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Review

Digestibility of calcium in feed ingredients and requirements of digestible calcium for growing pigs

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Abstract. Efforts to reduce phosphorus (P) excretion from pigs have increased during the past few decades and it has been recognised that interactions among dietary P, calcium (Ca), phytate, and microbial phytase exist. However, limited research has been reported on Ca digestibility, but to optimise the use of both Ca and P, digestibility values of Ca are needed. Due to endogenous losses of Ca, values for standardised total tract digestibility (STTD) of Ca in different Ca supplements and feed ingredients have been determined, and these values may be used to formulate mixed diets. Phytate may bind intrinsic Ca in feed ingredients of plant origin as well as extrinsic Ca from ingredients of animal origin or Ca supplements, but not all forms of Ca in Ca supplements will bind to phytate. Therefore, the effect of phytase on the STTD of Ca may vary depending on the amount of Ca bound to phytate and in some cases microbial phytase will result in increased STTD of Ca from animal proteins or Ca supplements. Dietary fibre may increase the STTD of Ca, but particle size and soybean oil do not influence the STTD of Ca. Requirements for digestible Ca by growing pigs has not yet been determined, but with the availability of values for the STTD of Ca in most commonly used feed ingredients, the basis for determining such values has been prepared. In conclusion, data for the STTD of Ca and the effects of microbial phytase in many feed ingredients have been determined and future research will be directed at determining the requirements for digestible Ca by different groups of pigs.

Additional keywords: microbial phytase, phytate, pig.

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Introduction

Concerns about phosphorus (P) scarcity and P pollution have increased during the past decades. The main concerns are that most of the P that is fed to livestock is from phosphate rock, which is a limited resource and expensive, and the P from manure is a potential pollutant in water and may cause eutrophication (Sims *et al*. 1998; Carpenter and Bennet 2011; FAO 2011). During the past decades, scientists have conducted research to identify solutions to optimise the use of P and also to reduce P excretion. As a result, one of the most common practices in the feeding of pigs and poultry is the inclusion of microbial phytase in the diet, which increases the digestibility of P (Akinmusire and Adeola 2009; Almeida and Stein 2010; Rodríguez *et al*. 2013) and may improve growth performance of pigs (Patience *et al*. 2015). However, results of some experiments indicate that the activity of microbial phytase is reduced as dietary calcium (Ca) concentration increases (Lei *et al*. 1994; Lantzsch *et al*. 1995; Brady *et al*. 2002; Selle *et al*. 2009). Excess dietary Ca also results in reduced digestibility of P (Clark 1969; Stein *et al*. 2011) and it is, therefore, important that the concentration of Ca in the diets does not exceed the requirement. However, Ca supplements such as limestone and Ca carbonate are inexpensive and because of a lack of data demonstrating the exact requirements for Ca, it is possible that Ca is sometimes included in excess of the requirement.

Values for the standardised total tract digestibility (STTD) of P in most ingredients used in swine diets have been determined in recent years and the requirements for STTD P by growing pigs and sows have been reported (NRC 2012). However, for Ca, only total Ca values in ingredients and total Ca requirements for growing pigs and sows are available, but because it has been demonstrated that endogenous losses of Ca exist in pigs (González-Vega *et al*. 2013), it will be more accurate to express requirements for dietary Ca as STTD Ca. As a consequence, using values for STTD of Ca and P in diet formulations will optimise the use of both Ca and P and will reduce the excretion of P. Therefore, the aim of this contribution is to review recent work from our laboratory on determination of digestibility of Ca in feed ingredients and data for the requirement of digestible Ca in diets fed to growing pigs.

