

## 15 Bioavailability of Minerals and Vitamins in Feedstuffs

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### Introduction

In commercial pig production, the main objective of diet formulation and feeding strategy is to maximize profits, which does not necessarily imply maximal animal performance. To maximize economic efficiency, supplying indispensable nutrients as close as possible to meeting, but not exceeding, the requirements of the pig is advantageous. In addition, this will have a positive impact on today's environmentally conscious society by reducing the excretion of unutilized nutrients. Such optimum feeding strategies involve consideration of a multitude of factors, but one concept that may contribute greatly is the formulation of diets based on available nutrients.

Unfortunately, unlike energy-containing nutrients, there is much less information on the bioavailability of minerals and vitamins. Part of the difficulty is identifying appropriate response variables or depleting the animals sufficiently to obtain a desired response. Obviously, further progress must be made in assessing true nutritional values of feedstuffs, which are necessary for the formulation of efficient and environmentally friendly diets. The objective of this chapter is to review briefly the bioavailability of minerals and vitamins, which can contribute greatly to successful and sustainable pig production. The bioavailability of energy-yielding nutrients is discussed at length in Chapter 14.

### Mineral Bioavailability

Several excellent reviews on comparative mineral bioavailability are available (Nelson and Walker, 1964; Ammerman and Miller, 1972; Peeler, 1972; Cantor et al., 1975a,b; Cromwell, 1992; Ammerman et al., 1995). Most of these reviews have dealt with the relative bioavailability of inorganic rather than organic (i.e., feed) sources of mineral elements. Estimates of true absorption efficiencies of mineral elements will be given in this review, and data for absorption efficiencies will be extrapolated from human, chick, or rat data if reliable pig data are not available. It is deemed important to have some perspective of true absorption efficiency so that data for relative absorption efficiency can be properly evaluated. Also, to assess the effects of supplemental mineral salts on acid-base balance properly, one needs to have knowledge of true absorption efficiencies.



### Calcium

The true absorption efficiency of Ca for humans consuming a mixed diet of animal and plant sources has been estimated at 30% (Groff et al., 1995). There is little definitive information on relative Ca bioavailability in swine. It is, however, assumed that limestone, oyster shell, gypsum, marble dust, and aragonite are essentially 100% available as sources of Ca, relative to a  $\text{CaCO}_3$  precipitate used as a standard (Cromwell et al., 1989a). Calcium bioavailability in dolomitic limestone, however, was lower, ranging from 51% to 78%. Ross et al. (1984) also established that particle size of the Ca sources evaluated had no influence on the bioavailability of Ca. Calcium in dehydrated alfalfa meal, however, was only 21% available, relative to Ca in the  $\text{CaCO}_3$  precipitate standard (Cromwell et al., 1983).

Bohlke et al. (2005) used growing pigs to estimate both the apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of Ca in corn, low-phytate corn, and dehulled soybean meal. Values for AID and ATTD were similar and averaged 49% for corn, 70% for low-phytate corn, and 49% for soybean meal. These values are in good agreement with previous estimates of Ca digestibility in a corn-soybean meal diet (Spencer et al., 2000; Veum et al., 2001). Most practical diets fed to swine contain not only corn and soybean meal, but also inorganic Ca from limestone and monocalcium phosphate or dicalcium phosphate. The ATTD of Ca in inorganic Ca is greater than the ATTD of Ca in organic sources, and the ATTD of Ca in a diet based on corn, soybean meal, limestone, and monocalcium phosphate or dicalcium phosphate is between 62% and 70% (Stein et al., 2008; Almeida and Stein, 2010).

Based on poultry data reviewed by Peeler (1972), the relative bioavailability of Ca is 100% in dicalcium phosphate, tricalcium phosphate, defluorinated rock phosphate, Ca gluconate, Ca citrate, Ca lactate, Ca sulfate, and bone meal. More recently, Augspurger and Baker (2004) used chick bone ash to establish that no differences existed in the relative bioavailability of Ca (relative to reagent-grade  $\text{CaCO}_3$ ) in feed-grade limestone, calcium citrate, calcium citrate malate, and oyster shell. It is generally assumed that Ca availability in meat meal and fish meal is equivalent to that in feed-grade limestone.

The absorption efficiency of Ca may be increased by: (1) low intakes, (2) presence of lactose in the diet, (3) pregnancy and lactation, and (4) young age (Groff et al., 1995). Some experiments have also shown that the digestibility of Ca may be increased by addition of phytase to the diet (Almeida and Stein, 2010). In contrast, several factors reduce the efficiency of Ca absorption: (1) consumption without food, (2) presence of phytates or oxalates in the diet, (3) excesses of dietary Mg or P, and (4) fat malabsorption problems that cause steatorrhea.

### Phosphorus

The true absorption efficiency of P from a mixed diet for humans has been estimated at between 70% and 90% (Groff et al., 1995). A summary of P bioavailability estimates in various inorganic and organic feed ingredients for swine and poultry is contained in the review by Soares (1995). Phosphorus bioavailability estimates for swine per se have been published by Cromwell (1992). Relative to commercial mono-dicalcium phosphate, that is,  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ , P availability in commercial dicalcium phosphate fed to turkey is about 90%, and that in defluorinated rock phosphate is about 80% (Waibel et al., 1984). Feed-grade dicalcium phosphates are actually mixtures of  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  and  $\text{CaHPO}_4$ , and both compounds are variables in water of hydration (e.g.,  $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$  or  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ; Baker, 1989). Thus, Ca or P analysis is perhaps the only accurate means of

establishing the ratio of  $\text{CaHPO}_4$  (23% Ca, 18.5% P) to  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  (16% Ca, 21% P). A rule of thumb for swine formulation would consist of setting the P bioavailability in the purchased feed-grade phosphate source at 100% (regardless of whether labeled dicalcium or mono-dicalcium phosphate), and then assuming that P bioavailability in commercial defluorinated phosphate is 90% (Cromwell, 1992). Coffey et al. (1994), however, suggested that P bioavailability (relative to  $\text{NaH}_2\text{PO}_4$ ) in defluorinated P sources for chicks and pigs averaged 85%. Recently, it was reported that the ATTD of P in  $\text{NaH}_2\text{PO}_4$  is approximately 92%, whereas in  $\text{CaHPO}_4$  and  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  the ATTD of P is approximately 82% when fed to growing pigs (Petersen and Stein, 2006). Stein et al. (2008) also worked with growing pigs and included different levels of  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  in a corn-soybean meal based diet and reported an average value for ATTD of P of 84%.

Plant-source P is relatively unavailable (Nelson, 1967) although some feed ingredients (e.g., wheat and barley) contain phytase, which increases P bioavailability. Cromwell (1992) estimated the bioavailability of P in corn to be only 14% and that in soybean meal to be 23–31% relative to  $\text{CaHPO}_4$ . He further estimated that the P in wheat and wheat by-products was 29–49% bioavailable; that in rice bran, 25%; cottonseed meal, 1%; and peanut meal, 12%. He considered the P contributed by dried whey, blood meal, fish meal, and alfalfa meal to be close to 100% bioavailable, but his P bioavailability estimate for meat-and-bone meal was only 67%. High-moisture corn has been estimated to contain up to four times more bioavailable P than dry yellow dent corn (Cromwell, 1992).

