



## Review Article

# Methodological aspects of determining phosphorus digestibility in swine: A review

Yue She, Defa Li, Shuai Zhang\*

State Key Laboratory of Animal Nutrition, Ministry of Agriculture Feed Industry Centre, China Agricultural University, Beijing 100193, China

## ARTICLE INFO

## Article history:

Received 7 December 2016  
 Received in revised form  
 7 February 2017  
 Accepted 10 February 2017  
 Available online 21 February 2017

## Keywords:

Digestibility  
 Methodology  
 Phosphorus  
 Pigs

## ABSTRACT

The role of phosphorus (P) in swine nutrition has been taken on new significance in recent years. Methods to determine the available phosphorus (AP) content of swine feeds include relative bioavailability (RBV), apparent total tract digestibility (ATTD), standardized total tract digestibility (STTD), and true total tract digestibility (TTTD). The RBV of P is determined by measuring bone ash or bone P, whereas the ATTD of P is determined by calculating the difference between P intake and P excretion in feces. Recent research has shown that the use of ATTD of P underestimates the AP due to the existence of endogenous P in feces and digesta. The STTD can be calculated from ATTD by taking basal endogenous phosphorus losses (EPL) into consideration. The basal EPL in pigs can be measured by feeding a P-free diet. Values for STTD of P are believed to be additive in mixed diets but not for ATTD of P. The regression method is a common approach to determine total EPL and TTTD of P, which measures the linear relationship between fecal P excretion and the dietary intake of total P. In addition, *in vitro* methods such as the bionic enzymatic method are being increasingly utilized because they can be done quickly and simply. Several dietary factors such as P and Ca concentrations, phytate, Ca to P ratio and vitamin D may affect AP. This review summarizes the evolution of methods to measure AP and factors that can affect AP, which may provide information to formulate swine diet more accurately. Moreover, the knowledge about AP may help to reduce the P waste in swine production and thus decrease its impact on the environment.

© 2017, Chinese Association of Animal Science and Veterinary Medicine. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

Phosphorus (P) is the second most abundant mineral in the body following calcium (Ca), and is an essential macro element which must be supplemented in diets fed to pigs (Zhang et al., 2010). An excess or deficiency of P in the diet may induce diseases such as rickets or osteomalacia (Bühler et al., 2010).

Most of the P in plants is bound to phytate which is poorly digested by monogastric animals due to the lack of endogenous phytase to break down phytate (Ajakaiye et al., 2003). As a result,

the organic P in plant ingredients is commonly ignored when determining the levels of available phosphorus (AP) in diets fed to pigs (Ketaren et al., 1993). However, previous studies have indicated that the P in plant ingredients such as wheat co-products is highly digestible when included in diets fed to pigs (Kima et al., 2005; Widyaratne and Zijlstra, 2007). Therefore, it is important to have precise knowledge of the AP content of feed ingredients fed to swine for economic and environmental considerations.

## 2. Methodology for determining phosphorus digestibility

### 2.1. Relative bioavailability of phosphorus

Traditionally, the AP was determined by measuring its relative bioavailability (RBV). The RBV of different P sources is determined by measuring bone strength, bone ash weight, or percentage bone ash of pigs that are fed various sources of test P and comparing them with the same bone parameters of pigs fed a standard P source (Cromwell, 1992). The RBV of a standard P source is normally given a

\* Corresponding author.

E-mail address: [zhangshuai@mafic.ac.cn](mailto:zhangshuai@mafic.ac.cn) (S. Zhang).

Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.



value of 100%, and the bioavailability of test P is estimated as a relative percentage to the standard P source using the slope ratio method (Petersen et al., 2011). Gillis et al. (1954) was the first to conduct an experiment to determine AP among different phosphate sources using the slope ratio method. In that study, a basal diet was formulated and graded percentages of the test ingredient were added to the basal diet to provide diets containing graded concentrations of AP. Pigs were euthanized after being fed the test diets for 4 weeks. Gillis et al. (1954) assumed that there was a linear relationship between the AP in the standard P source and the P in the test ingredient. The regression procedure was used to measure the slope of the response criteria for pigs fed the test ingredient and the standard P source. The RBV was then calculated using the ratio of the slope of the test ingredient to the slope of the standard P source.

