all 10 combinations of application regime, incubation time, and soil type factors are presented in Table 77. This table illustrates the same trends discussed above. Low regime applications of manures of any type did not produce statistically significant changes in the Mehlich III-extractable Cu content of native soils. High regime applications of manure enhanced statistical differences in plant-available Cu content between control and manured soils. However, high regime manure applications still did not lead to any statistically significant differences in Mehlich III extractability of soil Cu due to differences in the type of manure applied.

Table 76: Mehlich III-Soluble Cu in Manured Soils, Soil Type Comparison

Low Application Regime, Both Incubation Times					
Groseclose Soil		Cecil Soil		Mahan Soil	
Treatment	mg Cu kg^{-1}	Treatment	mg Cu kg^{-1}	Treatment	mg Cu kg^{-1}
HAP	0.82 a†	HAP+Phyt	0.73 a	HAP	1.13 a
NPA+Phyt	0.80 a	HAP	0.69 a	HAP+Phyt	1.11 a
HAP+Phyt	0.79 a	NPA	0.66 a	NPA+Phyt	1.09 a
NPA	0.75 a	NPA+P Nbasis	0.65 a	NPA	1.07 a
NPA+P Nbasis	0.72 a	NPA+Phyt	0.60 a	NPA+P Nbasis	0.89 b
NPA+P Pbasis	0.38 b	NPA+P Pbasis	0.27 b	NPA+P Pbasis	0.61 c
Control	0.23 c	Control	0.10 b	Control	0.30 d

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05. Statistical analyses were conducted separately for each of the three soil types.

Table 77: Mehlich III-soluble Cu in Manured Soils, Comparison of All Factor Combinations

Low Manure Application Rate				High Manure Application Rate			
4-Day Incubation		28-Day Incubation		4-Day Incubation		28-Day Incubation	
Groseclose Soil							
Treatment	mg kg^{-1}	Treatment	mg kg^{-1}	Treatment	mg kg^{-1}	Treatment	mg kg^{-1}
NPA+P Nbasis	3.10 a†	HAP+Phyt	2.47 a	HAP+Phyt	3.16 a	NPA+P Pbasis	2.44 a
NPA+Phyt	2.87 a	NPA+Phyt	2.44 a	NPA	3.03 ab	HAP+Phyt	2.44 a
NPA+P Pbasis	2.85 a	NPA+P Pbasis	2.43 a	HAP	3.02 ab	HAP	2.42 a
NPA	2.79 a	HAP	2.41 a	NPA+Phyt	2.99 ab	NPA+P Nbasis	2.42 a
HAP+Phyt	2.78 a	NPA	2.38 a	NPA+P Nbasis	2.94 ab	NPA	2.40 a
HAP	2.67 a	NPA+P Nbasis	2.38 a	NPA+P Pbasis	2.73 ab	NPA+Phyt	2.37 a
control	2.66 a	control	2.36 a	control	2.66 b	control	2.36 a
Cecil Soil							
Treatment	mg kg^{-1}	Treatment	mg kg^{-1}	Treatment	mg kg^{-1}	Treatment	mg kg^{-1}
NPA+P Nbasis	1.83 a	NPA+Phyt	1.84 a	NPA+Phyt	2.22 a	NPA+P Nbasis	2.13 a
HAP+Phyt	1.78 a	NPA+P Nbasis	1.84 a	HAP+Phyt	2.22 a	HAP+Phyt	1.95 ab
HAP	1.78 a	NPA	1.82 a	NPA+P Nbasis	2.17 a	HAP	1.95 ab
NPA	1.75 a	NPA+P Pbasis	1.81 a	NPA	2.14 ab	NPA	1.94 ab
control	1.73 a	HAP+Phyt	1.80 a	HAP	2.12 ab	NPA+Phyt	1.89 ab
NPA+Phyt	1.71 a	HAP	1.79 a	NPA+P Pbasis	1.93 bc	NPA+P Pbasis	1.80 bc
NPA+P Pbasis	1.68 a	control	1.63 a	control	1.73 c	control	1.63 c
Mahan Soil							
Treatment	mg kg^{-1}	Treatment	mg kg^{-1}				
HAP+Phyt	2.44 a	NPA+Phyt	2.14 a				
HAP	2.37 a	control	2.14 a				
NPA+P Nbasis	2.36 a	NPA+P Pbasis	2.13 a				
NPA	2.35 a	HAP+Phyt	2.13 a				
NPA+Phyt	2.33 a	HAP	2.13 a				
NPA+P Pbasis	2.33 a	NPA+P Nbasis	2.10 a				
control	2.28 a	NPA	2.07 a				

†Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05. Statistical analyses were conducted separately for each of the 10 different combinations of factors.

Summary

Mehlich III extraction of manured soils revealed essentially no meaningful differences in solubility of soil Cu related to dietary treatments. It is suggested that combining manure and soil promoted chelation and/or adsorption of manure Cu to form some type of acid-insoluble compounds. This is proposed to help explain why the Mehlich III extractant did not solubilize much more Cu from manured soils than from the untreated control soils. Note that increasing incubation time appeared to heighten this chelation or adsorption effect.

Regardless of the underlying mechanisms involved, soils amended with NPA+P and NPA+Phyt manures on an equal-Cu basis did not differ significantly in Mehlich III-extractable Cu content. Moreover, P-based application of NPA+P and NPA+Phyt manures did not produce statistically significant differences in Mehlich III-solubility of soil P.

3.3.3.c. Olsen Extraction of Soil Cu

Effect of Manure Treatments

An overall summary of the results of Olsen extraction of Cu from manured soils is presented in Table 78. The Olsen-soluble Cu means in Table 78 reflect data from 262 individual extractions. All nine combinations of application regime, incubation time, and soil type factors that were subjected to extraction with $NaHCO_3$ are represented. These results indicate that Olsen solution was a more effective overall extractant of soil Cu than the 0.01 M $CaCl_2$, but less effective than the Mehlich III extractant. The Olsen-soluble Cu data in Table 78 are similar to water-soluble Cu results in terms of numerical ranking among treatment means. However, Olsen extraction of manure soils did not reveal the degree of statistical differences seen in the water extracts.

Table 78: Olsen-soluble Cu in Manured Soils, Overall Comparison

Treatment	mg Cu kg^{-1}
HAP+Phyt	1.02 a†
HAP	1.01 a
NPA+Phyt	0.98 a
NPA	0.94 ab
NPA+P Nbasis	0.89 b
NPA+P Pbasis	0.52 c
Control	0.19 d

