Calcium and phosphorus digestibility: 
Metabolic limits

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Primary Audience: Nutritionists, Researchers, Veterinarians

SUMMARY

Calcium and phosphorus are the two most abundant macro minerals required for proper performance, growth, and production of poultry. Feed ingredients from plant sources are inadequate in meeting requirements for these minerals. To meet Ca and P requirements, inorganic sources of Ca and P are usually added to poultry diets. The potentially negative effects of excessive mineral excretion, especially P, have necessitated the need to minimize the amount of P that is excreted into the environment. One of the ways this has been addressed is through source reduction from exogenous phytase supplementation to diets that are marginally deficient in these minerals. A better understanding of the mechanisms involved in Ca and P metabolism in poultry is essential. Such information will help in formulating diets that closely meet the Ca and P needs of the bird with minimal excretion into the environment. This, however, will require a slightly different approach. One approach is diet formulation on a digestible Ca and P basis. Unlike for amino acids where digestion and absorption is minimally affected by physiological factors, parathyroid hormone and vitamin D₃ play important roles in Ca and P metabolism. The critical roles played by vitamin D₃ in Ca and P metabolism are enormous, and taking advantage of this could enhance Ca and P utilization. Therefore, a need to generate critical data that could expand our understanding of the important role vitamin D₃ plays in Ca and P metabolism and the site of their absorption in the gut exists. Finally, the metabolic limits of Ca and P utilization in poultry will depend on many dietary and physiological factors that influence digestion, absorption, retention, and excretion of these minerals. The potential metabolic limit is expected to keep changing with the genetic improvement of birds.

Key words: calcium, phosphorus, metabolism, vitamin D₃

INTRODUCTION

Today’s birds (laying hens, broilers, turkeys, and ducks) are completely different from their counterparts before domestication. They grow faster, are heavier at market weight, produce more eggs per year, and use nutrients more efficiently. Additionally, they have less access to exercise and less exposure to sunlight; despite this, little attention has been given to redefining the Ca, P, and vitamin D₃ needs of these birds.

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Factors that may affect Ca and P digestion, absorption, and metabolism include the diet’s phytic acid contents, Ca to P ratio, sodium, and vitamin D3 level, as well as the amount of room provided for the bird to exercise [1–3]. Legislation and propositions that would ban cages or mandate a substantial increase in cage space allocation to birds will lead to an increase in room for exercise, resulting in an increase in bone mineral density that would invariably lead to an increase in Ca and P deposition in bones. The increasing use of alternative feed ingredients, such as dried distillers’ grain with solubles, meat and bone meal, and bakery byproducts, further makes attaining an accurate estimation of dietary Ca and P difficult. The accurate estimation of Ca and P requirements is not only important for birds’ performance and productivity, it is also a welfare issue (especially in cases of severe deficiencies) and is critical for environmental stewardship (i.e., reduction in nutrient excretion).

Calcium and P requirements for different classes of poultry have been estimated using different methods, ages of birds, and inclusion levels of both Ca and P. To adequately determine Ca and P requirements for today’s birds, some critical issues, including streamlining terminologies (e.g., available P vs. nonphytate P), have to be addressed. The objectives of this paper are to examine the roles of different factors that affect Ca and P metabolism, explore ways in which some of these factors could be controlled to enhance efficiency of Ca and P utilization, and proffer suggestions for improvement.