More recent estimates of P digestibility in corn, soybean meal, and corn-soybean meal diets indicate greater values than the relative bioavailability estimates of Cromwell (1992). Bohlke et al. (2005) used growing barrows to estimate both AID and ATTD of P in corn, low-phytate corn, and soybean meal. The estimated values were surprisingly similar for AID and ATTD with average values being 29%, 56%, and 38% for corn, low-phytate corn, and dehulled soybean meal, respectively. These values for corn and soybean meal agree reasonably well with the Stein et al. (2008) estimate of 38.4% for the ATTD of P in a corn-soybean meal diet fed to growing pigs. The ATTD of P in dried distillers grains with solubles (DDGS) is much greater than in corn and soybean meal, and ATTD values between 50% and 70% have been reported (Pedersen et al., 2007; Stein et al., 2009; Almeida and Stein, 2010). Likewise, the ATTD of P in high-protein dried distillers grains (HP DDG) is close to 60% (Widmer et al., 2007). The reason for the much greater ATTD of P in DDGS and in HP DDG than in corn is believed to be that these ingredients have been fermented, which presumably results in a partial breakdown of the phytate bonds. In contrast, corn germ, which has not been fermented, has an ATTD of P of less than 30% (Widmer et al., 2007). The ATTD of P in field peas fed to growing pigs is approximately 55% if no phytase is used, but this value increases to 65.9% if 500 units of microbial phytase are added to the diet (Stein et al., 2006).

Fermentation-derived P is highly bioavailable because much of the P in yeast, DDGS, etc., is in the form of nucleic-acid P, most of which is RNA. Research using chicks has established that the P in RNA is close to 100% bioavailable relative to  $\text{KH}_2\text{PO}_4$ , which is used as a standard (Burns and Baker, 1976). The same is true for P existing in ingredients as phospholipids (D. H. Baker, unpublished data).

### Phytate

There is much confusion regarding effects of phytates on not only P bioavailability, but also on the Ca and trace-mineral status of animals. Phytates added to various research diets are generally provided as phytic acid or as the Na or Ca salt of phytic acid. These compounds are not the same as the mixed Ca-Mg-K salt of phytic acid generally found in plant-source feed ingredients (Nelson, 1967; Erdman, 1979). Moreover, the negative effects of added phytates on trace mineral utilization are affected significantly by dietary Ca level (O'Dell et al., 1964; Hendricks et al., 1969; Bafundo



et al., 1984b). Research at the University of Illinois using chicks, for example, has shown that the Zn-antagonizing effect of 1.2% supplemental Na phytate when added to a typical corn-soybean meal broiler diet is evident only when Ca is provided in the diet in a quantity at least twice the level recommended by the NRC (1994; Bafundo et al., 1984b).

Knowledge of where phytate occurs in plant-source feedstuffs is important for proper interpretation of effects of such ingredients on mineral bioavailability (Erdman, 1979). Corn is unique among the cereal grains because 90% of the phytate is located in the germ portion of the kernel. Wheat and rice germ also contain considerable phytate, but the hull portion is also rich in phytate. Thus, wheat bran and rice bran have high concentrations of phytate (Halpin and Baker, 1987). Soybeans are different from most other oilseeds in that phytic acid is contained in protein bodies distributed throughout the seed. Hence, protein isolates are richer in phytate than soybean meal (Erdman, 1979). Phytic acid in peanuts, cottonseed, and sunflower seeds is concentrated only in crystalloid and globoid substructures.

Dietary addition of microbial or feed-derived phytase significantly improves the utilization of phytate P by pigs and poultry (Almeida and Stein, 2010). Phytase also markedly improves the utilization of Ca, Zn, Mn, and Fe, but it does not improve the utilization of either Cu (Aoyagi and Baker, 1995) or Se. Certain organic acids (citrate, lactate, and formate) also increase phytate P utilization, and they may also function additively with phytase. The 1 $\alpha$ -hydroxylated vitamin D<sub>3</sub> compounds markedly improve phytate P utilization in broiler chicks, but interestingly, efficacy has not been observed in pigs or laying hens (Biehl et al., 1995; Biehl and Baker, 1996; Snow et al., 2003). Low-phytate corn varieties have bioavailable P levels that are up to three times greater than those in conventional corn varieties. A newer approach has involved corn varieties that express an *E. coli*-derived phytase gene (Nyannor et al., 2007; Nyannor and Adeola, 2008). This approach, as well as the novel approach of producing a phytase-transgenic pig that secretes phytase in its saliva (Golovan et al., 2001), has proven effective in markedly improving dietary phytate P utilization.

### Sodium and Chloride

True absorption efficiency of Na and Cl is thought to be close to 100% (Groff et al., 1995). Using information from requirement studies, one can generalize that NaCl, Na<sub>2</sub>SO<sub>4</sub>, NaHCO<sub>3</sub>, Na acetate, and Na citrate are equal in bioavailable Na and that NaCl, KCl, NH<sub>4</sub>Cl, and CaCl<sub>2</sub> · 2H<sub>2</sub>O are equivalent in bioavailable Cl. Because Na and Cl (and also K) are so intimately involved in acid-base balance and in urinary excretion of cations and anions, suitable bioassays of availability are difficult to develop. Defluorinated phosphates are generally rich in Na, and this ingredient is common in swine diets. Miller (1980) estimated that Na in defluorinated P is 83% available relative to the Na in NaCl. The Na in aluminosilicate (e.g., zeolite) products is thought to be absorbed as efficiently as the Na in NaCl (Fethiere et al., 1988).

### Magnesium

It has been estimated that the Mg supplied by feed-grade sources of MgO, MgSO<sub>4</sub>, and MgCO<sub>3</sub> is 100% bioavailable for swine, relative to the Mg in reagent-grade MgO, and grains and concentrates have Mg bioavailability of 50–60% compared with MgO (Miller, 1980). In data obtained with rats it was also established that Mg in oxide, chloride, carbonate, phosphate, sulfate, and silicate salts of Mg are equally bioavailable (Cook, 1973). The true absorption efficiency by chickens of Mg

from soybean meal is 60%, which is about the same as that estimated for MgSO<sub>4</sub> · 7H<sub>2</sub>O (Guenther and Sell, 1974). There are, however, no data obtained with swine that have established the relative bioavailability values for Mg in vegetable and animal feed ingredients.

### Potassium

Definitive information on K bioavailability from various K sources is lacking. Corn-soybean meal diets for swine are rich in K, making K bioavailability a subject of primarily academic interest. Peeler (1972) predicted that K<sub>2</sub>CO<sub>3</sub>, KHCO<sub>3</sub>, K<sub>2</sub>HPO<sub>4</sub>, K acetate, and K citrate would be 100% available, relative to the K in KCl. Groff et al. (1995) have suggested that more than 90% of ingested K is absorbed by humans.

Among response criteria that have been used to examine the bioavailability of K are blood K, urinary K, and K retention, but only K retention (balance trials) seems to respond linearly to K intake (Combs and Miller, 1985). Based on slope ratio methodology and within the realm of experimental error, K from K<sub>2</sub>CO<sub>3</sub>, KHCO<sub>3</sub>, corn, and soybean meal was judged essentially 100% bioavailable relative to K acetate, which was used as the standard (Combs and Miller, 1985; Combs et al., 1985).

### Copper

The true absorption efficiency of Cu from a mixed diet consumed by humans (Groff et al., 1995) has been estimated to range from 25% (high intakes) to 50% (low intakes). Copper bioavailability, like that of Zn, is difficult to quantify accurately because Cu accumulation in tissues (primarily liver) increases only slightly (and curvilinearly) between deficient levels and a dietary level of about 250 mg Cu/kg diet. Beyond this level, Cu accumulates rapidly and generally in a linear fashion. Relative to CuSO<sub>4</sub> · 5H<sub>2</sub>O, Miller (1980) suggested good utilization of the Cu in CuCl<sub>2</sub> and CuCO<sub>3</sub>, and poor utilization in CuS. The pig work by Cromwell et al. (1989b) indicated that Cu from CuO is almost totally unavailable in the intestinal tract, and similar observations with chickens have been reported (Baker et al., 1991; Aoyagi and Baker, 1993a; Baker and Ammerman, 1995a). Aoyagi and Baker (1993a,b, 1994) and Aoyagi et al. (1995) established a bioavailability assay for Cu in chicks fed Cu either above the requirement (liver Cu accumulation) or below the requirement (gall bladder Cu accumulation). Relative bioavailability (RBV) of Cu for various inorganic and feed-ingredient sources of Cu was in good agreement between the two methods. Relative to analytical-grade CuSO<sub>4</sub> · 5H<sub>2</sub>O, RBV values for Cu were 145% for analytical-grade CuCl and 95–115% for feed-grade CuSO<sub>4</sub> · 5H<sub>2</sub>O, Cu-lysine, Cu<sub>2</sub>(OH)<sub>3</sub>Cl, and Cu-methionine. Other RBV values were 0% for both analytical-grade and feed-grade CuO, 115% for analytical-grade Cu(OAc) · H<sub>2</sub>O, 100% for analytical-grade Cu<sub>2</sub>O, and 100% for analytical-grade CuCO<sub>3</sub> · Cu(OH)<sub>2</sub>. Among animal- and plant-source proteins, RBV values ranged from 0% for pork liver to 115% for chicken liver. Intermediate values were obtained for poultry by-product meal (90%), beef liver (80%), corn-gluten meal (50%), peanut hulls and soy mill run (45%), cottonseed meal and dehulled soybean meal (40%), and rat liver (20%; Aoyagi et al., 1993).