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Endogenous losses of Ca

Digestibility of a nutrient can be equivalent to the availability of the nutrient if the nutrient that disappears in the gastrointestinal tract is absorbed and used by the animal (Ammerman 1995; Stein *et al*. 2007). Digestibility can be defined as apparent, standardised or true digestibility, and the difference among these expressions is related to the way the endogenous losses of the nutrients are considered in the calculations (Stein *et al*. 2007). Apparent digestibility values of a nutrient are not expected to always be additive in mixed diets because these values may vary with the concentration of the nutrient in the diet if there are endogenous losses of the nutrient. Specifically, if the digestibility is determined in an ingredient with a low concentration of that nutrient, and the ingredient is used in a mixed diet with a greater concentration of the nutrient, values for apparent digestibility obtained in the individual ingredients are not additive in the mixed diet (Stein *et al*. 2005; NRC 2012). However, values for standardised or true digestibility are additive in mixed diets because these values are corrected for endogenous losses, as has been demonstrated for amino acids (Stein *et al*. 2005). There are two types of endogenous losses, namely, basal endogenous losses, which are diet-independent, and specific endogenous losses, which are diet-dependent, and the total endogenous loss of a nutrient, therefore, is the sum of the basal and specific endogenous losses (Stein *et al*. 2007). If apparent digestibility values are corrected for the basal endogenous loss, the standardised digestibility is calculated, but if apparent digestibility values are corrected for total endogenous losses, the true digestibility is calculated. Values for standardised and true digestibility are expected to be additive in mixed diets because values are not influenced by the nutrient concentration in the ingredients included in the diet. It is, therefore, recommended that mixed diets be formulated using values for standardised or true digestibility (NRC 2012). Total endogenous losses can be determined by using the regression procedure (González-Vega *et al*. 2013), but to determine basal endogenous losses, a Ca-free diet may be used (González-Vega *et al*. 2014, 2015*a*, 2015*b*). Because it is easier to determine values for basal endogenous losses than values for total endogenous losses, it is usually more practical to formulate diets on the basis of standardised digestibility. Values for the basal endogenous loss of Ca may be determined after feeding a Ca-free diet and have been reported in the range from 123 mg per kg dry matter intake (González-Vega *et al*. 2015*a*) to 670 mg per kg dry matter intake (González-Vega *et al*. 2014) and there is evidence that diet composition may influence the basal endogenous loss of Ca (González-Vega *et al*. 2015*b*). Due to the high variability of basal endogenous loss of Ca among published experiments, it is suggested that a Ca-free diet is included in all experiments that aim at determining STTD of Ca. This approach is similar to the approach suggested for determining values for standardised ileal digestibility of amino acids (Stein *et al*. 2007).

Digestibility of Ca in feed ingredients

Due to the relatively low concentrations of Ca in feed ingredients of plant origin, Ca from animal proteins or inorganic sources of Ca, which have high concentrations of Ca, are usually added to commercial diets for pigs. For most feed ingredients, only the total concentration of Ca has been determined and, in the most recent version of 'Nutrient Requirements of Swine', no digestibility values for Ca were reported due to the lack of data (NRC 2012). However, recent work in our laboratory has focussed on determining the STTD of Ca in most commonly used sources of Ca (Table 1).

Dietary factors that may affect digestibility of Ca

Ca concentration

The apparent total tract digestibility (ATTD) of Ca is not affected by the concentration of dietary Ca if dietary Ca is between 50% and 150% of the requirement (Stein *et al*. 2011), but it is possible that concentrations of dietary Ca outside this range will affect ATTD of Ca. Indeed, if the concentration of dietary Ca is less than 50% of the requirement, the ATTD of Ca is reduced because the endogenous losses of Ca represent a greater proportion of the faecal Ca output (González-Vega *et al*. 2013). If dietary Ca is above the requirement, the ATTD of P will be reduced (Stein *et al*. 2011), which may result in a reduction of growth performance of pigs (González-Vega *et al*. 2015*c*).

Phytate

Phytate is naturally present in most plant ingredients and is a molecule that not only binds P but also may bind other minerals or nutrients. Thus, the digestibility of Ca and P may be limited because pigs do not secrete phytase, which releases the Ca and P that are bound to phytate. However, addition of microbial phytase to the diet may increase the ATTD and STTD of Ca not only in plant ingredients, but also in animal proteins and in Ca supplements (González-Vega *et al*. 2013, 2015*a*, 2015*b*). This latter observation indicates that the phytate in plant ingredients binds not only the intrinsic Ca in the ingredient, but also some of the Ca from other ingredients in the diet. However, not all forms of Ca are bound to phytate and Ca in monocalcium phosphate and dicalcium phosphate is less likely to be bound to phytate than is Ca from calcium carbonate (González-Vega *et al*. 2015*a*). Responses to microbial phytase on the digestibility of Ca are, therefore, variable among feed ingredients, depending on how much of the Ca in that ingredient is bound to phytate, which in turn is affected by the total concentration of phytate in the diet.