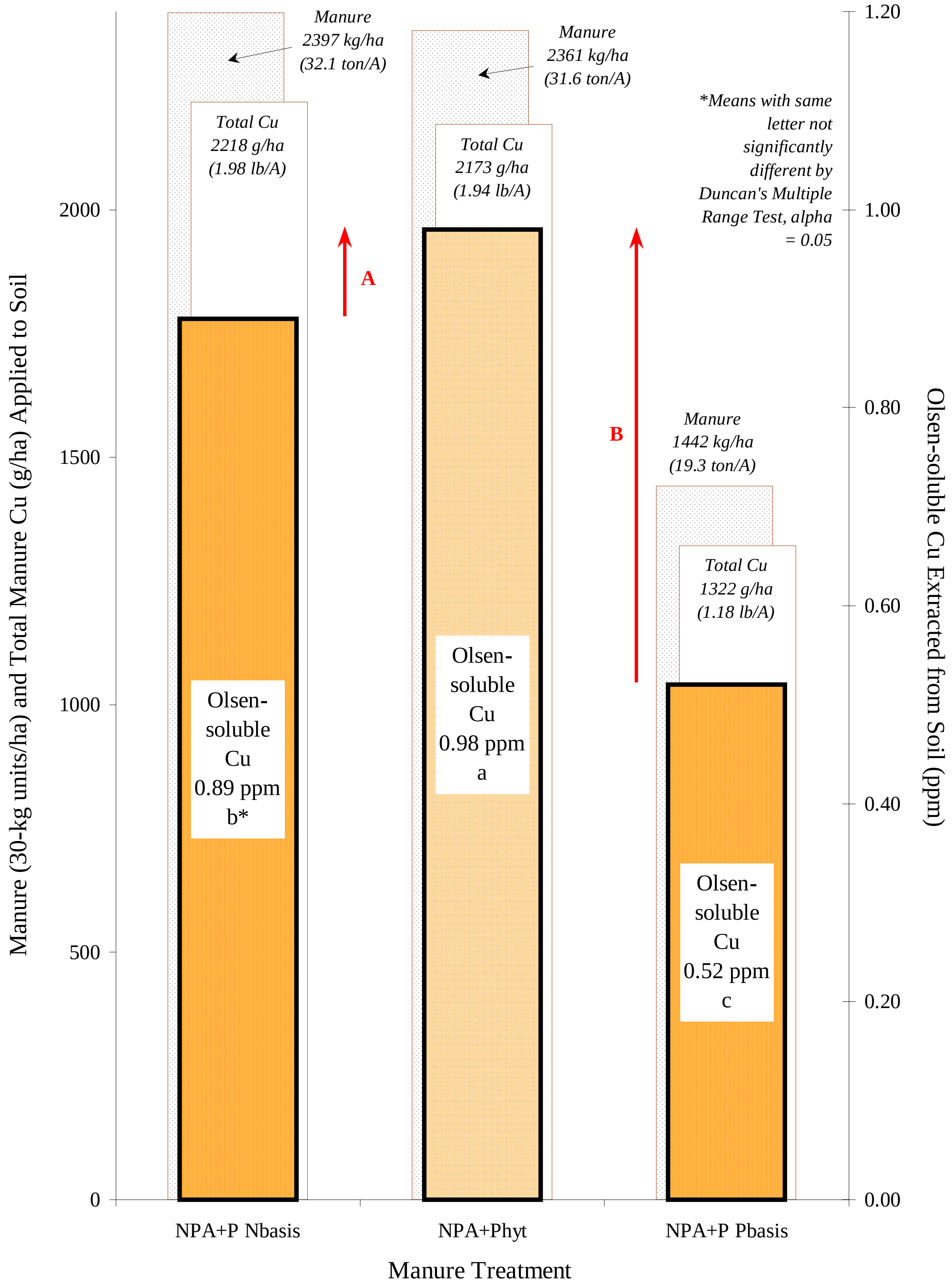
† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05

Consider first the key question of how switching from the conventional NPA+P diet to the alternative NPA+Phyt diet affected Olsen solubility of soil Cu. In order to more clearly illustrate the effect of this dietary shift under both N- and P-based nutrient management, the NPA+P Nbasis, NPA+Phyt, and NPA+P Pbasis results from Table 78 are presented in Figure 25. Background columns in this figure compare the total amounts of manure and manure Cu applied to soils as a result of the NPA+P Nbasis, NPA+Phyt, and NPA+P Pbasis treatments. The foreground columns show the amount of Olsen-soluble Cu that the soils contained after application of the manure treatments. The values in the foreground columns are drawn directly from Table 78.

Soils amended with the NPA+Phyt treatment contained significantly more Olsen-soluble Cu than soils amended with the NPA+P Nbasis treatment (0.98 mg kg^{-1} versus 0.89 mg kg^{-1}). Similar amounts of manure Cu were applied to both soils. Therefore, Olsen extractability of soil Cu increased by 10% as a result of applying the alternative NPA+Phyt manure instead of the NPA+P manure to soil. This statistically significant increase is represented by line segment A in Figure 25. Consider next the P-based nutrient management scenario, illustrated by the NPA+P P basis and NPA+Phyt treatments. In this situation, converting to a phytase diet caused a 64% increase in the total amount of manure Cu applied to soils. This dietary change also caused the amount of Olsen-soluble Cu extracted from soils to increase by a statistically significant 88%, from 0.52 mg Cu kg^{-1} to 0.98 mg Cu kg^{-1} (line segment B).

Why did soils amended with the NPA+Phyt manure contain a significant 10% more Olsen-soluble Cu than soils mixed with NPA+P manure on an equal-Cu basis? The data in Table 78 do not suggest whether the difference was caused primarily by the supplemental defluorinated calcium phosphate in the NPA+P feed or by the supplemental phytase in the NPA+Phyt feed. The NPA-amended soils did not differ significantly in Olsen-soluble Cu content from either the NPA+P Nbasis- or the NPA+Phyt-treated soils. The data in Table 78 also show statistically similar Olsen-soluble Cu levels for soils amended with NPA, HAP, and HAP+Phyt manures. Although the calcium phosphate, phytase, and HAP effects were not statistically significant, they did produce numerically meaningful differences in Mehlich III solubility of soil Cu. Note that these effects were not consistent with the results of Olsen extraction of wet or dry composite manures. This suggests yet again that reactions between manure and soil components significantly impacted the form and solubility of Cu derived from manure. Finally, note that control and manured soils differed statistically and numerically in Olsen-soluble Cu content. Therefore, chelation or adsorption reactions that equalized Mehlich III solubility of Cu among all soils did not produce the same dramatic effect on Olsen solubility of Cu.

Figure 25: Olsen-soluble Cu Comparison, Soils Treated with NPA+P and NPA+Phyt Manures



Influence of Application Regime, Incubation Time, and Soil Type Factors

The influence of changing manure application regime on Olsen extractability of Cu from manured soils are presented in Table 79. Recall that high application regime treatments were not used on any Mahan soils. In addition, Groseclose soils treated with high regime manure applications and incubated for 28 d were not extracted with Olsen solution. Therefore, Table 79 only shows the results of Olsen extraction of Groseclose and Cecil soils after 4 d of incubation. As the amount of manure applied to these soils doubled, Olsen-soluble P levels rose across all treatments. For each application regime, Table 79 shows no statistically significant differences in Olsen-soluble Cu content between soils amended on an equal-Cu basis with any of the five different types of manure. With increasing manure loading, differences between the NPA+P Pbasis and control soils became statistically significant.

Table 79: Olsen-Soluble Cu, Application Regime Comparison

Groseclose and Cecil Soils, 4-Day Incubation Time			
Low Application Regime		High Application Regime	
Treatment	mg Cu kg^{-1}	Treatment	mg Cu kg^{-1}
HAP	0.83 a†	HAP+Phyt	1.34 a
HAP+Phyt	0.81 a	NPA+Phyt	1.32 a
NPA+Phyt	0.79 a	HAP	1.29 a
NPA	0.74 a	NPA	1.24 a
NPA+P Nbasis	0.70 a	NPA+P Nbasis	1.17 a
NPA+P Pbasis	0.30 b	NPA+P Pbasis	0.74 b
Control	0.15 b	Control	0.15 c

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05. Statistical analyses were conducted separately for each of the two application regimes.

Olsen-soluble Cu means for manure-amended soils incubated for 4 d and 28 d prior to extraction are compared in Table 80. Note that only low application regime results are included. Increasing incubation time decreased Olsen extractability of Cu across all soils to which manure was applied on an equal-Cu basis (i.e., all soils other than the NPA+P Pbasis and control soils). It is suggested that overall Cu solubility dropped on these soils because longer incubation time promoted stronger bonding between Cu and reactive soil surfaces. Increasing incubation time also produced a statistically significant difference in Olsen-soluble Cu content between the NPA+P Nbasis soil and both the HAP+Phyt- and HAP-treated soils. For both incubation times, NPA+Phyt soils contained more Olsen-soluble P than NPA+P Nbasis soils numerically, but not statistically.

Table 80: Olsen-Soluble Cu, Incubation Time Comparison

All Soils, Low Application Regime			
4-Day Incubation		28-Day Incubation	
Treatment	mg Cu kg^{-1}	Treatment	mg Cu kg^{-1}
HAP	0.91 a†	HAP+Phyt	0.84 a
HAP+Phyt	0.90 a	HAP	0.84 a
NPA+Phyt	0.88 a	NPA	0.80 ab
NPA	0.83 a	NPA+Phyt	0.78 ab
NPA+P Nbasis	0.77 a	NPA+P Nbasis	0.73 b
NPA+P Pbasis	0.39 b	NPA+P Pbasis	0.43 c
Control	0.16 c	Control	0.26 d

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05 level. Statistical analyses were conducted separately for each of the two incubation times.

Means presented in Table 81 show the influence of soil type on Olsen-solubility of Cu from manured soils. Only low application regime data are included in the comparison. The three native soils (prior to manure addition) differed in Olsen-soluble Cu content. These differences in native or background Cu help explain why the manured Mahan soils contained the most base-soluble Cu across all treatments. On Groseclose and Cecil soils, no statistically significant differences in Olsen solubility of Cu were observed between soils amended with the five Cu basis manure treatments. On Mahan soils, the NPA+P Nbasis soils contained significantly less water-soluble Cu than soils amended with the other equal-Cu basis treatments. This is the only statistically meaningful treatment-related Cu solubility difference presented in Table 81.