Copper ingested from fecal material is utilized no better than 30% relative to that provided as CuSO<sub>4</sub> · 5H<sub>2</sub>O (Izquierdo and Baker, 1986). Copper absorption from the gut is reduced substantially if Na<sub>2</sub>S (Barber et al., 1961; Cromwell et al., 1978), roxarsone (Czarnecki and Baker, 1985; Edmonds and Baker, 1986), or reducing agents such as cysteine or ascorbic acid (Baker and Czarnecki-Maulden, 1987; Aoyagi and Baker, 1994) are included in the diet.



### Iodine

Little definitive work on iodine (I) availability has been conducted in any species. What does exist is primarily rat research in which bioavailability assessment was not the primary objective. Nonetheless, Miller (1980) estimated that (relative to NaI) the I in KI,  $\text{Ca}(\text{IO}_3)_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{KIO}_3$ , and  $\text{CuI}$  was 100% bioavailable. The I in ethylenediamine dihydriodide ( $\text{C}_2\text{H}_8\text{N}_2 \cdot 2\text{HI}$ ) is also considered to have an RBV of at least 100% (Miller and Ammerman, 1995). It seems also reasonable to assume that the I present in iodized salt is 100% available relative to NaI.

### Iron

Baby pigs are uniquely susceptible to Fe deficiency anemia because of their rapid growth rate, confinement rearing (little access to soil), and lack of placental or mammary Fe transfer from dam to offspring. Thus, it is standard practice to give newborn pigs Fe injections during the first few days of life. Over 90% of the Fe from Fe-dextran (100- or 200-mg injections) is incorporated into hemoglobin (Braude et al., 1962; Miller, 1980). Fe-dextran doses administered orally during the first 12 hours after birth (prior to gut closure) also promote efficient Fe incorporation into hemoglobin (Harmon et al., 1974; Thoren-Tolling, 1975; Cornelius and Harmon, 1976).

There is no effective means of increasing placental or mammary transfer of Fe from the sow to her offspring. Work at Cornell University indicated that whether Fe sources are administered to dams orally or via injection, neither pig Fe stores at birth nor Fe concentration in milk is increased sufficiently to prevent anemia in the offspring (Pond et al., 1961). Iron sources fed to lactating sows at high levels will elicit hemoglobin responses in the nursing pigs, but this has been shown to be more due to Fe consumption from the Fe in the dam's feces than to an increased concentration of Fe in the dam's milk.

Pig studies on Fe bioavailability have revealed essentially 100% relative bioavailability of Fe in  $\text{FeSO}_4 \cdot 2\text{H}_2\text{O}$ ,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , Fe citrate, and Fe choline citrate relative to Fe in  $\text{FeSO}_4 \cdot \text{H}_2\text{O}$ . The chick and rat data by Fritz et al. (1970) have largely confirmed these findings (Harmon et al., 1967; Ullrey et al., 1973; Furugouri and Kawabata, 1975). Chick and rat data indicate that Fe ammonium citrate,  $\text{FeCl}_2$ , Fe fumarate, and Fe gluconate are also 100% bioavailable, whereas  $\text{FeCl}_3$ ,  $\text{Fe}_2(\text{SO}_4)_3$ , and Fe carbonate are less available. Iron in oxides of Fe is almost totally unavailable, whereas Fe in carbonates is variable in bioavailability, depending on where the carbonates are mined (Harmon et al., 1969; Henry and Miller, 1995).

Commercial dicalcium and defluorinated phosphates are rich in Fe, containing 2.5–3.0%  $\text{FePO}_4 \cdot 2\text{H}_2\text{O}$  (Baker, 1989). Bioavailability of Fe in these products generally ranges from 35% to 85% (Ammerman and Miller, 1972; Kornegay, 1972; Deming and Czarnecki-Maulden, 1989).

In human nutrition, Fe sources are usually categorized as heme or nonheme in nature. However, this categorization often leads to misinterpretation, because some nutritionists unwittingly conclude that all animal sources of Fe are heme Fe, when, in fact, they are generally a mixture of heme (highly available), ferritin (less available), and hemosiderin (poorly available; Layrisse et al., 1975; Bogunjoko et al., 1983). To illustrate the confusion, liver (fresh or dried) is rich in Fe, but the Fe present therein is about 10% heme Fe and 90% nonheme Fe. Thus, liver Fe is lower in bioavailability than the Fe in poultry by-product meal or meat meal (Chausow, 1987). While Fe in commercial blood meal would be expected to be highly available, values ranging from 40% to 50% (relative to  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) were provided by Miller (1980) for flash-dried

blood meal, and only 22% was reported by Chausow and Czarnecki-Maulden (1988a). Therefore, it is probable that the drying process influences the bioavailability of Fe in dried-blood meals.

The Fe in grains and oilseed meals may be largely bound to or complexed with phytate, fiber, or protein. As such, its bioavailability would be expected to be lower than that of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ . Data on availability of Fe in these sources are limited. Without definitive data, one should assume that cereal grain and oilseed Fe sources are no more than 50% available, relative to  $\text{FeSO}_4 \cdot \text{H}_2\text{O}$ .

Chausow (1987) and Chausow and Czarnecki-Maulden (1988a,b) evaluated the utilization of several Fe sources (relative to  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) in chicks, cats, and dogs. Based on their chick bioavailability results where hemoglobin repletion of Fe-depleted chicks was regressed on Fe intake, Fe bioavailability was 22% in dried-blood meal, 48% in meat-and-bone meal, 68% in poultry by-product meal, 39% in feather meal, and 32% in fish meal. Among plant-source feed ingredients evaluated, Fe relative bioavailabilities were 96% for sesame meal, 77% for rice bran, 65% for alfalfa meal, 45% for dehulled soybean meal, and 20% for yellow corn (Chausow and Czarnecki-Maulden, 1988a,b).

Boling et al. (1998) used a hemoglobin depletion-repletion assay in chicks to estimate the bioavailability of several Fe sources, relative to either analytical-grade  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  or feed-grade  $\text{FeSO}_4 \cdot \text{H}_2\text{O}$ . The Fe in analytical-grade ferric sulfate,  $\text{Fe}_2(\text{SO}_4)_3 \cdot 7\text{H}_2\text{O}$ , was only 37% bioavailable and that in cottonseed meal was 56% available. Two by-products of the galvanizing industry, mixtures of  $\text{FeSO}_4 \cdot \text{H}_2\text{O}$  and  $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ , were also evaluated, and the Fe in these products was as bioavailable as that in the ferrous sulfate standard.