CALCIUM AND PHOSPHORUS

The bulk of Ca and P absorbed by birds are required primarily for bone formation. Calcium, the most abundant mineral in the body, exists in 3 different forms: hydroxyapatite \([\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]\) of the bone in the ratio of 2:1 with P, in the extracellular matrix as ionized Ca, and Ca bound to protein or as Ca bound to anions. Calcium is also found intracellularly [4]. Phosphorus is the mineral with the second highest requirement (after Ca) in birds, with about 80% stored in the skeleton (1:2 ratio with Ca in bone-hydroxyapatite). A significant amount of phosphate (Pi) is found either in inorganic form or bound to organic compounds such as protein, lipid, and DNA or RNA. Cellular and extracellular Pi levels are tightly maintained within a narrow range, as significant fluctuations in Pi concentration could negatively affect many of the important biochemical processes (bone development and integrity, energy metabolism, cell signaling, and so on) in the body [5]. The importance of intestinal Pi absorption on Pi homeostasis has been established in sodium-dependent phosphate transporter (NaPi-IIb) in knockout mice [6] and in chicks [7]. Although NaPi-IIb gene has been shown to play significant roles in Pi absorption, this transporter is regulated by vitamin D3 and dietary P (concentration of Pi in the intestinal lumen, [7–9]). In a recent study in our laboratory, we have also shown the importance of gut health in the expression of NaPi-IIb gene [10]. Twenty-six-day-old broiler chickens that were challenged (orally gavaged) with mild (5× normal dose) coccidial vaccine had significantly higher expression of NaPi-IIb gene than the control group, despite being on the same diet that was adequate in nonphytate P (nPP) with a Ca to nPP ratio of 2.0 [10]. Additionally, the NaPi-IIb gene was found to be expressed throughout the small intestine of broiler chicks (d 0–14). In a study by Olukosi et al. [11], the NaPi-IIb gene expression was upregulated in the jejunum and ileum of broiler chicks on a diet that was severely deficient in nPP (2.5 g of nPP/kg) relative to those on marginally deficient (3.5 g of nPP/kg), adequate (4.5 g of nPP/kg), and excess (5.5 g of nPP/kg) nPP diets.

Intestinal Ca and P Absorption

It has been reported that Ca is absorbed across the intestinal wall via 2 pathways: the transcellular and paracellular routes [12–15]. Transcellular active transport occurs predominantly in the upper section of the small intestine, especially in the duodenum and upper jejunum, in several animal species including mice, rats, and chicken. About 10% of Ca absorption via this route takes place in the colon of rat [12]. Transcellular Ca absorption occurs via 3 components: the epithelial Ca channels from the intestinal lumen into the enterocyte [16], intracellular calbindins for trans-cytosolic diffusion [17], and the adenosine triphosphate-activated basolateral membrane Ca pump [18]. Vitamin
D₃ is involved in each of these steps [19–21]; the role of vitamin D₃ in transcellular Ca absorption have been extensively studied and documented [19, 22].

Unlike the transcellular pathway, the paracellular pathway is nonsaturable, and absorption occurs throughout the small intestine [12, 17]. Until recently, it was widely believed that Ca absorption in the ileum occurred predominantly via the vitamin D₃-independent paracellular (passive diffusion) pathway, and that it is concentration dependent [22, 23]. Based on recent data using mice colon and ileum and in caco-2 cell lines [15], although this process is paracellular, it is nonetheless vitamin D₃ driven (facilitative diffusion).

The absorption of Ca and P via the transcellular pathway occurs predominantly in the upper portion (duodenum and upper jejunum) of the small intestine [24]. In some studies (both in vitro and in vivo) we have gained insight into the mechanisms involved in Ca and P absorption in the small intestine. These studies [15, 25, 26] did not contradict the abundance of the respective transporters for Ca in the upper segment of the small intestine, but showed that the paracellular route of Ca absorption, especially in the ileum, plays a significant role in the amount of Ca that is absorbed (Table 1). Paracellular absorption occurs throughout the entire small intestine. The length of the distal jejunum and ileum, which is significantly longer that the duodenum and upper jejunum, allows greater surface area for absorption of Ca and P through the vitamin D₃-driven paracellular route [15]. Furthermore, the duration of time digesta spend in the jejunum and ileum provides another reason why paracellular absorption of Ca and P may contribute significantly to the amount of absorbed Ca and P. In a recent study, the P digestibility [27] between the upper and lower ileum were significantly different, with greater disappearance of P in the distal ileum. The location of active Pi absorption has been shown to be different between mice and rats. For example, Marks et al. [28] reported that Pi absorption occurs throughout the mouse intestine, with the highest rate of absorption in the ileum, a section of the small intestine with the highest levels of NaPi-IIb mRNA and protein. In rats, however, the greatest absorption occurs in the duodenum and upper jejunum [28, 29]. Although, NaPi-IIb mRNA expression has been observed throughout the entire small intestine of the broiler chicken [11], the relative contribution of each section to overall Pi absorption has not be determined.