Base-soluble Cu results from all nine combinations of application regime, incubation time, and soil type factors subjected to Olsen extraction are presented in Table 82. These results reveal few statistically significant differences in Cu solubility among soils amended with different manures on an equal-Cu basis. However, they confirm that soils amended with NPA+P manure on an equal-Cu basis generally released the lowest amounts of Olsen-soluble Cu, while soils treated with phytase- and HAP-derived manures generally released the highest.

Table 81: Olsen-Soluble Cu, Soil Type Comparison

Low Application Regime, Both Incubation Times					
Groseclose Soil		Cecil Soil		Mahan Soil	
Treatment	mg Cu kg^{-1}	Treatment	mg Cu kg^{-1}	Treatment	mg Cu kg^{-1}
HAP	0.82 a†	HAP+Phyt	0.73 a	HAP	1.13 a
NPA+Phyt	0.80 a	HAP	0.69 a	HAP+Phyt	1.11 a
HAP+Phyt	0.79 a	NPA	0.66 a	NPA+Phyt	1.09 a
NPA	0.75 a	NPA+P Nbasis	0.65 a	NPA	1.07 a
NPA+P Nbasis	0.72 a	NPA+Phyt	0.60 a	NPA+P Nbasis	0.89 b
NPA+P Pbasis	0.38 b	NPA+P Pbasis	0.27 b	NPA+P Pbasis	0.61 c
Control	0.23 c	Control	0.10 b	Control	0.30 d

† Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05. Statistical analyses were conducted separately for each of the three soil types.

Table 82: Olsen-Soluble Cu in Manured Soils, Comparison of All Factor Combinations

Low Manure Application Rate				High Manure Application Rate			
4-Day Incubation		28-Day Incubation		4-Day Incubation		28-Day Incubation	
Groseclose Soil							
Treatment	mg kg^{-1}	Treatment	mg kg^{-1}	Treatment	mg kg^{-1}		
NPA+Phyt	0.97 a†	HAP	0.65 a	HAP+Phyt	1.56 a		
HAP	0.96 a	HAP+Phyt	0.65 a	HAP	1.47 a		
HAP+Phyt	0.94 a	NPA+Phyt	0.63 a	NPA+Phyt	1.40 a		
NPA	0.87 a	NPA	0.62 a	NPA	1.37 a		
NPA+P Nbasis	0.86 a	NPA+P Nbasis	0.61 a	NPA+P Nbasis	1.26 a		
NPA+P Pbasis	0.41 b	NPA+P Pbasis	0.34 b	NPA+P Pbasis	0.75 b		
control	0.21 b	control	0.30 b	control	0.21 c		
Cecil Soil							
Treatment	mg kg^{-1}	Treatment	mg kg^{-1}	Treatment	mg kg^{-1}	Treatment	mg kg^{-1}
HAP	0.70 a	HAP+Phyt	0.84 a	NPA+Phyt	1.24 a	HAP+Phyt	1.16 a
HAP+Phyt	0.69 a	NPA+P Nbasis	0.81 a	HAP+Phyt	1.13 a	HAP	1.13 a
NPA	0.61 a	NPA	0.78 a	HAP	1.11 a	NPA+Phyt	1.13 a
NPA+Phyt	0.61 a	HAP	0.68 ab	NPA	1.10 a	NPA+P Nbasis	1.05 a
NPA+P Nbasis	0.58 a	NPA+Phyt	0.58 b	NPA+P Nbasis	1.08 a	NPA	1.02 a
NPA+P Pbasis	0.20 b	NPA+P Pbasis	0.43 c	NPA+P Pbasis	0.74 b	NPA+P Pbasis	0.70 b
control	0.08 b	control	0.19 d	control	0.08 c	control	0.19 c
Mahan Soil							
Treatment	mg kg^{-1}	Treatment	mg kg^{-1}				
HAP	1.34 a	HAP	1.05 a				
NPA+Phyt	1.31 a	HAP+Phyt	1.04 a				
HAP+Phyt	1.30 a	NPA+Phyt	1.00 a				
NPA	1.29 a	NPA	0.98 a				
NPA+P Nbasis	1.07 b	NPA+P Nbasis	0.82 b				
NPA+P Pbasis	0.82 c	NPA+P Pbasis	0.53 c				
control	0.35 d	control	0.28 d				

†Means with same letter not significantly different by Duncan's Multiple Range Test, alpha = 0.05. Statistical analyses were conducted separately for each of the nine different combinations of factors.

Summary

Olsen extraction of manured soils revealed a similar Cu solubility pattern to that produced by water extraction. Although calcium phosphate and phytase effects were not significant individually, their combined impact was a statistically meaningful difference in Cu solubility between soils amended with NPA+P and NPA+Phyt manures on a equal-Cu basis. As a result, under N-based manure management, conversion from the conventional NPA+P diet to the NPA+Phyt diet produced a significant 10% increase in base-extractable Cu content of manured soils. Under P-based manure management, feeding the alternative ration increased loadings of total manure Cu by 64% and produced a 88% increase in the amount of Olsen-soluble Cu extracted from soils.

On average, soils amended with HAP and HAP+Phyt manures yielded 7% to 8% more Cu to the Olsen extractant than did soils amended with NPA manure on an equal-Cu basis. However, these HAP and HAP-plus-phytase effects were not statistically significant overall. Changes in manure application regime, incubation time, and soil type produced few meaningful changes in the overall pattern of Olsen solubility of soil Cu.

3.4. CONCLUSIONS

3.4.1. PRIMARY CONCLUSIONS

Extraction of manure-amended soils with 0.01 M $CaCl_2$ showed that, in a P-limited manure application scenario, switching from the conventional NPA+P diet to the alternative, phytase-containing NPA+Phyt diet resulted in a statistically significant increase in the amount of water-soluble P in those soils. For example, when data from all 10 combinations of application regime, incubation time, and soil type factors used in the study were considered together, water-soluble soil P levels increased by 29% (from 9.2 mg kg^{-1} to 11.9 mg kg^{-1}) as a result of converting to the phytase-amended diet. This increase in water solubility of soil P was enhanced by lengthening incubation time prior to extraction. After 4 d of incubation, the NPA+Phyt-amended soils contained 26% more water-soluble P than the NPA+P Pbasis soils. After 28 d of incubation, the same NPA+Phyt soils contained 36% more water-soluble P than the NPA+P Pbasis soils. Statistical differences were not affected by this incubation-time effect. Neither changing manure application regime nor changing soil type notably affected these NPA+P Pbasis versus NPA+Phyt comparisons. It is critical to note that, when wet manures were applied to soil on an equal-N and equal-manure-weight basis, the conversion from the NPA+P diet to the alternative NPA+Phyt diet produced a statistically significant 14% decrease in the amount of $CaCl_2$ extractable P in those soils. This decrease in water-soluble soil P was relatively modest, considering that the same dietary conversion resulted in a 41% decrease in the total amount of manure P loaded on the soils.

Extraction of manure-amended soils with Mehlich III showed that, in a P-based manure application scenario, switching from the conventional NPA+P diet to the alternative NPA+Phyt diet did not significantly affect the amount of plant-available P in those soils. Data from all 10 combinations of application regime, incubation time, and soil type factors used in the study showed that Mehlich III solubility of soil P increased by only 2% (from 280.6 mg kg^{-1} to 286.8 mg kg^{-1}) as a result of converting to the phytase-amended diet. Increasing incubation time prior to Mehlich III extraction enhanced differences in plant availability of P between soils treated with conventional and phytase-derived manures. After 4 d of incubation, conversion to the NPA+Phyt diet caused plant availability of soil P to increase by a statistically insignificant 1%. After 28 d of incubation, switching to the NPA+Phyt diet produced a statistically significant 4% increase in plant-available P levels. Neither changing manure application regime nor changing soil type notably affected the NPA+P Pbasis versus NPA+Phyt comparison. It is very important to note that, when wet manures were applied to soils on an equal-N and equal manure-weight-basis, switching from the NPA+P diet to the alternative NPA+Phyt diet produced a statistically significant 24% decrease in plant-available soil P levels. The same dietary conversion produced a 41% decrease in the total loadings of manure P to soils.