The true absorption efficiency of Fe by humans consuming a mixed animal- and plant-based diet is assumed to be about 15% (Groff et al., 1995), but many factors can affect the efficiency of Fe absorption. It is well established that dietary ascorbic acid, cysteine, and organic acids such as citrate or lactate can almost double the efficiency of Fe absorption. On the other hand, dietary phytate, oxalates, and excess dietary Zn (in the presence of phytate) decrease the absorption of Fe to less than half that occurring without these antagonizing factors. Also, absorption efficiency is much greater when consumed by Fe-deficient animals than by Fe-adequate animals.

### Manganese

Manganese deficiency is a greater problem in diets fed to poultry than in diets fed to swine. The bioavailability of manganese has, therefore, been extensively studied using avian models (Southern and Baker, 1983a,b; Black et al., 1984, 1985; Henry, 1995; Baker et al., 1986; Halpin and Baker, 1986a,b; Halpin et al., 1986; Halpin and Baker, 1987). Tissues (primarily bone) accumulate Mn linearly, and this fact can be used as a basis for assessing Mn bioavailability. Relative to  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , Mn bioavailability is approximately 100% in  $\text{MnCl}_2$ , 75% in  $\text{MnO}$ , 55% in  $\text{MnCO}_3$ , and 30% in  $\text{MnO}_2$  (Henry, 1995). Availability of Mn in a protein-Mn or a methionine-Mn complex is similar to that in  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  (Baker et al., 1986; Fly et al., 1989). The Mn in corn, soybean meal, wheat bran, and fish meal is considered totally unavailable for both poultry and swine (Baker et al., 1986), and the considerable Mn present in rice bran is only minimally bioavailable. In practice, excesses of either Fe or cobalt in corn-soybean meal diets have minimal effects on Mn utilization, although excesses of Mn reduce Fe absorption from the gut. Excess dietary P (inorganic or phytate) reduces Mn utilization substantially (Baker and Wedekind, 1988; Wedekind and Baker, 1990a,b; Wedekind et al., 1991a,b; Baker and Oduho, 1994).



Even with a highly available source of Mn such as  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , gut absorption of Mn by chicks is only about 2–3% of the ingested dose (Wedekind et al., 1991a,b). Elimination of all fiber and phytate from the diet nearly doubles the absorption efficiency of Mn (Halpin et al., 1986).

### Selenium

Selenium in  $\text{Na}_2\text{SeO}_3$  and  $\text{Na}_2\text{SeO}_4$  is well utilized, and, based on Se accumulation in tissues, it has been reported that Se in selenomethionine is more than 100% bioavailable relative to Se in  $\text{Na}_2\text{SeO}_3$  (Mathias et al., 1967; Cantor et al., 1975a,b; Mahan and Moxon, 1978). Selenium seems to be well utilized from cereal grains (60–80%) and extremely well utilized from alfalfa meal (> 100%), but utilization is poor from fish and poultry by-product meals (10–40%). Poultry data have provided Se bioavailability estimates in soybean meal of only 18% for restoration of glutathione peroxidase activity but 60% for prevention of exudative diathesis (Cantor et al., 1975b). The bioavailability of Se in animal-derived feed ingredients fed to chicks averages 28% relative to  $\text{Na}_2\text{SeO}_3$ , whereas plant-derived feed ingredients have Se bioavailability of 47% (Wedekind et al., 1998). Selenium-enriched yeast has a bioavailability of 159% relative to  $\text{Na}_2\text{SeO}_3$ . Mahan and Parrett (1996) also compared Se-enriched yeast to  $\text{Na}_2\text{SeO}_3$  as Se sources for grower–finisher pigs. Based on Se retention in the body, the Se in Se-enriched yeast was more bioavailable than the Se in  $\text{Na}_2\text{SeO}_3$ , but the reverse was true when serum GSH was used as the criterion for bioavailability.

Selenium absorption from the gut is relatively efficient and 63% of an administered dose was absorbed by pigs (Wright and Bell, 1966). A variety of arsenic compounds as well as cysteine, methionine, copper, tungsten, mercury, cadmium, and silver have been reported to decrease the efficiency of inorganic Se absorption from the gut (Baker and Czarnecki-Maulden, 1987; Lowry and Baker, 1989a).

### Zinc

True absorption of Zn from a mixed diet consumed by humans is considered to be about 20% (Groff et al., 1995). This estimate presumes an absorption efficiency of less than 10% for the Zn in plant-based foods, but an absorption efficiency of 30% in animal-based food products. The Zn in edible meat products such as pork loin and hamburger is efficiently absorbed (Hortin et al., 1991; 1993), and it is thought that cysteine (and cysteine present as glutathione) present in meat products is responsible for the efficient absorption of Zn.

Little swine data exist on the relative bioavailability of Zn in Zn-containing supplements. Miller et al. (1981) reported that the bioavailability of Zn in Zn dust (99.3% Zn) was high for pigs relative to analytical-grade ZnO. Feed-grade ZnO for pigs, however, has a bioavailability of only 56–68% (Hahn and Baker, 1993; Wedekind et al., 1994), relative to that in a feed-grade  $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$  standard.

Many of the same factors that affect the efficiency of Fe utilization also apply to Zn. Thus, low intakes of Zn and dietary-reducing agents, such as ascorbic acid and cysteine, increase Zn absorption, whereas high-Zn intakes and the presence of phytate or oxalate in the diet decrease Zn absorption. Stress or trauma or both (e.g., surgery, burns) also decrease the efficiency of Zn absorption in humans (Groff et al., 1995).

Because high levels (2,000–3,000 mg Zn/kg) of feed-grade ZnO are now routinely used in the United States for growth promotion in weanling pigs (Hahn and Baker, 1993; Hill et al., 2000), the issue of bioavailability of Zn in ZnO products has become more important. Early chick work on Zn

bioavailability in ZnO indicated that the Zn in reagent-grade ZnO was as bioavailable as Zn in a reagent-grade  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  standard (Edwards, 1959). However, in chickens it was indicated that the bioavailability in feed-grade ZnO, the principal Zn source used in the feed industry, is about 50% relative to feed-grade  $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$  (Wedekind and Baker, 1990c; Wedekind et al., 1992), but there is great variability in the bioavailability of Zn among different sources of feed-grade ZnO (Edwards and Baker, 1999).

Based on the review by Baker and Ammerman (1995b) it may be concluded that the Zn in  $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ ,  $\text{ZnCO}_3$ ,  $\text{ZnCl}_2$ , analytical-grade ZnO, Zn methionine, and Zn acetate is highly bioavailable relative to analytical-grade  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , and all of these sources may, therefore, supply Zn in diets fed to swine. Weight gain and bone Zn accumulation of animals fed Zn-deficient diets are the best measures of bioavailability of Zn (Wedekind et al., 1992). Soft-tissue Zn, plasma Zn, and plasma alkaline phosphatase activity generally give poor fits when regressed against supplemental Zn intake.

Zinc, like many other trace elements, is poorly utilized by nonruminant animals fed conventional corn–soybean meal diets. Indeed, the dietary requirement for Zn is three to four times greater in animals fed these diets than in those fed phytate-free (e.g., egg white) diets. Also, in the presence of phytate and fiber, excess Ca decreases Zn utilization. Whereas excess Zn can exacerbate Cu and Fe deficiency, excesses of either Cu or Fe have minimal effects on Zn utilization (Southern and Baker, 1983c; Bafundo et al., 1984a).

The Zn in soy products is poorly utilized (Edwards and Baker, 2000). Soybean meal, soy-protein concentrate, and soy-protein isolate have Zn bioavailabilities (relative to  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ) of 34%, 18%, and 25%, respectively. Utilization of Zn is greater in animal-source ingredients than in plant-source ingredients, but some animal-source products may contain factors that antagonize Zn utilization (Baker and Halpin, 1988).

### Chromium

Chromium became of interest in swine nutrition when Page et al. (1993) reported that Cr tripicolinate supplementation increased carcass merit in finishing pigs. Subsequently, Lindemann et al. (1995, 2004) observed that Cr addition to gestation diets may increase litter size. Relative to Cr tripicolinate, the bioavailability of Cr in Cr propionate, Cr methionine, and Cr yeast is 13.1%, 50.5%, and 22.8%, respectively, in the growing pig (Lindemann et al., 2008).