When 2 levels of Ca (normal = 1%, or low = 0.02%) were fed for 14 d to adult male Wistar rats, the Ca flux, from the mucosal-to-serosal, in the ileum of rats on the low-Ca diet was twice that of rats on the normal diet [12]. This information confirmed that only cellular Ca flux was stimulated due to a lack of changes in sodium or water flux in the in situ model or mannitol flux in the in vitro model [12]. A recent study examining the role of vitamin D₃ in intestinal tight junction proteins (claudin-2 and -12) has highlighted the importance of intestinal Ca absorption through the paracellular route [15]. Calcium absorption is physiologically and nutritionally regulated, and vitamin D₃ plays an important role; whereas calcitonin, parathyroid hormone, and vitamin D₃ significantly regulate Ca metabolism.

### Vitamin D₃

Vitamin D₃ is a steroid hormone that is required in diets of poultry. The critical role of vitamin D₃ and its metabolites in Ca and P metabolism in laboratory animals and poultry species have been reported [1, 5, 30–35]. Researchers have reported the important roles dietary Ca, P, and vitamin D₃ play in intestinal Ca and Pi ab-

### Table 1. Proportion of calcium absorbed in the 3 sections of the small intestine

<table>
<thead>
<tr>
<th>Species</th>
<th>Year</th>
<th>Duodenum, %</th>
<th>Jejunum, %</th>
<th>Ileum, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat¹</td>
<td>1962</td>
<td>8</td>
<td>4</td>
<td>88</td>
</tr>
<tr>
<td>Rat²</td>
<td>1959</td>
<td>7</td>
<td>17</td>
<td>65</td>
</tr>
<tr>
<td>Dog²</td>
<td>1959</td>
<td>4</td>
<td>16</td>
<td>80</td>
</tr>
</tbody>
</table>

¹Marcus and Lengemann (1962) [25].
²Cramer and Copp (1959) [26].
The action of vitamin D₃ on genes responsible for intestinal active Pi transport has been reported to be age dependent in rats [35] and inhibited by the antibiotic actinomycin. Unlike the action of actinomycin, lipophilic polyene antibiotic (filipin) treatment of vitamin D₃-deficient intestinal chick tissue resulted in an immediate increase in the rate of active Ca transport, but same treatment for intestinal tissue from vitamin D₃-treated birds did not result in any change in active Ca absorption [36]. This observation shows that the vitamin D₃ status of a bird may influence the effect of dietary components or supplements on intestinal Ca and Pi absorption. Furthermore, intravenous injection of vitamin D₃ into vitamin D₃-deficient chickens resulted in a tendency to increase plasma membrane Ca pump within 2 h postinjection [33]. Hurwitz and Bar [30] reported that the effects of vitamin D₃ on Pi absorption may be independent from that on Ca absorption. Adequate vitamin D₃, with or without phytase supplementation, was shown to enhance P utilization in broilers [1].