Differences in water-soluble P content between soils treated with conventional NPA+P manure and alternative NPA+Phyt manure were related to the fact that the NPA+Phyt feed contained less defluorinated calcium phosphate than the conventional NPA+P feed. Overall, when phytase enzyme was added to the baseline NPA diet without modification in feed calcium phosphate levels, $CaCl_2$-extractable P levels in manured soils were not significantly affected (12.2 mg kg^{-1} for NPA versus 11.9 mg kg^{-1} for NPA+Phyt). Meanwhile, simple addition of defluorinated phosphate to the baseline NPA diet caused a statistically significant reduction in water-soluble P levels in manured soils (12.2 mg kg^{-1} for NPA versus 9.2 for NPA+Phyt). This solubility-depressing calcium phosphate effect was enhanced by increasing soil-manure incubation time prior to extraction, apparently leading to the overall change in water solubility of soil P with incubation time described above. These observations strongly suggest that dietary defluorinated calcium phosphate played a critical role in controlling water extractability of P in manured soils.

It appears that the Mehlich III extractant readily solubilized the manure-derived calcium phosphate compounds that caused differences in water solubility of P between soils mixed with NPA+P and NPA+Phyt manures. This is not surprising, considering the low pH of the Mehlich III solution and the acid solubility of calcium phosphates. Note that neither addition of defluorinated calcium phosphate nor addition of phytase enzyme to the baseline diet significantly affected Mehlich III solubility of P in the manure-amended soils (280.0 mg kg^{-1} for NPA versus 280.6 mg kg^{-1} for NPA+P versus 286.8 for NPA+Phyt). Finally, it should be emphasized that similar P solubility patterns to those described above were observed when wet manures were extracted with $CaCl_2$ and Mehlich III solution prior to mixing with soils. This suggests that reactions between manures and soil did not notably affect the water solubility of calcium phosphate compounds in the original wet manures.

3.4.2. SECONDARY CONCLUSIONS

When soils were amended with equal quantities of manure Cu (i.e., amended on an equal-manure-weight and equal-N basis), extraction with 0.01 M $CaCl_2$ showed that switching from the conventional NPA+P diet to the alternative NPA+Phyt diet produced a modest, yet statistically significant increase in the amount of water-soluble Cu in those soils. Data from all 10 combinations of application regime, incubation time, and soil type factors showed that the dietary change increased the amount of water-soluble Cu in soils by 6% (from 0.65 mg kg^{-1} for NPA+P Nbasis to 0.69 mg kg^{-1} for NPA+Phyt). This increase can again be linked to a solubility-depressing effect associated with supplemental calcium phosphate in the NPA+P feed, since simply feeding phytase without modifying dietary inorganic P levels did not significantly affect soil Cu solubility, while addition of defluorinated phosphate to the baseline NPA diet caused a significant drop in water extractability of soil Cu. It should be noted that this calcium phosphate effect was not observed in water extracts of wet manures prior to mixing with soil, suggesting that soil-manure reactions played an important role in determining the ultimate availability of soil Cu to the $CaCl_2$ extractant. While increasing manure loadings enhanced differences in water solubility of Cu between NPA+P Nbasis and NPA+Phyt soils, Cu solubility comparisons were not notably affected by changing incubation time or soil type. Finally, it must be emphasized that converting from the conventional NPA+P feed to the alternative NPA+Phyt feed under P-based manure management caused a statistically significant 92% increase in the amount of water-soluble Cu extracted from manured soils. The dramatic nature of this increase can be directly attributed to the fact that feeding the alternative diet produced a 67% rise in the amount of total manure Cu applied to soils.

When soils were amended with equal quantities of manure Cu, combined results from all Mehlich III extractions of manured soils revealed no statistically significant differences in plant availability of soil Cu related to dietary treatments. For example, 2.33 mg kg^{-1} plant-available Cu were extracted from soils subjected to the NPA+P Nbasis manure treatment, while 2.28 mg kg^{-1} plant-available Cu were extracted from soils treated with NPA+Phyt manures on an equal-Cu basis. Surprisingly, the Mehlich III extractant did not solubilize much more Cu from manured soils (2.33 to 2.23 mg kg^{-1}) than from the untreated control soils (2.16 mg kg^{-1}). It is suggested that combining manure and soil promoted chelation and/or adsorption of manure Cu to form acid-insoluble compounds, with the net result that most Cu extracted by the Mehlich III solution originated from native soils, not from manure. This hypothesis helps explain why P-based application of NPA+P and NPA+Phyt manures did not produce statistically significant differences in Mehlich III-solubility of soil P (2.23 mg kg^{-1} for NPA+P Pbasis versus 2.28 mg kg^{-1} for NPA+Phyt), even though the former treatment loaded 67% more total manure Cu on soils than did the latter. It should be noted that a very different pattern of Cu solubility was revealed upon extraction of wet composite manures with Mehlich III, which lends further support to the idea that significant reactions occurred between soil particles and Cu in manures.

When manures were applied to soils on an equal-P basis, switching from the conventional NPA+P diet to either the alternative HAP or the alternative HAP+Phyt feed produced statistically significant increases in water solubility of soil P. Analysis of data from all 363 $CaCl_2$ extractions shows that the NPA+P Pbasis soils contained 9.2 mg kg^{-1} water-soluble P while the HAP and HAP+Phyt soils contained 12.5 mg kg^{-1} and 13.7 mg kg^{-1} water-soluble P, respectively. As explained previously, simply reducing the percentage of feed P in calcium phosphate form resulted in an increase in the $CaCl_2$ extractability of P in manured soils. Since conversion from the conventional turkey feed to both the alternative HAP diet and the alternative HAP-plus-phytase diet involved a significant reduction in dietary defluorinated phosphate levels, it follows that this calcium phosphate effect contributed to the differences in water-soluble P content between the NPA+P-, HAP-, and HAP+Phyt-amended soils. In the case of conversion to the HAP feed, it appears that only the dietary calcium phosphate effect contributed significantly to the soil P solubility increase. There was no significant increase in water extractability of P in manured soils with simple substitution of HAP corn in the baseline NPA diet. In the case of conversion to the HAP+Phyt feed, both an HAP-plus-phytase effect and the aforementioned calcium phosphate effect contributed to the soil P extractability increase. When soils were amended with equal amounts of manure and manure N, conversion from the conventional diet to either feed containing HAP corn produced a drop in total P loadings to soil of roughly 40%. The result was that HAP-treated soils contained significantly less water-soluble P than the NPA+P Nbasis soils. However, the HAP+Phyt-treated soils did not differ significantly in $CaCl_2$-extractable P content from the NPA+P Nbasis-amended soils (13.8 mg kg^{-1} for NPA+P Nbasis versus 13.7 mg kg^{-1} for HAP+Phyt).

When soils were treated with equal quantities of manure Cu (as well as equal amounts of manure and manure N), extraction with $CaCl_2$ revealed significant increases of 12% and 15% in water solubility of soil Cu as a result of switching from the conventional NPA+P diet to the alternative HAP and HAP+Phyt diets. These increases in Cu solubility were due in part to reduced dietary calcium phosphate levels in the NPA+P feed, since soils amended with the baseline NPA manure contained significantly more water-soluble Cu than soils amended with the conventional NPA+P manure (0.70 mg kg^{-1} for NPA versus 0.65 for NPA+P Nbasis). A HAP-corn effect was also involved, since modification of the baseline diet with both HAP corn and HAP corn plus phytase enzyme significantly increased water-soluble soil Cu levels (0.70 mg kg^{-1} for NPA versus 0.73 mg kg^{-1} for HAP and 0.75 for HAP+Phyt). Under P-based manure management, converting from the conventional NPA+P feed to the alternative HAP and HAP+Phyt feeds caused statistically significant increases of 103% and 108% in the amount of water-soluble Cu extracted from manured soils. The dramatic nature of these increases was directly linked to the fact that feeding the alternative diet produced an increase of approximately 67% in the amount of total manure Cu applied to soils.