### Vitamin Bioavailability

There are two primary concerns regarding vitamin bioavailability in modern swine diets and premixes: (1) stability in vitamin and vitamin-mineral premixes, as well as in diets and supplements; and (2) utilization efficiency from plant- and animal-source feed ingredients. Readers are referred to the reviews of Wornick (1968), Zhuge and Klopfenstein (1986), Baker (1995), and Baker (2001) for details of factors affecting the stability of crystalline vitamins in diets and premixes. Regarding vitamin bioavailability in feed ingredients, a paucity of pig research data exist, and even considering chick and rat data, few feed ingredients have been evaluated.

There are many pitfalls in vitamin (and mineral) bioavailability assessment. Body stores often preclude developing a distinct deficiency during the course of a conventional growth trial. Even if a frank deficiency can be produced, one must deal with the vexing question of whether the



responding criterion (usually weight gain) increased because of the vitamin being supplied or perhaps because of increased diet intake that results from adding the unknown ingredient to an often less-than-voraciously palatable purified diet. Because water-soluble B vitamins respond better insofar as growth is concerned, they are, in many respects, easier to evaluate than fat-soluble vitamins.

Certain conclusions seem evident concerning the proper bioassay methodology for maximum efficacy and extrapolative value of results for assessing vitamin bioavailability: (1) pretest periods to obtain desired deficiency states are generally necessary, (2) activity of a key enzyme of which the vitamin is a component or cofactor is generally a less-desirable dependent variable than weight gain, (3) precursor materials (e.g., methionine for choline and tryptophan for niacin) must be carefully considered, and (4) use of specific vitamin inhibitors may assist in establishing the veracity of assessed bioavailability values.

### Vitamin A

Vitamin nomenclature policy (Anonymous, 1979) dictates that the term *vitamin A* be used for all B-ionone derivatives, other than pro-vitamin A carotenoids, exhibiting the biological activity of all-*trans* retinol (i.e., vitamin A alcohol or vitamin A<sub>1</sub>). Esters of all-*trans* retinol should be referred to as retinyl esters.

Vitamin A is present in animal tissues, whereas most plant materials contain only pro-vitamin A carotenoids, which must be split in the intestinal tract to form vitamin A. In blood, vitamin A is transported as retinol, but it is stored, primarily in the liver, as retinyl palmitate. Absorption efficiency of vitamin A is relatively constant over a wide range of doses, but higher doses of carotenoids are absorbed much less efficiently than lower doses (Erdman et al., 1988).

Vitamin A esters are more stable in feeds and premixes than retinol. The hydroxyl group, as well as the four double bonds on the retinol side chain, is subject to oxidative losses. Thus, esterification of vitamin A alcohol does not totally protect this vitamin from oxidative losses. Current commercial sources of vitamin A are generally "coated" esters (e.g., acetate or palmitate) that contain an added antioxidant such as ethoxyquin or butylated hydroxytoluene (BHT).

The water content of premixes and feedstuffs has a negative effect on vitamin A stability. Moisture causes vitamin A beadlets to soften and become more permeable to oxygen. Thus, both high humidity and presence of free-choline chloride (hygroscopic) enhance vitamin A destruction. Trace minerals also exacerbate vitamin A losses in premixes exposed to moisture. For maximum retention of vitamin A activity, premixes should be as moisture-free as possible and should be made to have a pH above 5. Low pH causes isomerization of all-*trans* vitamin A to less potent *cis* forms and also results in de-esterification of vitamin A esters to retinol (DeRitter, 1976). Likewise, heat processing, especially extrusion, can reduce vitamin A bioavailability (Baker, 2001).

Crystalline  $\beta$ -carotene is absorbed from the gut more efficiently than  $\beta$ -carotene existing in foods and feeds (Rao and Rao, 1970). Some of the  $\beta$ -carotene in foods is complexed with protein. Fiber components of feeds, especially pectins, reduce  $\beta$ -carotene absorption from the gut in chicks (Erdman et al., 1986).

Ullrey (1972) reviewed the bioavailability aspects of vitamin A precursor materials for swine and reported that pigs were far less efficient than rats in converting carotenoid precursors to active vitamin A. Thus, bioefficiencies (wt/wt) ranging from 7% to 14% were observed for corn carotenes in pigs relative to all-*trans* retinyl palmitate. Thus, at best, carotenoid precursors in corn (also corn gluten meal) have no more than 261 IU/mg vitamin A activity when consumed by swine.

This is decidedly less than the theoretical potency of 1,667 IU/mg (assuming all the carotenoids are all-*trans*- $\beta$  carotene), which is assumed for the rat. Corn carotenoids consist of about 50% cryptoxanthin, 25%  $\beta$ -zeacarotene, and 25%  $\beta$ -carotene (Ullrey, 1972). Quantification of vitamin A bioavailability is difficult. Accumulation of vitamin A in the liver may be the most acceptable method (Erdman et al., 1988; Chung et al., 1990).

### Vitamin D

The term *vitamin D* is appropriate for all steroids having cholecalciferol biological activity. Cholecalciferol itself is synonymous with vitamin D<sub>3</sub>, as distinguished from ergocalciferol, which is also called vitamin D<sub>2</sub>. Commercially, vitamin D<sub>3</sub> is available as a spray-dried product or (frequently in combination with vitamin A) as gelatin-coated beadlets; one international unit is equal to 0.025  $\mu$ g of cholecalciferol (Anonymous, 1979). These products are quite stable if stored as the vitamin itself at room temperature. In complete feeds and mineral-vitamin premixes, vitamin D activity losses of up to 20% can occur after four to six months of storage at room temperature (Baker, 2001).

Vitamin D precursors are present in plant (ergosterol) and animal (7-dehydrocholesterol) feedstuffs, but they require ultraviolet irradiation for conversion into active D<sub>2</sub> and D<sub>3</sub>, respectively. Although D<sub>2</sub> and D<sub>3</sub> have long been considered equal in biological activity for pigs, observations by Horst et al. (1982) indicate that D<sub>3</sub> may be more bioactive than D<sub>2</sub>. Hydroxylated forms of cholecalciferol [25-OH D<sub>3</sub>, 1 $\alpha$ -OH D<sub>3</sub>, 1,25(OH)<sub>2</sub> D<sub>3</sub>], particularly 1 $\alpha$ -hydroxylated products, contain more D<sub>3</sub> bioactivity than D<sub>3</sub> itself.

### Vitamin E

Vitamin E is the generic term for all tocol and tocotrienol derivatives having  $\alpha$ -tocopherol biological activity. There are eight naturally occurring forms of vitamin E:  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols and  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocotrienols. Among these, D- $\alpha$ -tocopherol possesses the greatest biological activity (Bieri and McKenna, 1981). An international unit of vitamin E is the activity of 1 mg of DL- $\alpha$ -tocopheryl acetate. All racemic (i.e., DL- $\alpha$ -tocopherol) has about 70% of the activity of pure  $\alpha$ -tocopherol. Bieri and McKenna (1981) consider  $\beta$ -tocopherol and  $\gamma$ -tocopherol to have only 40% and 10% of the activity, respectively, of  $\alpha$ -tocopherol. The only other natural form to possess activity is  $\alpha$ -tocotrienol, which, on the rating scale used previously, was listed by Bieri and McKenna (1981) as containing a biopotency of 25%.

Plant-source ingredients are richer in vitamin E bioactivity than animal-source feed ingredients. Plant oils are particularly rich in bioactive vitamin E, although corn and corn oil contain about six times more  $\gamma$ -tocol than  $\alpha$ -tocol (Ullrey, 1981). Fat-extracted soybean meal has very little vitamin E activity.