There are several forms of vitamin D, with vitamins D₃ and D₂ (ergocalciferol) being the most common types [4]. Ergocalciferol is the less potent of the 2 (about 10% potency) [37]. Physiologically, vitamin D₃ stimulates intestinal Ca absorption, a process mediated by a metabolite of vitamin D₃ (1-α,25-dihydroxyvitamin D₃). The administration of 1-α,25-(OH)₂D₃ has been reported to increase the rate of active Ca transport in the duodenum of vitamin D₃-deficient chicks. It is important to note that vitamin D₃ from an animal source is better available (about 60–70%) to the birds than vitamin D₃ from plant sources. One of the functions of vitamin D₃ is to enhance paracellular Ca absorption through the upregulation of tight junction proteins leading to the formation of calcium-specific pores [15]. The most potent and commonly used metabolite of vitamin D₃ in broilers is 25-OH D₃ [38–42]. The role of vitamin D₃ in ameliorating tibal dyschondroplasia as a result of low dietary Ca, as well as increasing tibia ash, has been documented [41, 43, 44].

**Intestinal Phosphate and Phosphate Homeostasis**

Body Pi homeostasis is determined by the intestinal uptake of dietary Pi, renal Pi reabsorption and excretion, and the exchange of Pi between the extracellular and bone storage pool [5, 32]. Active transport of Pi occurs via the NaPi-IIb (type II transporters) and the expression of NaPi-IIb at the brush border membrane is the rate-limiting step for the transcellular uptake of Pi. Type III transporters (PiT1 and PiT2), which are also sodium dependent, have also been discovered (Table 2) [5]. The role of type III transporters in Pi transport in laboratory animals is currently being elucidated; early studies showed that Pi transport in the intestine could either be by sodium-dependent or sodium-independent processes [45, 46].

Based on the available information that a significant amount of Ca is absorbed in the ileum [25] and that the passage rate of digesta, especially from highly viscous feed ingredients such as rye, triticale, oats, and barley, may influence

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**Table 2. Characteristics of phosphate transporters**

<table>
<thead>
<tr>
<th>Phosphate transporter</th>
<th>Location</th>
<th>Preference</th>
<th>Inhibited by phosphonoformic acid</th>
<th>Electrical activity</th>
<th>Stoichiometry</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type II transporter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaPi-IIa</td>
<td>Kidney</td>
<td>Divalent Pi (HPO₄²⁻)</td>
<td>Yes</td>
<td>Electrogenic</td>
<td>Na⁺:HPO₄²⁻ (3:1)</td>
</tr>
<tr>
<td>NaPi-IIb</td>
<td>Intestine</td>
<td>Divalent Pi (HPO₄²⁻)</td>
<td>Yes</td>
<td>Electrogenic</td>
<td>Na⁺:HPO₄²⁻ (3:1)</td>
</tr>
<tr>
<td>NaPi-I-</td>
<td>Kidney</td>
<td>Divalent Pi (HPO₄²⁻)</td>
<td>Yes</td>
<td>Electroneutral</td>
<td>Na⁺:HPO₄²⁻ (2:1)</td>
</tr>
<tr>
<td><strong>Type III transporter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PiT1</td>
<td>Intestinal BBM</td>
<td>Monovalent Pi (H₂PO₄⁻)</td>
<td>No</td>
<td>Electrogenic</td>
<td>Na⁺:H₂PO₄⁻ (2:1)</td>
</tr>
<tr>
<td>PiT2</td>
<td>Renal BBM</td>
<td>Monovalent Pi (H₂PO₄⁻)</td>
<td>No</td>
<td>Electrogenic</td>
<td>Na⁺:H₂PO₄⁻ (2:1)</td>
</tr>
</tbody>
</table>

¹Data from Marks et al. (2010) [28].
²BBM = brush border membrane.
ileal nutrient (including Ca and P) absorption [47], particular attention should be placed on the feed ingredient components of diets. In early studies with laboratory animals, it was reported that Pi transport, age of the bird, and sources of Ca may also determine the rate of Ca absorption in the small intestine of poultry [48]. For example, a diet with most of its Ca coming from limestone or mono- or dicalcium phosphate would result in a significant amount of its Ca being absorbed in the duodenum and upper jejunum. However, if a significant amount of dietary Ca comes from a source that is not highly available in the gastrointestinal tract (GIT), fewer Ca may be absorbed in the duodenum.