Phytase

Phytase is the enzyme needed to hydrolyse the bond between phytate and P, which will subsequently result in increased solubility of P in the intestinal tract. Inclusion of microbial phytase in swine diets not only increases the digestibility of P (Akinmusire and Adeola 2009; Almeida and Stein 2010; Rodríguez *et al*. 2013), but also the digestibility of Ca (Rodríguez *et al*. 2013; González-Vega *et al*. 2013, 2015*a*). Negative effect of excess dietary Ca on the efficacy of phytase has been observed in several experiments (Lei *et al*. 1994; Lantzsch *et al*. 1995; Brady *et al*. 2002). However, it is not clear how Ca affects the efficacy of phytase, but there are three possible explanations, including the following: (1) insoluble Ca–phytate complexes may be formed in the small intestine, which may reduce the ability of microbial phytase to liberate the P in the diet (Wise 1983; Fisher 1992); (2) excess Ca in the

Table 1. Digestibility of calcium (Ca) in feed ingredients
ATTD, apparent total tract digestibility; STTD, standardised total tract digestibility; TTTD, true total tract digestibility

Ca source	ATTD of Ca (%)		STTD of Ca (%)		TTTD of Ca (%)	
	No phytase	With phytase	No phytase	With phytase	No phytase	With phytase
Inorganic sources						
Calcium carbonate[A]	57.98	70.62	60.43	73.07	–	–
Calcium carbonate[B]	60.90–70.90	–	–	–	–	–
Calcium carbonate[C]	69.96–74.29	–	74.13–78.45	–	–	–
Dicalcium phosphate[A]	75.29	76.39	77.80	78.90	–	–
Lithothamnium calcareum[A]	62.54	66.24	64.98	68.67	–	–
Monocalcium phosphate[A]	82.76	83.24	85.86	86.34	–	–
Sugar beet co-product[A]	66.18	63.18	68.41	65.41	–	–
Plant sources						
Canola meal[D]	33.71–42.96	45.89–65.91	–	–	46.60	70.30
Corn[E]	49.60	–	–	–	–	–
Soybean meal[E]	46.70	–	–	–	–	–
Animal sources						
Fish meal (cornstarch-based diet)[F]	40.42–51.22	57.27	45.64–53.87	60.07	–	–
Fish meal (corn-based diet)[F]	73.07–84.24	84.01	76.21–88.99	86.88	–	–
Meat and bone meal[G]	53.00–81.00	–	–	–	–	–

[A]González-Vega *et al*. (2015*a*).
[B]Stein *et al*. (2011).
[C]Merriman and Stein (2015).
[D]González-Vega *et al*. (2013).
[E]Bohlke *et al*. (2005).
[F]González-Vega *et al*. (2015*b*).
[G]Sulabo and Stein (2013).

gastro-intestinal tract increases gastric and (or) intestinal pH, which reduces the efficacy of microbial phytase (Sandberg *et al*. 1993); and (3) dietary Ca may compete with phytase for the active site on phytate, which will reduce the efficiency of hydrolysing the phytate-P bond (Qian *et al*. 1996).

Particle size of Ca carbonate

In laying hens, the effect of particle size on the digestibility of Ca is variable (Scheideler 1998; Araujo *et al*. 2011), but in pigs, a particle size between 0.10 and 0.54 mm does not affect the relative bioavailability of Ca (Ross *et al*. 1984). Recently, it was also reported that a particle size between 200 and 1125 microns does not affect the ATTD or STTD of Ca in Ca carbonate included in phytate-containing diets based on corn and potato protein isolate (Merriman and Stein 2015). Therefore, it appears that, at least for Ca carbonate, the particle size does not influence the digestibility of Ca.

Pelleting

Thermal treatments, such as extrusion, may increase absorption of some minerals in broilers (Hafeez *et al*. 2014) and rats (Alonso *et al*. 2001), because of a reduction in the concentration of phytate-bound P due to heating and possibly a reduction of other antinutritional factors (Alonso *et al*. 2001). But if diets are subjected to high temperatures with moisture, Maillard reaction products may be formed, which may reduce mineral bioavailability (O'Brien *et al*. 1989). Although, pelleting of diets for suckling piglets increased absorption of Ca in Caco-2 cells (Delgado-Andrade *et al*. 2010), further research is needed to determine the effect of thermal treatments on Ca digestibility in pigs.

Site of absorption

The place where Ca is absorbed may be influenced by the type of diet that is fed to pigs (Partridge 1978) and the source of Ca in the diet (González-Vega *et al*. 2014). Most Ca is absorbed in the small intestine (Moore and Tyler 1955*a*, 1955*b*; Partridge 1978; Liu *et al*. 2000; Schröder and Breves 2006), but some Ca may be absorbed very early in the duodenum (González-Vega *et al*. 2014). Although, results of several experiments have indicated that no absorption of Ca takes place in the large intestine (Bohlke *et al*. 2005; González-Vega *et al*. 2014), data indicating that Ca may be absorbed in the colon, but not in caecum, have also been reported (Liu *et al*. 2000).