Vitamin E is subject to destruction by oxidation, and this process is accelerated by heat, moisture, unsaturated fat, and trace minerals. Losses of 50–70% may occur in alfalfa hay stored at 32°C for 12 weeks; losses up to 30% may occur during dehydration of alfalfa (Livingston et al., 1968). Treatment of high-moisture grains with organic acids also greatly enhances vitamin E destruction (Young et al., 1975, 1977, 1978). However, even mildly alkaline conditions of vitamin E storage are very detrimental to vitamin E stability. Thus, finely ground limestone or MgO coming in direct contact with vitamin E can markedly reduce its bioavailability.



### Vitamin K

Vitamin K is also a fat-soluble vitamin and exists in three series: phyloquinones ( $K_1$ ) in plants, menaquinones ( $K_2$ ) formed by microbial fermentation, and menadiones ( $K_3$ ), which are synthetic. All three forms of vitamin K are biologically active. Only water-soluble forms of menadione are used to supplement swine diets. The commercially available forms of  $K_3$  supplements are menadione sodium bisulfite (MSB), menadione sodium bisulfate complex (MSBC), and menadione dimethyl pyrimidinol bisulfite (MPB). These forms contain 52%, 33%, and 45.5% menadione, respectively. Stability of these  $K_3$  supplements in premixes and diets is impaired by moisture, choline chloride, trace elements, and alkaline conditions, and MSBC and MPB can lose almost 80% of bioactivity if stored for three months in a vitamin-trace mineral premix containing choline, but losses are far less if stored in a similar premix containing no choline (Baker, 2001). Coated  $K_3$  supplements are generally more stable than uncoated supplements. Bioactivity of MPB is greater than either MSB or MSBC for chicks (Griminger, 1965; Charles and Huston, 1972). Seerley et al. (1976) also observed that MPB is effective for swine. Oduho et al. (1993) compared menadione nicotinamide bisulfite (MNB; 45.7% menadione, 32% nicotinamide) to MPB as a source of vitamin K activity for young chicks. Based on prothrombin time, MNB was reported to be equal to MPB in vitamin K activity. Although certain feed ingredients are rich in vitamin K activity for swine (e.g., alfalfa meal; Fritschen et al., 1971), little quantitative information exists on the bioavailability of vitamin K in swine feedstuffs.

### Biotin

Commercial D-biotin has no specific unit of activity. Thus, 1 g of D-biotin equals 1 g of activity. Pelleting, or heat, has little effect on biotin activity in feeds, but oxidative rancidity severely reduces biotin bioavailability. Much of the biotin in feed ingredients exists in a bound form,  $\epsilon$ -N-biotinyl-L-lysine (biocytin), which is a component of protein. Crystalline biotin is absorbed well from the small intestine, but the bioavailability of biotin in biocytin varies widely and is dependent on the digestibility of the proteins that are found (Baker, 1995). Avidin, a glycoprotein in egg albumen, binds biotin and makes it totally unavailable. Proper heat treatment of egg white will denature avidin and prevent it from binding biotin. Based on bioassay results using biotin-depleted chicks, it is apparent that among the cereal grains, bioavailability of biotin in corn is high (> 100%), whereas in wheat, barley, and sorghum bioavailability is about 50% (Anderson and Warnick, 1970; Frigg, 1976; Anderson et al., 1978). Bioavailable biotin concentrations of 0.11 mg/kg in corn, 0.08 mg/kg in barley, 0.09 mg/kg in sorghum, and 0.04 mg/kg in wheat were estimated by Anderson et al. (1978). Feedstuff ingredient tables generally list the biotin concentration in soybean meal as 0.30 mg/kg. Buenrostro and Kratzer (1984) reported that biotin is 100% available in soybean meal and 86% available in meat-and-bone meal for laying hens. Hence, with considerable bioavailable biotin present in corn-soybean meal diets, grower-finisher pigs fed such diets have generally not responded to supplemental biotin. With sows, Bryant et al. (1985) provided evidence that under some conditions supplemental biotin increased conception rate, decreased the weaning-to-estrus interval, and improved both foot health and hair coat, particularly in advanced parities. Lewis et al. (1991) reported that addition of 0.33 mg biotin/kg to a corn-soybean meal diet throughout gestation and lactation increased the number of pigs weaned, but did not improve foot health. In a similar study, however, Watkins et al. (1991) observed no benefit from adding 0.44 mg biotin/kg to a corn-soybean meal diet.

Biotin may be the only vitamin for which a good test exists to assess the veracity of the bioavailability assay. Thus, growth responses to ingredients added to a biotin-free purified diet can be measured in the presence and absence of crystalline avidin. Thus, growth rate of biotin-depleted chicks is doubled by supplementation with 20% corn, but it is not significantly increased when the same quantity of corn is fed in the presence of 3.81 mg avidin/kg diet (Anderson et al., 1978). Similar results occurred when barley was evaluated (Anderson et al., 1978). Therefore, these results provide convincing evidence that the growth responses observed from cereal-grain supplementation of the biotin-free purified diet result from the available biotin per se furnished by the grains.

### Choline

In animal nutrition, choline remains in the B-vitamin category, even though the quantity required far exceeds the "trace organic nutrient" definition of a vitamin. Choline is absorbed primarily in the small intestine and is required by the body for: (1) phospholipid synthesis, (2) acetylcholine formation, and (3) transmethylation of homocysteine to methionine. When a choline deficit is produced experimentally by feeding a choline-free diet to chicks, phospholipid synthesis or acetylcholine formation or both seem to have priority over transmethylation of homocysteine to methionine, in that betaine (the methylated product of choline oxidation) does not elicit a growth response, whereas choline does. When about one-half to two-thirds of the dietary choline needed for maximal growth is supplied as choline, as in practical diets, then synthetic choline and betaine are equally efficacious (Lowry et al., 1987; Dilger et al., 2007).

In mammalian, but not in avian species, the dietary need for choline can be replaced by excess methionine. In crystalline form, choline chloride (74.6% choline) is hygroscopic, and, therefore, it is considered a stress agent to other vitamins in a vitamin-mineral premix; choline is usually supplied via a separate premix and not via the general vitamin-mineral premix. Crude plant oils (e.g., corn and soybean oil) contain choline as phospholipid-bound phosphatidyl choline. The bioavailability of choline in this form is at least 100% (Emmert et al., 1996). Refined plant oils generally have been subjected to alkaline treatment and "bleaching," and these processes almost totally remove phospholipids, including phospholipid-bound choline.

Choline bioavailability (relative to crystalline choline chloride) in oilseed meals for chicks has been estimated at 83% in soybean meal (Molitoris and Baker, 1976a; Emmert and Baker, 1997), 76% in peanut meal, and only 24% in canola meal (Emmert and Baker, 1997). Also in chicks, excess dietary protein increases the dietary requirement for choline (Molitoris and Baker, 1976b). Minimizing liver lipid content may require a greater level of dietary choline than that required to maximize rate and efficiency of weight gain (Anderson et al., 1979).

As with niacin, for which tryptophan serves as a precursor, choline bioavailability assessment is difficult, if not impossible, in pigs, because all common feed ingredients supply both choline and methionine. Therefore, it is difficult to separate responses from one another, although use of the transmethylation inhibitor ethionine or the inhibitor of methylation of aminoethanol by methionine in choline biosynthesis (i.e., 2-amino-2-methyl-1-propanol) might prove useful in this endeavor (Molitoris and Baker, 1976a; Anderson et al., 1979; Lowry et al., 1987).

Corn-soybean meal diets for growing and finishing pigs often do not respond to choline supplementation, probably because soybean meal is so rich in choline content (NCR-42, 1980). Swine pregnancy, however, benefits from choline addition to corn-soybean meal diets (Kornegay and Meacham, 1973; Stockland and Blaylock, 1974; NCR-42, 1976). Failure of corn-soybean meal



for swine and poultry grower diets to respond to vitamin supplementation is not unique to choline (among those generally supplemented). Unpublished work from the University of Illinois has shown that these diets also fail to respond consistently to either nicotinic acid or pantothenic acid. Choline, nicotinic acid, and pantothenic acid should nonetheless be included in vitamin mixtures for swine to provide a margin of safety against environmental and stress conditions that might manifest in a swine-production operation.