**Dynamic of Ca and P Metabolism**

Unlike many other minerals, Ca absorption in the gut and metabolism is strictly regulated by many factors and its intestinal absorption is highly regulated. The Ca status of the birds determines the level of Ca absorption at any given time. This is regulated largely by the parathyroid hormone and vitamin D₃. In determining the Ca and P requirements in poultry, particular attention should be paid to the vitamin D₃ status of the bird. A good understanding of Ca and P metabolism will require a good understanding of the interaction between Ca, P, and vitamin D₃. This should be tailored to different breeds or strains of poultry, age, feed ingredients, and phytase inclusion levels (if used in the diet).

The expression of NaPi-IIb gene and active Pi absorption in rat decreases with age [35]. The administration of vitamin D₃ resulted in an increase in Pi in the intestinal brush border membrane vesicle both in suckling rat (~2.5-fold) and in adult rats (~2.1-fold). Vitamin D₃ treatment also resulted in the upregulation of NaPi-IIb mRNA abundance in 14-d-old rats, but not in adult rats [35].

The quantity of Ca and P that is excreted is a function of many factors, including the source of the nutrients (which affects digestibility or availability), the dietary concentration of Ca, P, and vitamin D₃, the parathyroid hormone status of the bird, reproductive status, and blood pH. In a healthy bird under normal conditions, domestic chickens excrete less than 2% of filtered Ca in urine because of the role of the parathyroid hormone in stimulating the kidney to reabsorb most of the filtered Ca. Details of the role of the kidney in Ca and P metabolism in birds have been reported [32].

**Ca and P Ratio**

Dietary Ca to P ratio could influence digestion and absorption of Ca and P in the GIT of poultry. Depending on their ratios, different physiological responses could be triggered. Hence, proper care must be taken to minimize interactions between Ca and P that may be detrimental to their absorption. High dietary Ca could result in high digesta pH, and may negatively affect the efficacy of phytase and the absorption of other minerals [2, 3, 49, 50, 51].

To minimize variation in the Ca to P ratio in diets, it is necessary to be consistent with the definition of terms used in diet formulation. The terms available P (aP) and nPP have been used interchangeably [52, 53]. However, these terms are different and may be misleading because between 30 and 43% (broiler) and 8 and 26% (laying hen) of the phytate-P (IP₆) is available to broilers and laying hens fed corn, soybean meal, or soybean meal-based diets [Table 3; 3, 54–56]. These values increased to between 52 and 75% with the addition of phytase to the diet. This shows that available P level would be higher than nPP in feeds because poultry have the capacity to release some phytate-bound P in cereals and oilseeds. Essentially, the 2 to 1 ratio of Ca to aP may be underestimating the amount of P that is available to the bird. It is likely the Ca in such diets could be underrepresented. To minimize the potential for errors and confusion, the need for further clarifications of terms exists. For example, it may be necessary to move away from comparing the Ca to P ratio to terms such as digestible or retainable Ca and P. This will take into account the digestible portion of the phytate-bound P.

**Other Factors Affecting Ca and P Metabolism**

Several other factors may affect Ca and P metabolism; these could include dietary, physiological, or animal factors. Most plant sources of feed ingredients have low levels of Ca and P, and a substantial amount of P from cereal and...
grains [58–59] is found in the form of phytate (myo-inositol hexakisphosphate or IP6), which is unavailable, for most part, to the birds due to a lack of adequate endogenous phytase enzyme. Phytate-P is generally about 2.5 to 4.0 g/kg in a conventional poultry diet, of which less than one-half is available to the bird [60]. Formulating poultry diets on digestible Ca and P basis will help to minimize the negative effect of too high or too low concentrations of Ca relative to that of P, which might lead to precipitation in the gut.