As indicated previously, the acidic Mehlich III extractant effectively solubilized manure-derived calcium phosphate compounds that contributed to differences in water solubility of soil P. This helps explain why the calcium phosphate effect did not contribute to any differences in plant availability of P among soils treated on an equal-P basis with conventional NPA+P manures and alternative HAP and HAP+P manures. When all available data were combined for analysis, the NPA+P Pbasis and HAP soils did not differ significantly in Mehlich III-soluble P content (280.6 mg kg^{-1} for NPA+P Pbasis versus 286.6 mg kg^{-1} for HAP). However, a statistically significant increase in Mehlich III extractability of soil P was observed as a result of simply feeding HAP corn plus phytase enzyme (without modifying dietary calcium phosphate). Therefore, converting from the conventional to the HAP+Phyt diet significantly increased plant-available P levels by 8% in soils manured on an equal-P basis (280.6 mg kg^{-1} for NPA+P Pbasis versus 303.2 for HAP+Phyt). Under N-based manure management, switching from the conventional feed to either alternative diet containing HAP corn caused total P loadings to soils to drop. In this scenario, converting to the HAP and HAP+Phyt diets caused statistically significant decreases of 20% and 24% in plant-available soil P levels (376.7 mg kg^{-1} for NPA+P Nbasis versus 286.6 mg kg^{-1} for HAP and 303.2 mg kg^{-1} for HAP+Phyt).

As stated above, Mehlich III extraction of manured soils revealed no statistically significant differences in plant availability of soil Cu related to dietary treatments. Statistically similar amounts of plant-available Cu were extracted from soils treated on an equal-Cu (and equal-N and manure-weight basis) with conventional NPA+P, alternative HAP, and alternative HAP+Phyt manures. Combining manures and soil promoted chelation and/or adsorption of manure Cu in such a manner that most Cu extracted by the Mehlich III solution originated from native soils, not from manure. This hypothesis helps explain why P-based application of the conventional NPA+P manure and the alternative HAP and HAP+Phyt manures did not produce statistically significant differences in Mehlich III-solubility of soil P (2.23 mg kg^{-1} for NPA+P Pbasis versus 2.26 mg kg^{-1} for HAP and 2.32 mg kg^{-1} for HAP+Phyt), even though the former treatment loaded roughly 67% more total manure Cu on soils than did the latter two treatments.

CHAPTER 4: SUMMARY AND CONCLUSIONS

4.1. PHOSPHORUS

4.1.1. WATER SOLUBILITY OF P

Feeding the alternative, phytase-amended NPA+Phyt diet instead of the conventional NPA+P diet to turkeys reduced overall P excretion by roughly 40%. The conventional NPA+P manure contained 1.46% total P, while the alternative NPA+Phyt manure contained 0.88% total P. Although feeding the phytase-amended diet reduced the overall amount of P excreted, it increased the relative water solubility of the P that persisted in the NPA+Phyt manure. For example, extraction of wet (i.e., point of excretion) individual pen manures with 0.01 M $CaCl_2$ solubilized 78.5% of total P in the NPA+Phyt manure and 51.8% of total P in the conventional NPA+P manure. This particular solubility difference was statistically significant at the $p = 0.05$ level. Note that P in the alternative NPA+Phyt manure was more soluble on a percentage basis, but that a greater overall quantity of P was extracted from the conventional NPA+P manure on an equal-manure-weight basis. A total of 7568 mg P was extracted per kg of NPA+P manure versus a total of 6929 mg P per kg of NPA+Phyt manure. Most of the dissolved P extracted from the wet manures with $CaCl_2$ was in molybdate-reactive orthophosphate form. For example, 67.9% of total P in the wet individual pen NPA+Phyt manures was solubilized into dissolved orthophosphate form, compared with 43.6% for the wet individual pen NPA+P manures. This particular orthophosphate solubility difference was also statistically significant. Composite manure samples were produced by combining subsamples of manures from individual pens. Extraction of P from wet composites with 0.01 M $CaCl_2$ revealed statistically significant differences between conventional and phytase-derived manures similar to those described above. For example, 86.1% of total P in the wet NPA+Phyt composite was dissolved in the $CaCl_2$ extractant, compared with 60.0% for the wet NPA+P composite.

The significant differences in water solubility of manure P observed in the wet conventional and phytase-derived manures carried through to soils amended with those manures. Soils of three types were amended with wet composite manures under two application regimes and allowed to incubate for four or 28 d prior to extraction. Overall, 29% more P was extracted with 0.01 M $CaCl_2$ from soils amended with wet NPA+Phyt manures than from soils amended with wet NPA+P manures on an equal-P basis (11.9 mg kg^{-1} for NPA+Phyt soils versus 9.2 mg kg^{-1} for NPA+P Pbasis soils). These results suggest that switching from conventional to phytase-amended poultry rations has the potential to increase water-soluble P loss from soils managed under P-based nutrient management. It is important to note that, when wet manures were applied to soils on an equal-N basis, soils treated with the alternative NPA+Phyt manure contained 14% less $CaCl_2$-extractable P than did the NPA+P-amended soils. In this case, the NPA+Phyt-amended soils contained 11.9 mg kg^{-1} water-soluble P, compared with 13.9 mg kg^{-1} for the NPA+P Nbasis-amended soils. It should be emphasized that in this N-based management scenario, the NPA+Phyt soils were loaded with 41% less total manure P than the NPA+P soils. Therefore, under N-based nutrient management, switching from conventional to phytase diets has the potential to reduce total P loadings to soil so dramatically that a net reduction in land application of water-extractable soil P is also achieved. This underlines the importance of considering factors other than simple extractability of manure or soil P when assessing the impact of diet changes on the P pollution potential of turkey manures.

Two factors differed in the formulation of the NPA+Phyt and NPA+P diets. The NPA+Phyt feed was supplemented with phytase enzyme and also contained less defluorinated calcium phosphate than the NPA+P feed. Overall, this study provides extremely convincing evidence that many of the key differences in water solubility of P between the conventional NPA+P and alternative NPA+Phyt manures, as well as between soils amended with those manures, were caused primarily by differences in feed calcium phosphate content. This assertion could not have been made if the study had not included the negative control NPA treatment, which provided a key baseline for assessing the effect of each dietary modification individually. For example, across all combinations of soil type, application regime, and incubation time, soils amended with the baseline NPA manure contained significantly more $CaCl_2$-extractable P than did soils amended with conventional NPA+P manure on an equal-P basis (12.2 mg kg^{-1} for NPA versus 9.2 mg kg^{-1} for NPA+P Pbasis). This means that simple addition of deflourinated calcium phosphate to the baseline NPA diet caused water solubility of P in manure-amended soils to drop by about 25%. It must be emphasized that these soils were loaded with equal amounts of manure P. It is proposed that greater amounts of calcium phosphate in the conventional commercial feed led to an increase in the proportion of manure P in water-insoluble calcium phosphate forms. This in turn led to a relative decrease in the water solubility of P in that manure.

It is important that the manure P solubility differences caused by changes in feed defluorinated calcium phosphate content were not diminished after mixing with soils. Even more intriguing is the fact that increasing soil incubation time prior to extraction actually enhanced the solubility-depressing calcium phosphate effect described above. After 4 d of incubation, the NPA+P-amended soils contained 18% less water-soluble P than the NPA-treated soils. After 28 d of incubation, the NPA+P-treated soils contained 27% less water-soluble P than soils amended with the baseline NPA manure (solubility differences were statistically significant for both incubation times). It is also important to note that drying wet composite manures at moderate temperature (40 °C) for 4 d enhanced the way in which supplemental dietary calcium phosphate depressed water solubility of manure P. For example, after drying, 57% less P was extracted with $CaCl_2$ from the conventional NPA+P manure than from the baseline NPA manure. Taken together, these results suggest that the calcium phosphate effect (and consequently differences in the form and solubility of P between conventional and alternative manures) has true potential to persist after the moment of excretion by the bird and perhaps even after the point of land application. For instance, consider the fact that, despite the great variability in the way commercial producers handle and store poultry manure, most waste excreted by commercial poulty is transformed into a dry, alkaline litter before it is removed from production facilities for land application or other uses. It is likely that the calcium phosphate effects observed in this experiment would persist in such a dry, alkaline material. This hypothesis that a manure calcium phosphate effect can persist for long periods is supported by previous research. For example, Sharpley and Smith (1995) found that multi-year applications of manures greatly enhanced the proportion of soil P in acid-soluble, calcium-bound forms.