### Folacin

The term folacin is the accepted generic term for folic acid and related compounds exhibiting folacin activity. More than 150 forms of folacin exist in foods. Chemically, folic acid consists of a pteridine ring, *para*-aminobenzoic acid (PABA), and glutamic acid. Animal cells cannot synthesize PABA or attach glutamic acid to pteric acid (i.e., pteridine attached to PABA). Thus, folic acid must be supplied in the diet of nonruminant animals. The folacin present in feeds and foods exists largely as polyglutamates. In plants, folacin exists as a polyglutamate conjugate containing a  $\gamma$ -linked polypeptide chain of (primarily) seven glutamic acid residues. Intestinal proteases do not cleave the glutamate residues from this compound. Instead, a group of intestinal enzymes known as conjugases (folyl polyglutamate hydrolases) remove all but the last glutamate residue. Only the monoglutamyl form is thought to be absorbed into the enterocyte. Most of the folic acid taken up by the brush border is reduced to tetrahydrofolate ( $FH_4$ ) and then methylated to  $N^5$ -methyl- $FH_4$ , the predominant form of folate in blood plasma. The majority of the  $N^5$ -methyl- $FH_4$  in plasma is bound to protein.

Like thiamin, folic acid has a free amino group (on the pteridine ring), and this makes it very sensitive to losses in activity due to heat treatment, particularly if heat is applied to foods or feeds containing reducing sugars, such as lactose or glucose. Whether the free amino group of folacin (or thiamin) can bind to the free aldehyde moiety of pyridoxal or pyridoxalphosphate is not known. Intestinal conjugase inhibitors may be present in certain beans and pulses, and these may impede folacin absorption (Krumdieck et al., 1973; Bailey, 1988). Storage of feeds and premixes results in loss of folacin activity (Verbeeck, 1975).

Growing pigs fed conventional corn-soybean meal diets generally do not respond to folacin supplementation. Hence, it is not generally provided at supplemental levels in such diets (Easter et al., 1983). For gestating-lactating sows, however, improvements in reproductive performance as a result of folacin supplementation have been reported (Lindemann and Kornegay, 1989; Matte et al., 1992), whereas other experiments resulted in no response (Pharazyn and Aherne, 1987; Easter et al., 1983; Harper et al., 1994).

### Niacin

The term *niacin* is the generic descriptive term for pyridine 3-carboxylic acid and derivatives delivering nicotinamide activity. Thus, pyridine 3-carboxylic acid per se is properly referred to as nicotinic acid (Anonymous, 1979). Niacin is a very stable vitamin when added to feed or premixes, being little affected by heat, oxygen, moisture, or light. In plant-source feed ingredients, much of the niacin activity, mostly nicotinamide nucleotides, is bound and, therefore, unavailable (Yen et al., 1977). Ghosh et al. (1963) estimated that 85–90% of the niacin activity in cereal grains and 40% in oilseeds is in a bound and unavailable form. Alkaline hydrolysis is the only means by which niacin

can be efficiently released from its bound state in these ingredients. Meat and milk products, on the other hand, contain no bound niacin, but instead contain free nicotinic acid and nicotinamide.

There is no good way to assess the bioavailability of niacin, per se, because excess tryptophan is converted to nicotinic acid and all common feed ingredients contain tryptophan, as well as nicotinic acid. Thus, 50 mg of tryptophan yields 1 mg of nicotinic acid (Baker et al., 1973; Czarnecki et al., 1983). Does excess leucine in corn-soybean meal swine diets antagonize tryptophan or nicotinic acid, or does it impair the metabolic conversion of tryptophan to nicotinic acid? This subject is controversial (Anonymous, 1986) and data exist to support both views. Data from chick studies indicate that excess leucine has no effect on either tryptophan conversion to niacin or niacin bioavailability (Lowry and Baker, 1989b). Iron, on the other hand, is required in two metabolic reactions in the pathway of tryptophan to nicotinate mononucleotide. Oduho et al. (1994) established that Fe deficiency in chicks will reduce the conversion efficiency of tryptophan to niacin (i.e., from 42:1 to 56:1, weight:weight).

Niacin activity can be purchased as either free nicotinic acid or free nicotinamide. Niacin bioavailability in nicotinamide is roughly 120% as bioavailable, as in nicotinic acid (Baker et al., 1976; Oduho and Baker, 1993). However, it also has been suggested that niacin and nicotinamide are equal in biopotency for chicks (Bao-Ji and Combs, 1986; Ruiz and Harms, 1988).

### Pantothenic Acid

Pantothenic acid is generally sold as either D- or DL-Ca pantothenate, and only the D-isomer has bioactivity (Staten et al., 1980). Thus, 1 g of D-Ca pantothenate equals 0.92 g pantothenic acid (PA) activity, and 1 g DL-Ca pantothenate equals 0.46 g of PA activity. Crystalline PA is relatively stable when exposed to heat, oxygen, and light, but it can rapidly lose activity when exposed to moisture.

Feed ingredients contain PA in the form of coenzyme A, and in this form it may not be fully available for intestinal absorption. Chick bioassay work indicates that PA in corn and soybean meal is 100% bioavailable, whereas PA in barley, wheat, and sorghum is about 60% bioavailable (Southern and Baker, 1981). Processed feed ingredients may exhibit losses in PA bioavailability, although definitive animal data are lacking on this subject. The PA in a typical diet for adult Americans is only 50% bioavailable and processing (freezing, canning, refining, etc.) may decrease bioavailability further (Saubertlich, 1985).

### Riboflavin

Riboflavin is relatively labile, being reduced in bioactivity by light, alkali, and oxygen. In feedstuffs, it exists primarily as nucleotide coenzymes, in which form the bioavailability is probably less than 100%. Chung and Baker (1990) estimated that riboflavin bioavailability in a corn-soybean meal diet is 60% for chicks, relative to crystalline riboflavin. Zhuge and Klopenstein (1986) reported that crystalline riboflavin loses activity in vitamin-mineral premixes over time and that high-temperature storage enhances the loss.

Saubertlich (1985) suggested that several factors may reduce the bioavailability of riboflavin in foods. Among the suggested factors antagonizing riboflavin were excess dietary levels of tetracycline, Fe, Zn, Cu, ascorbate, and caffeine. Patel and Baker (1996) used chick growth bioassays to evaluate dietary excesses of Fe (420 mg/kg), Zn (448 mg/kg), Cu (245 mg/kg), ascorbic acid



(1000 mg/kg), caffeine (200 mg/kg), or chlortetracycline (500 mg/kg), which were added to riboflavin-deficient soy-isolate semipurified diets. None of these supplements decreased the utilization of crystalline riboflavin.

### Thiamin

Thiamin is available to the food and feed industries as thiamin · HCl (89% thiamin) or thiamin · NO<sub>3</sub> (92% thiamin). These compounds are stable up to 100°C and are readily soluble in water (NRC, 1987). An international unit of thiamin activity is equivalent to 3 µg of crystalline thiamin · HCl. Because thiamin contains a free amino group, heat processing can rapidly destroy thiamin bioactivity via the Maillard reaction. Similarly, any processing procedure that involves alkaline treatment leads to loss of thiamin activity. The thiamin contained in swine feed ingredients is present largely in phosphorylated forms, either as protein-phosphate complexes or as thiamin mono-, di-, or triphosphates. Some raw ingredients (e.g., fish) contain thiaminase, which can destroy thiamin in diets to which it may be added. Although thiaminase is of particular concern in the nutrition of cats and fur-bearing animals, it is of little consequence in modern swine feeding. Thiamin in fish meal is lost to the fish solubles fraction when fish meal is produced. Thus, fish meal contains essentially no bioavailable thiamin. Similarly, as a result of the high-temperature processing, meat meals contain very little bioavailable thiamin activity.