Fibre

The hydroxyl and carboxyl groups associated with fibre may bind some minerals at neutral pH, reducing the availability of these minerals in the small intestine (Debon and Tester 2001; Miyada *et al*. 2011), but these complexes may become available in the colon if the fibre is fermented (James *et al*. 1978). Synthesis of short-chain fatty acids by fermentation of dietary fibre reduces intestinal pH (Wong *et al*. 2006; Rose *et al*. 2007), which may enhance the solubility and absorption of minerals in rats (Ohta *et al*. 1995), humans (Coudray *et al*. 1997) and pigs (Bird *et al*. 2000). Butyrate may also increase the absorption of minerals because butyrate may stimulate the growth of epithelial cells in the intestines (Montagne *et al*. 2003). Increased ATTD and STTD of Ca in diets containing synthetic cellulose or corn compared with synthetic diets containing no fibre have been observed (González-Vega *et al*. 2015*b*). This observation indicates that not only synthesis of short-chain fatty acids may influence Ca digestibility, but other factors such as transit time, gut motility or mineral precipitation may also be involved (González-Vega *et al*. 2015*b*).

Fat

Fat may reduce the rate of passage, which may increase the digestibility of amino acids (Cervantes-Pahm and Stein 2008; Kil and Stein 2011). In humans, reduction of body fat may be caused by high concentrations of dietary Ca, because high concentrations of Ca may increase excretion of fat (Bendsen *et al*. 2008; Soares *et al*. 2012), indicating that dietary Ca may bind to fat in the intestinal tract and form Ca soaps, and thereby reduce absorption. However, the effect of fat on the digestibility of Ca is variable and depends on the type of fatty acids in the diet (Boyd *et al*. 1932; Agnew and Holdsworth 1971; Wargovich *et al*. 1984). In pigs, inclusion of 7% soybean oil did not affect the digestibility of Ca and P (González-Vega *et al*. 2015*b*). However, the effect of other types of oil on the digestibility of Ca and P has not been reported and current research at the University of Illinois is directed at determining the influence of type of oil on the digestibility of Ca in diets fed to pigs.

Vitamin D

Homeostasis of Ca is mainly regulated by two hormones, parathyroid hormone and calcitonin. If the concentration of Ca in plasma is low, parathyroid hormone is secreted, which leads to the activation of vitamin D to its active form 1α,25 dihydroxycholecalciferol, but if Ca plasma concentration is high, calcitonin is secreted, and activation of vitamin D is prevented (Costanzo 2006). Active absorption of Ca from the small intestine is increased by 1α,25 dihydroxycholecalciferol (Kaune 1996) because of increased expression of calbindin, intra-cellular calcium transporters and Ca-ATPases (van Abel *et al*. 2003; Kutuzova and DeLuca 2004). Results of recent studies have indicated that 1α,25 dihydroxycholecalciferol may also increase passive absorption of Ca from the small intestine (Kutuzova and DeLuca 2004; Christakos 2012).

Digestible Ca requirement

The requirements of Ca for pigs are expressed as total Ca requirements because no data for the requirement of digestible Ca have been reported (NRC 2012). However, recent work in our laboratory attempted to determine the requirement for digestible Ca in 11–25 kg pigs. Preliminary results of this work indicated that there is a large negative effect on growth performance of including digestible Ca in excess of the requirement in the diets (González-Vega *et al*. 2015*c*). One of the reasons for the negative effect on the pig growth performance and feed conversion rate may be the negative effect of excess Ca on the digestibility of P (Stein *et al*. 2011), and the negative effects of Ca may, therefore, be a result of insufficient concentrations of P available for formation of soft tissue. However, further research needs to be conducted to determine the requirement for digestible Ca by pigs.

Conclusions

Dietary concentrations of Ca should be considered in formulation of diets for pigs because excess dietary Ca has negative effects on the digestibility of P and the efficacy of phytase. Values for STTD of Ca in Ca supplements and feed ingredients have been determined and may be used in diet formulation and it has been demonstrated that the effect of microbial phytase may vary among ingredients. Fibre may increase the digestibility of Ca, but particle size and soybean oil do not affect the digestibility of Ca. Requirements for digestible Ca by pigs have not been established, but preliminary data from weanling pigs indicated that excess dietary Ca has significant negative effects on growth performance of pigs. Therefore, it is necessary that the exact requirements for digestible Ca by different groups of pigs be determined and future research will be conducted to determine requirements for digestible Ca by pigs.

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