Given the key role that feed calcium phosphate content played in controlling solubility of manure P, it is important to remember that the NPA+Phyt diet in this experiment contained less inorganic P than would be used in most commercial settings. The NPA+Phyt diet was purposely formulated to contain total nonphytate P below NRC-recommended levels in order to maximize differences in P content between alternative and conventional manures. A commercial turkey producer would probably raise defluorinated phosphate levels in the alternative NPA+Phyt diet in order to meet NRC (1994) recommendations for nonphytate P. This implies that conventional and alternative manures from commercial sources would probably show fewer differences in P solubility than were observed in this study. It must be emphasized, however, that an alternative diet containing phytase would never be formulated to contain as much inorganic P as a phytase-free conventional diet. This would defeat the whole purpose of feeding phytase enzyme. Therefore, some increase in water solubility of manure P would be expected when feeding alternative rations formulated with phytase.

How did feeding phytase enzyme, the true alternative ingredient in the alternative NPA+Phyt diet, affect water solubility of manure and soil P? Feeding phytase actually had a less consistent and smaller impact on water solubility of manure P than did changes in feed calcium phosphate content. Simple addition of phytase enzyme to the baseline diet did result in statistically significant increases in the percentage of manure P dissolved in 0.01 M $CaCl_2$. For example, extraction of wet individual pen manures showed that the P in the phytase-amended NPA+Phyt manure was 14% more water-extractable than the P in the baseline NPA manure. For wet composite manures, a phytase-induced solubility increase of 10% was observed. Both of these increases in manure P solubility were statistically significant. It is proposed that addition of phytase enzyme to the baseline diet decreased the proportion of manure P in insoluble phytate form by transforming it into readily soluble plant-derived nonphytate P. Whatever the exact mechanism, a significant phytase effect did contribute to differences in P water solubility between the conventional NPA+P alternative NPA+Phyt manures at the point of excretion.

After wet manures were applied to soils, phytase-induced increases in water solubility of manure P disappeared. Overall, NPA-treated soils contained 12.2 mg kg^{-1} water-soluble P, compared to 11.9 mg kg^{-1} for NPA+Phyt-treated soils. This similarity in water-soluble P content between NPA- and NPA+Phyt-amended soils was not noticeably affected by changes in manure application regime, incubation time, or soil type. Drying wet manures at 40 °C for 4 d also erased any phytase-induced increases in water solubility of manure P. The P in the dried NPA+Phyt manure was significantly less $CaCl_2$-soluble than the P in the dried baseline NPA manure (75.8% for dried NPA+Phyt versus 79.5% for dried NPA). These results show that differences in water-soluble P content between soils amended with wet NPA+P and NPA+Phyt manures on an equal-P basis were caused by differences in feed and manure calcium phosphate content. The same can be said for the differences in water solubility of P between dried NPA+P and NPA+Phyt manures. Finally, these results may also indicate that differences in water solubility of manure P caused solely by feeding phytase enzyme are short-lived and have little potential to significantly increase losses of water-soluble P from manured lands.

How did feeding HAP corn and HAP corn plus phytase in combination affect the water solubility of P in manures and manure-amended soils? Much like addition of phytase enzyme, substitution of HAP corn for normal corn in the baseline NPA diet significantly increased the water solubility of P in wet manures. This HAP-induced increase in water solubility of manure P also did not persist after manure drying or application of manures to soil. Simultaneous feeding of phytase enzyme and HAP corn produced statistically significant and longer-lasting increases in extractability of manure P. Extraction of wet manures with 0.01 M $CaCl_2$, Mehlich III, and Olsen all revealed statistically significant HAP-plus-phytase-induced increases in manure P solubility. Extraction of manure-amended soils with each of the three solutions also revealed this HAP-plus-phytase effect, which increased with increasing soil-manure incubation time in a number of cases. It is interesting to note that 4 d of drying at moderate temperature (40 °C) reduced or actually reversed the solubility-enhancing HAP-plus-phytase effect. This dramatic drying influence makes it more difficult to predict how feeding phytase enzyme plus HAP corn in a commercial situation might alter the potential for loss of water-soluble P from manured soils.

The previous paragraph has addressed the question of how, in the absence of dietary calcium phosphate adjustment, adding HAP corn or a combination of HAP corn plus phytase enzyme to feeds affected the solubility of P in manure and in manure-amended soils. It must be emphasized again that formulating an actual HAP or HAP plus phytase diet would always involve a reduction in inorganic feed P. Therefore, when assessing the true impact of converting to an alternative diet, calcium phosphate effects as well as HAP and HAP-plus-phytase effects must be considered. The ultimate conclusion is that switching from a conventional NPA+P-style diet to a diet containing HAP corn or HAP corn plus phytase enzyme may increase the potential for water-soluble P losses from manured soils, if only due to the impact of changing dietary calcium phosphate levels. Recall that HAP-containing diets are not currently in commercial use, due to the limited availability of this type of corn.

This study has shown that use of phytase enzyme and/or HAP corn in turkey feed reduces total P excretion, but can increase the relative water solubility of the P that is excreted, both before and after application to soils. Phytase feeding is widely embraced as a solution to the P pollution problems associated with concentrated poultry production. Is it possible that conversion to phytase feeding could actually increase the potential for water-soluble P loss from soils amended with poultry manure? Given the limited scope of this study, a conclusive response certainly cannot be provided at this time. In fact, it is suggested that an across-the-board “yes” or “no” may never be possible. That is because, depending on a number of site-specific factors, the consequences of increased water-solubility of manure P may vary significantly. For example, if manure is applied without incorporation to a soil already saturated with P and presenting significant run-off risk factors (excessive slope, hydrologic connectivity to receiving waters, etc.), increasing levels of water-soluble P in the manure might exacerbate a soluble-P-loss problem. Under another set of circumstances, the fact that manure from phytase-fed poultry contains elevated levels of water-soluble P could simply enhance the manure’s value as a soil amendment. For example, if manure is applied to soils that are not saturated with P and that present low soluble-P-runoff risk factors, enhanced water solubility of manure P may simply translate into faster availability of manure P to actively growing crops. Remember that commercial P fertilizer sources are formulated with the goal of maximizing readily soluble P content. Such an enhancement in manure plant-food value could encourage the movement of poultry litter out of environmentally sensitive areas for land application as a substitute for commercial fertilizer. Regardless of one's situation or perspective, the results of this study show that these issues warrant further research.

4.1.2. POTENTIAL PLANT AVAILABILITY OF P

Raw manures as well as manure-amended soils were extracted with the acidic, chelate-containing Mehlich III solution. The Mehlich III extraction procedure was originally designed to assess the plant availability of P and other nutrients in soils, not manures. Nevertheless, wet and dried manure samples as well as soils in this study were extracted with Mehlich III. It was hoped that comparing the results of water and acid extraction of manures would provide insight into the different forms of P in the manures. Overall, Mehlich III extraction of wet manure samples revealed relatively few differences in potential plant availability of P between the conventional NPA+P and the alternative NPA+Phyt manures. For example, the P in the wet individual pen NPA+Phyt manures was only 8% more Mehlich III-soluble than the P in the wet individual pen NPA+P manure (67.6% of total P in the NPA+P manure was dissolved versus 73.2% for the NPA+Phyt treatment). This solubility difference was not statistically significant at the $p = 0.05$ level. Note that, although these manures did not differ in Mehlich III solubility on a percentage basis, more P was extracted from the NPA+P manure (9868 mg P per kg manure) than from an equal quantity of the NPA+Phyt manure (6455 mg P per kg manure). After wet individual pen manures were combined to make composites, a statistically significant difference in Mehlich III solubility of P was observed between the NPA+P and NPA+Phyt manures. However, neither the NPA+P nor the NPA+Phyt manures differed significantly in P solubility percentage from the baseline NPA manure (75.0% of total P was soluble for NPA+P versus 82.2% for NPA and 90.0% for NPA+Phyt). Since calcium phosphates are more soluble in acidic extractants than in near-neutral solutions such as 0.01 M $CaCl_2$, these results lend further support to the hypothesis that calcium phosphate compounds were largely responsible for differences in water solubility between the conventional and alternative manures.