Pelleting likely also results in some loss of thiamin activity. Retention of thiamin activity was 48% and 95%, respectively, when stored in the form of the HCl and NO<sub>3</sub> in a premix for 21 days at 40°C and 85% relative humidity (Baker, 2001). In a complete feed stored under similar conditions, thiamin · HCl retained only 21% of its activity, whereas thiamin · NO<sub>3</sub> retained 97% of its activity. Thus, the mononitrate form of thiamin would seem to be the more stable form when storage in hot environments is anticipated. Grains and soybean meal are sufficiently rich in thiamin that, even with considerable losses of bioactivity due to heat or lengthy storage, seldom would there be a case where practical diets for swine would respond to supplementation with thiamin.

### Vitamin B<sub>6</sub>

Vitamin B<sub>6</sub> is not generally added in supplemental crystalline form to practical diets for swine because both corn and soybean meal are plentiful in this B vitamin. Work at the University of Illinois indicates that vitamin B<sub>6</sub> is about 40% bioavailable in corn and about 60% bioavailable in soybean meal (Yen et al., 1976). Moderate heat treatment (80°C–120°C) of corn seems to enhance B<sub>6</sub> bioavailability, whereas greater heat treatment (160°C) decreases availability. Most of the vitamin B<sub>6</sub> activity in corn exists as pyridoxal and pyridoxamine forms that are more heat-labile than is pyridoxine (Schroeder, 1971). Plant-source feedstuffs may contain B<sub>6</sub> as either pyridoxine glucoside or pyridoxalysine, and both of these compounds have minimal B<sub>6</sub> bioactivity (Gregory and Kirk, 1981; Trumbo et al., 1988). Even with the reduced bioavailability of vitamin B<sub>6</sub> in corn and soybean meal, relative to crystalline pyridoxine · HCl, a surplus of available B<sub>6</sub> is usually present in practical diets for swine, thus precluding a response to supplemental vitamin B<sub>6</sub> if it is added to these diets.

In premixes, vitamin B<sub>6</sub> can lose bioactivity, particularly when minerals in the form of carbonates or oxides are present (Verbeeck, 1975). High temperatures enhance loss of activity. Retention of B<sub>6</sub> activity after three months of storage at room temperature is 76%, but only 45% after three months

of storage at 37°C (Baker, 2001). Loss of B<sub>6</sub> activity in stored, pelleted complete feeds averages about 20% during three months of storage at room temperature (Baker, 2001).

### Vitamin B<sub>12</sub>

Cyanocobalamin, or vitamin B<sub>12</sub>, is available in crystalline form, where 1 U.S. Pharmacopeia (USP) unit is considered equivalent to 1 µg of the vitamin. Vitamin B<sub>12</sub> is essentially devoid in plant-source feed ingredients, existing instead in animal-source proteins and fermentation products, where it is considered (but not proved) to be 100% available.

Both animal- and fermentation-based feedstuffs contain B<sub>12</sub> as methylcobalamin or adenosylcobalamin, which are bound to protein. As in humans, but unlike in sheep and in horses, an "intrinsic" factor is required for gut absorption of B<sub>12</sub> in swine. Crystalline vitamin B<sub>12</sub> is quite stable in feeds and premixes.

### Vitamin C

There is little concern about the bioavailability of vitamin C (ascorbic acid) because swine are capable of synthesizing this vitamin. Nonetheless, vitamin C is often included in vitamin premixes for use in purified swine diets because of its antioxidant and putative antistress properties. Considerable losses of vitamin C activity in stored diets can occur. Coating ascorbate with ethylcellulose minimized the loss of potency. Both pelleting and extruding can markedly reduce the bioactivity of supplemental ascorbate added to feeds or premixes (Baker, 2001). Losses due to oxidation are well known, as ascorbic acid (reduced form) can be reversibly oxidized to dehydroascorbic acid, which, in turn, can be further irreversibly oxidized to diketogulonic acid. Both reduced and oxidized forms of ascorbate retain scurvy-preventing ascorbate activity, but diketogulonic acid has no activity. Both ascorbate and dehydroascorbate are heat labile, particularly when heat is applied in the presence of trace minerals such as Cu, Fe, or Zn.

### Summary

There is much less information about the bioavailability of minerals and vitamins than for the energy-containing nutrients. For many minerals and vitamins, it is challenging to establish the bioavailability because of difficulties identifying appropriate response variables or depleting the animals sufficiently to obtain a desired response. Therefore, use of the correct methodology is important when determining bioavailability of vitamins and nutrients, and because of the uniqueness of each mineral and vitamin, different methodologies are required depending on the compound being investigated.

For the macro minerals Ca and P, bioavailability in pigs may be estimated using conventional digestibility measurements, and there is now strong evidence that there is no difference between values for ileal and total tract digestibility of these minerals. Therefore, the total tract digestibility procedures may be used to estimate the bioavailability of Ca and P. There is, however, no information about the effectiveness of using digestibility procedures to estimate the bioavailability of other minerals. Conventional procedures based on slope ratio methodology are usually used to measure bioavailability of most minerals and all the vitamins. When interpreting data from these experiments, it is important to take bioavailability standards into account.



It is also evident that for some compounds, such as Se, values for bioavailability depend on the response variable that is used, which further complicates evaluation of the data. Values for the bioavailability of most minerals are also influenced by the dietary concentrations of other minerals and sometimes by the concentration of anti-nutritional factors, such as phytate and oxalate, in the diet. Therefore, care should always be taken when considering data for bioavailability of minerals and vitamins.

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## 16 Swine Nutrition and Environment

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### Introduction

Swine production in supplying pork as a major red meat contributes greatly to human health, as well as economic and social activities. Recorded human civilization can be traced back to the early domestication of a small number of farm-animal species, which included pigs, and animal production continues to play vital roles in the evolution of human physical and mental health, as well as social and economic development (Diamond, 2002; Fan et al., 2008a). The development of industrialized, intensive swine production, driven primarily by fossil fuels and technologies from both applied and fundamental animal-biology research, has been widely adopted at the global level in the past two decades.

Nevertheless, current intensive swine production practices are faced with several emerging sustainability issues. First, the risk of volatile markets, low profit margins, and poor economic and social viability associated with swine producers in rural areas is rising (Fan et al., 2008a). This is primarily due to increasing feed prices resulting from the demand for more direct consumption of vegetal food by the ever-increasing human population, extreme weather patterns affecting crop yields, and the increasing use of grains and oilseeds for producing biofuels in developed countries. Second, although increased consumption of animal products has improved the quality and longevity of human lives, this has also brought about some major health-management concerns such as obesity, cardiovascular disease, type 2 diabetes, chronic inflammatory bowel disease, and colorectal cancer. In spite of differences in lifestyles, living environments, and family genetics, increased consumption of animal products, especially red meats such as pork, is frequently associated with these increasing health concerns (e.g., Diamond, 2002). Without truly understanding and addressing the biological mechanisms for the linkage of consumption of animal products to the development of these chronic diseases, these human health concerns may become a major hurdle to maintenance and expansion of intensive animal production.

Third, poor efficiency of nutrient utilization is associated with the promotion of dietary practices that are primarily aimed toward maximal growth rate and lean yield, which enhance the profit margins in swine production. These practices contribute to several major environmental concerns (NRC, 1998, 2012) including emissions of greenhouse gases of methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O; Mackie et al., 1998); emission of acidifying and odor-causing ammonia (NH<sub>3</sub>; Rideout et al., 2004); leaching of nitrate (NO<sub>3</sub><sup>-</sup>) originated from swine manure (Jayasundara et al., 2010), runoff of trivalent chromium (Cr<sup>3+</sup>; Blowes, 2002; Ellis et al., 2002); increased risk of food-chain and