Furthermore, if the diet is supplemented with enzymes, especially phytase, the task becomes even harder. To minimize the problems associated with this, diet formulation on a digestible Ca and P basis becomes inevitable. The first step is to develop a consistent protocol for estimating endogenous Ca and P losses. This has been done for amino acids [61, 62]. This means endogenous ileal Ca and P have to be determined using the regression method. Apparent ileal Ca and P digestibility for each feed ingredient can then be standardized by correcting for endogenous Ca or P loss.

Some of the merits for formulating poultry diets on digestible Ca and P basis include better use of feed ingredients, especially the non-conventional feed ingredients; more research is needed in this area, however. The challenge is formulating diets that are completely devoid of these minerals; hence, the regression method may be the method of choice. In conducting such studies, it is recommended that a diet contain enough vitamin D3 to meet or exceed the birds’ requirements. It should be a short study (3 to 5 d), and the birds should not be in a deficient state for either Ca or P, depending on the mineral being investigated. Studies on Ca or P should be conducted one at a time. Conscious efforts must be made to minimize the physiological response to the experimental diets.

Gut health is another factor that may affect Ca and Pi transport in the GIT. Gut inflammation may affect nutrient transporters mRNA expression [10] and tight junction proteins. Passage rate could influence digesta resident time in the gut and decrease the rate of Ca and P digestion and absorption in the GIT. Digesta viscosity may influence digesta passage rate and high digesta viscosity may decrease nutrient and en-
ergy digestibility. The longer the ileum relative to the duodenum, the longer the digesta spends in the ileum, hence the higher the probability of a significant amount of Ca and P being absorbed in the lower jejunum and ileum. If this is the case, the paracellular route of Ca and P absorption may be important in poultry. This means adequate levels of vitamin D₃ are essential. Age, sex, production, and genetics (growth rate) of the birds could also influence Ca and P metabolism. To optimize Ca and Pi utilization by poultry, dietary vitamin D₃ recommendations for different classes of poultry by the NRC [53] should be re-evaluated for today’s birds. The reasons for this are compelling, especially if one considers the rate of growth and final mature weight of broilers and turkeys or the rate of egg production in laying hens and ducks. Re-evaluation may enhance Ca and Pi metabolism and will result in stronger and healthier bones. This is good not only for growth and performance but also for birds’ welfare and environmental stewardship.

CONCLUSIONS AND APPLICATIONS

1. The metabolic limit of Ca and P digestibility in poultry is a function of many factors that are limited by genetic capacity. This limit is not static, but will keep changing with improvement in poultry breeding and genetic resources. Also, the bird’s genetic potential cannot be completely realized, as there will be losses due to inefficiencies in nutrient and energy utilization and management practices.
2. Despite the potential for achieving certain metabolic heights in terms of Ca and P in poultry, there are many factors (dietary, physiological, and animal) interacting together. These interactions could either enhance or reduce the possibilities of attaining a high metabolic state. These factors could be harnessed for better Ca and P metabolic reality.
3. Unlike in other species (especially laboratory animals), where Ca and P metabolism has been extensively studied and documented, more research, applied and fundamental, is needed to fully understand Ca and P metabolism in poultry, especially at the GIT level.
4. There is a need to start formulating poultry diets on a digestible Ca and P basis, as is being done for amino acids. It is important to do this for complete diets as well as for major feed ingredients. The advantages of this are enormous; however, data are needed to build a large database from which a nutritionist can make an informed decision on diet formulation. This should be done with and without exogenous enzyme supplementation.
5. Vitamin D₃ is one of the most potent nutrients influencing Ca and P metabolism. It is important to generate more data in this area, as most of the available data on the role of vitamin D₃ in Ca and P metabolism are old and recent publications in this area are few.
6. When taken together, the advantages that would accrue from further research in Ca, P, and vitamin D₃ will span improvement in performance, productivity, bird welfare, and health. Additionally, nutrient excretion into the environment would be reduced.

REFERENCES AND NOTES