The basic Mehlich III P solubility pattern observed among the wet manures persisted after application of those manures with soils. When manures were applied to soils on an equal-P basis, switching from the conventional NPA+P diet to the alternative NPA+Phyt diet did not produce a statistically significant change in the amount of plant-available P in those soils. Analysis of all Mehlich III extraction data shows that, overall, soils amended with the NPA+P Pbasis manure treatment contained 280.6 mg kg^{-1} plant-available P, while soils treated with NPA+Phyt manure contained 286.8 mg kg^{-1} plant-available P. When manures were mixed with soils on an equal-N basis, the alternative NPA+Phyt treatment loaded 41% less total P on the soils than did the conventional NPA+P Nbasis manure treatment. The dietary shift also caused Mehlich-extractable P levels in the soils to drop by a statistically significant 24%, from 376.7 mg kg^{-1} for the NPA+P Nbasis soil to the 286.8 mg kg^{-1} for the NPA+Phyt soil. Changing manure application rate, incubation time, or soil type had essentially no influence on this pattern of P plant-availability in soils treated with NPA+P and NPA+Phyt manures.

Drying the composite manures at moderate temperature for 4 d increased differences in Mehlich III solubility of P between the conventional NPA+P and alternative NPA+Phyt manures. The net result was that P in the dry conventional manure became significantly more Mehlich III-soluble than was the P in the alternative NPA+Phyt manure. This shift most likely occurred because the drying process diminished the acid solubility of calcium phosphates in the NPA+P manure. This is the most likely explanation, since the NPA+P and baseline NPA manures differed significantly in P solubility percentage and the NPA and NPA+Phyt manures did not. Recall that extraction with $CaCl_2$ revealed a very similar reduction in the extractability of manure calcium phosphate compounds with drying. These results are a reminder that drying or other modifications to manure between the point of excretion and the point of land application can strongly influence the extent and persistence of P solubility differences between manure types. In this study, only wet manures were applied to soils. It is possible that combining dried manures with soils would have led to persistent and statistically significant differences in plant availability of P between soils treated with conventional and alternative manures.

Simple addition of phytase enzyme to the baseline NPA diet (i.e., without changing dietary calcium phosphate levels) did not produce statistically significant differences in Mehlich III solubility of P in wet manures, dried manures, or manured soils. The same can generally be said of substitution of HAP corn for normal corn in the baseline NPA diet. In contrast, the combined effect of HAP corn substitution plus phytase enzyme addition significantly enhanced the percentage of manure and soil P dissolved in the Mehlich III extractant. The net result of these trends is that wet manures derived from the alternative HAP diet did not generally differ in P Mehlich III solubility percentage from the conventional NPA+P manure. In contrast, HAP+Phyt manures did.

Given these Mehlich III extraction results, how will converting to phytase-based rations affect crop availability of P from manured soils? The experimental soil extraction data suggest that similar amounts of overall plant-available P will be present in fields amended with equal amounts of P, whether that P is derived from conventional manures or alternative manures. One question that remains to be answered, however, is how the increased water solubility of manure P fits into the plant-availability picture. Remember that soils treated with alternative, phytase-derived manure contained significantly more P in water-soluble form than did soils treated with conventional manure. Water-extractable P is generally considered the form most easily desorbed from soils and readily available for crop uptake. Does converting to a phytase-based diet affect plant availability of P or not? One possibility is that the greater water solubility of P in soils amended with alternative manures could result in more rapid release of plant-available P than if conventional manures were land-applied. This ready availability of P from alternative manures could be especially valuable to farmers when soil conditions do not favor the chemical and biological reactions which render soil P available for plant uptake, such as in early spring when soils are still cold. This initial fertilizer P advantage associated with the alternative manure would most likely be erased as the growing season progressed and soils warmed. The net result would be that, over the duration of the entire growing season, both the conventional and alternative manure amendments would provide similar amounts of P for crop uptake. This outcome would be consistent with the similarities in Mehlich III solubility of soil P observed in the study. A final point to keep in mind is that all experimental soils in this study were mixed with wet manures. Drying raw manures as well as increasing soil incubation time prior to extraction both decreased Mehlich III solubility of calcium phosphate compounds in manure. It is therefore suggested that manure drying or other processes enhancing Ca-P bonding could lead to significant differences in plant-availability of P from soils fertilized with conventional and alternative manures.

4.2. COPPER

4.2.1. WATER SOLUBILITY OF CU

Overall, extraction of Cu from manures and manure-amended soils produced much less clear-cut and consistent solubility differences between treatments than those seen during P extraction. Switching from the conventional NPA+P diet to the alternative NPA+Phyt diet did not significantly affect the water solubility of Cu in wet manures. For example, about 33% of total Cu in both the wet NPA+P manure and wet NPA+Phyt manure was solubilized by the 0.01 M $CaCl_2$ extractant. However, the results of this comparison changed as the manures underwent transformations after excretion. For instance, drying manures for 4 d at 40 °C caused a disproportionate increase in water-solubility of Cu for the NPA+P manure. As a result, Cu solubility in the dry NPA+P excreta was significantly greater than in the dry NPA+Phyt manure (Cu water solubility percentages were 48.5% for the dry NPA+P and 42.6% for the dry NPA+Phyt). Comparisons with the baseline NPA treatment suggest that this drying-induced shift in Cu solubility was related to the phytase enzyme in the NPA+Phyt feed. Differences in dietary calcium phosphate levels were apparently not involved.

Applying the wet NPA+P and NPA+Phyt manures to soils also changed Cu solubility relationships, although a different mechanism may have been involved. After composite manures were applied to soils on an equal-Cu (and equal-N and equal-manure-weight) basis, soils amended with wet NPA+Phyt manures released 6% more Cu during extraction with 0.01 M $CaCl_2$ than did soils amended with NPA+P manures. This Cu solubility difference was statistically significant. Comparisons with the baseline NPA treatment indicated that dietary calcium phosphate levels helped explain these differences in water extractability of Cu between NPA+P- and NPA+Phyt-amended soils. This increase in Cu solubility with increasing dietary calcium phosphate was enhanced by higher manure applications to soils, but was diminished by increasing incubation time prior to extraction. As indicated above, converting from the NPA+P to the NPA+Phyt feed under N-based manure management led to a significant 6% increase in water solubility of Cu in the manure-amended soils. In contrast, under P-based manure management, the same dietary shift produced a significant 92% increase in the quantity of water-soluble Cu extracted from manure-amended soils. The amount of Cu extracted increased so dramatically because conversion to the NPA+Phyt diet boosted total manure Cu loadings to soils by 67%.

Feeding phytase enzyme alone (i.e, without concurrent change to feed defluorinated phosphate levels) did not produce any changes in water solubility of Cu in manured soils. However, feeding HAP corn as well as HAP corn plus phytase caused statistically significant increases in water solubility of Cu in manure-amended soils of 4% and 7%, respectively. These solubility-enhancing HAP and HAP-plus-phytase effects increased in statistical significance with higher manure loading rates and increased in numerical significance with longer incubation time. The effects were observed on all three soil types, but both were significant only on the Groseclose. Note that these solubility-enhancing HAP and HAP-plus-phytase effects were not observed during $CaCl_2$ extraction of wet manures. This again suggests that transformations in manures after excretion played a key role in determining how dietary formulation ultimately affected water solubility of manure-derived Cu.

What will be the ultimate effect of switching to phytase-based feeds on water solubility of Cu from manured soils? The experimental results do not reveal consistent trends for both manures and soils. This study certainly found no evidence that feeding phytase will significantly increase the water solubility of Cu when equal quantities of manure are applied to the land. Perhaps the most important effect of conversion to phytase-based rations is simply the fact that, under P-based manure management, use of phytase can dramatically increase both the amount of total manure and consequently the total amount of metals like Cu that are applied to soils.

4.2.2. POTENTIAL PLANT AVAILABILITY OF CU

Mehlich III extraction of the wet manures showed that conversion from the conventional NPA+P ration to the alternative NPA+Phyt ration produced a statistically significant decrease in acid solubility of manure Cu. For example, the Mehlich III extraction dissolved 48.5% of total Cu in the wet NPA+P manure, but only 32.8% of total Cu in the wet NPA+Phyt manure. Across all five treatments, a direct linear relationship was observed between the percentage of total manure Cu that was soluble in Mehlich III and the percentage of the corresponding feed that was in nonphytate P forms (including inorganic as well as plant-derived nonphytate P). This suggests that Cu was less soluble in the NPA+Phyt manure because that manure was derived from a feed containing less nonphytate P. Exactly how this feed characteristic might have controlled Mehlich III solubility of manure Cu is not known. Note that under commercial conditions, conventional and phytase-amended rations would probably be formulated to contain equal nonphytate P levels. Drying of manures prior to extraction increased overall Mehlich III extractability of manure Cu. Drying also reduced the magnitude of the Cu solubility differences between the NPA+P and the NPA+Phyt manures. However, the dry NPA+P manure still contained a significantly higher Mehlich III Cu solubility percentage (53.8%) than the dry NPA+Phyt manure (43.5%).

Application of the wet NPA+P and NPA+Phyt manures to soils dramatically changed the Cu solubility relationship described above. Soils amended with NPA+P and NPA+Phyt manures on an equal-Cu (and equal-N) basis contained 2.33 and 2.28 mg Mehlich III-extractable Cu kg^{-1}, respectively. These concentrations were not statistically different. It is intriguing to note that the vast majority of Cu extracted from manured soils with Mehlich III appeared to be native soil Cu (i.e., present prior to manure application). For unknown reasons, the amount of Cu extracted from soils was affected very little when widely varying quantities of manure Cu were applied. Regardless of the mechanisms responsible for this phenomenon, soils amended with NPA+P and NPA+Phyt manures on an equal-Cu and -N basis contained very similar amounts of plant-available Cu. This held true across all variations in manure application regime, incubation time, and soil type. Conversion to a phytase diet did not affect plant availability of Cu in soils manured on an equal-N basis. Soils amended with the NPA+P and NPA+Phyt manures on an equal-P basis also yielded statistically similar amounts of Cu to the Mehlich III extractant (2.23 mg kg^{-1} and 2.28 mg kg^{-1}, respectively). This was unexpected, since 67% more manure Cu was applied to the NPA+Phyt soil than to the NPA+P soil. These results present no evidence that feeding alternative diets will significantly affect the potential plant availability of Cu in manured soils.

4.3. ZINC

4.3.1. WATER SOLUBILITY OF ZN

Extraction of wet manures with $CaCl_2$ showed that Zn in the alternative, phytase-derived NPA+Phyt manure was significantly more water soluble than Zn in the conventional NPA+P manure. This difference was dramatic, with only 11.0% of total Zn in the wet conventional manure solubilized compared to 61.5% for the wet NPA+Phyt manure. It is proposed that this major solubility difference occurred primarily because the NPA+P feed contained far more calcium phosphate than the other feeds. The result was relatively high levels of Ca and P in the NPA+P manure, which probably resulted in binding of Zn into water-insoluble forms. This hypothesis is supported by the fact that simple addition of supplemental defluorinated phosphate to the baseline NPA diet caused water solubility of Zn at the point of excretion to drop significantly, from 51.0% to 11.0%. In contrast, adding phytase enzyme, HAP corn, or HAP corn plus phytase to the baseline NPA diet all increased manure Zn water solubility percentages numerically, but not statistically.

The previous paragraph summarizes results of $CaCl_2$ extraction of manures from selected individual pens. These results do not reflect data associated with manures from the five individual turkey pens suspected of being contaminated with Zn. Comparison of Zn extraction results reflecting all available data (including data related to potentially contaminated samples) and results reflecting only selected data show very similar Zn water solubility patterns. Nevertheless, it is most appropriate to avoid drawing important conclusions based on results of analyses or procedures involving the samples suspected of contamination. For this reason, results of Zn extraction from composite manures must be viewed with caution. Furthermore, since soils in the soil incubation and extraction study were amended with composite manures, the manuring rates used did not result in equal loadings of Zn to soils. As a result, there was no value in analyzing or comparing the amounts of Zn extracted from the various soils.

Although it is not possible to compare the water-soluble Zn content of manure-amended soils, one can calculate (based on results of manure extraction) the relative quantities of readily extractable manure Zn that would be loaded on soils by the conventional and alternative manures under N- and P-based management. In a scenario involving land application of manures on an equal-weight or equal-N basis, it is suggested that wet NPA+Phyt-type manures would load soils with 6% less total Zn, but 423% more water-extractable Zn than would conventional manure of the NPA+P type. If the same manures were land-applied on an equal-P basis, the alternative manure would load soils with 55% more total Zn and 765% more water-soluble Zn than would the conventional manure. These percentages are impressive, but are only relevant if soil-manure reactions do not modify the relative extractability of the Zn from the conventional and alternative manure sources. How likely is it that Zn solubility differences observed in the NPA+Phyt and NPA+P manures will persist after land application? Is extraction of elements from raw manures a reliable predictor of the extractability of the same elements from soils amended with those manures? In the case of Cu, extraction of wet composite manures was generally not a reliable predictor of the Cu solubility pattern obtained after the same wet composite manures were land applied. In the case of P, however, manure extraction results were fairly reliable predictors of soil solubility patterns. The main reason for this was that the relatively low water solubility of calcium phosphate compounds in the NPA+P manures persisted long after application to soils. It seems reasonable to suggest that the significant differences in water solubility of Zn between the NPA+P and NPA+Phyt manures would persist after application of those manures to soil. This is because (1) the key factor causing Zn extactability difference among the manures was the relatively high manure Ca and P content of the NPA+P manure, and (2) this study has shown that calcium phosphates in the NPA+P manures remain relatively impervious to extraction with 0.01 M $CaCl_2$ after application to soils. These results indicate that further investigation of the impact of alternative poultry diets on water extractability of Zn from manured soils is warranted.

4.3.2. POTENTIAL PLANT AVAILABILITY OF ZN

Mehlich III extraction of Zn from the wet selected individual pen manure samples showed that the conventional NPA+P and alternative NPA+Phyt manures did not differ in plant availability of Zn. It is suggested that the acidic, chelate-containing Mehlich III solution dissolved the phosphate manure compounds which disproportionately limited $CaCl_2$ solubility of Zn in the NPA+P manure. If correct, this hypothesis explains why the wet individual pen NPA+P manures did not differ significantly from any of the other individual pen manures in potential plant availability of Zn. The Mehlich III extraction results also revealed an interesting pattern suggesting that phytate P levels in the manures controlled Mehlich III solubility of Zn to some degree. A direct linear relationship was observed between percentage of total manure Zn soluble in Mehlich III and percentage of feed P in total (inorganic plus plant-derived) nonphytate form. Nevertheless, the most important outcome of Mehlich III extraction of manure Zn was that nothing was found to suggest that long term plant availability of Zn from manured soils will be significantly affected by conversion to alternative poultry diets. Given the real threat that Zn phytotoxicity can pose to agronomic crops on manured lands, this is an especially important finding. The similarity in Mehlich III extractability of manure Zn between the alternative and conventional manures also may indicate that the manure Zn water solubility differences discussed above are grounds for further investigation rather than alarm. It is suggested that study of Zn availability from soils amended with alternative and conventional poultry manures be pursued to confirm that that use of alternative, phytase-based poultry diets will not result in excessive levels of plant-available Zn in manured soils.

CHAPTER 5: REFERENCES

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VITA

Christophe Lawrence was born in New York City on October 27, 1969. He was raised in part in New York City and in part in his mother's native village of Bournens in the French-speaking region of Switzerland. He spent a number of school years as well as most summers during his childhood living on his grandfather's farm in Bournens. He also lived and worked on that same farm every summer during his teenage years.

Christophe obtained his B.A. in History from Yale University in May, 1991. His experiences after Yale included working for the U.S. Forest Service in Idaho as a wildlife biology aide and firefighter. He also worked for three years in Arlington, Virginia for the consulting firm of Booz-Allen and Hamilton under contract to the U.S. Environmental Protection Agency (EPA). This work involved managing the RCRA/Superfund Hotline, EPA's national telephone information line on hazardous waste and site remediation regulations.

In January 1996, Christophe enrolled as an undergraduate at Virginia Tech in the Department of Crop and Soil Environmental Sciences (CSES). One year later, he switched to a Master's program with a focus on soil chemistry and fertility. He served as a graduate teaching assistant during 1997 and was a U.S. EPA Science to Achieve Results (STAR) Graduate Fellow during 1998. Christophe married Sarah Pearsall in Blacksburg, Virginia in August 1998.

In January 1999, Christophe began working for Virginia Cooperative Extension as a Crop and Soil Science Extension Agent serving the counties of King William and King & Queen in the eastern part of the state. He and Sarah currently reside in King William County.