The slope ratio method works well if the objective of the experiment is to compare the bioavailability of different P sources. However, there are some disadvantages of this procedure. The RBV method does not allow the calculation of the digestibility or the quantities of P absorbed from a specific ingredient because the digestibility of P in standardized phosphate sources is actually less than 100% (Petersen and Stein, 2006). It has been shown that the RBV of P in distillers dried grains with soluble (DDGS) overestimated the digestibility of P in DDGS, therefore, could not be accurately calculated from values for the RBV of P (Baker et al., 2013). Furthermore, the RBV of P among ingredients is not believed to be additive in mixed diets fed to pigs.

## 2.2. Apparent total tract digestibility of phosphorus

Research has demonstrated that P is mainly digested in the small intestine (Fan et al., 2000) and there were no significant differences in AP measured at the ileum and over the entire digestive tract (Fan et al., 2002). These findings have served as a basis for an alternative method for estimating AP. This method, apparent total tract digestibility (ATTD) of P is calculated as the difference between P intake and P excretion in feces (Adhikari et al., 2016). The total collection procedure and indicator procedure are the most commonly used approaches to determine the ATTD of P. If the total collection procedure is used, then the ATTD (%) of P in each diet can be calculated according to the following equation (Almeida and Stein, 2010):  $ATTD (\%) = [(P_i - P_f)/P_i] \times 100$ , where  $P_i$  is the total P intake (g) in the collection period and  $P_f$  is the total fecal P output (g) originating from the feed that is provided in the collection period. If the indicator procedure is used, then the ATTD (%) of P in each diet can be calculated according to the following equation (Wu et al., 2008):  $ATTD (\%) = [1 - [(I_m \times P_c)/(I_d \times P_d)]] \times 100$ , where  $I_m$  is the concentration of the digestibility marker in the assay diet (% as-fed basis),  $P_c$  is the P concentration in feces (% as-fed basis),  $P_d$  is the P concentration in the assay diet (% as-fed basis) and  $I_d$  is the concentration of digestibility marker in feces (% as-fed basis).

There are still some shortcomings when using values for ATTD of P to estimate AP. The estimation of ATTD of P for the same feed ingredient fed to pigs is variable among experiments (Bohlke et al., 2005; Hill et al., 2009, Table 1). In addition, values for the ATTD of P may not be additive in mixed diets (Almeida and Stein, 2010).

## 2.3. Standardized total tract digestibility of phosphorus

Endogenous P is constantly excreted from the pig's body (Fan et al., 2001). The endogenous P mainly comes from saliva and intestinal cells, as well as pancreatic and bile secretions that enter the digestive tract (Vitti and Da Silva Filho, 2010). However, values for ATTD of P do not quantify the proportion of endogenous P in the excreta (Almeida and Stein, 2010). It was observed that the ATTD of P underestimated the true digestive utilization of P in soybean meal

(SBM) for pigs by 25% (Fan et al., 2001; Pettey et al., 2006). Consequently, it is necessary to determine the extent of endogenous phosphorus losses (EPL).

The endogenous P excretion can be divided into basal EPL and total EPL (Fan et al., 2001). Basal EPL represent the minimum losses of P from the pig's body, whereas total EPL represent both basal EPL and EPL from the diet.

Three major approaches are available for determining EPL, including the use of a P-free diet, the tracer dilution technique using  $^{32}\text{P}$ -labeled phosphates and the regression method. The  $^{32}\text{P}$ -tracer technique was reported to overestimate the EPL due to rapid recycling of labeled nutrients within the gastrointestinal tract (Fan et al., 2001). In addition, in terms of safe handling of radioactive wastes, it is difficult to use tracer technique in whole-animal experiments.

Basal EPL can be measured using a P-free diet (Adhikari et al., 2015). The methodology for measuring basal EPL of P is similar to that used for measuring basal endogenous amino acid losses in pigs (Moter and Stein, 2004; Stein et al., 2005, 2007). A P-free diet typically includes cornstarch, gelatin, sucrose, soybean oil, ground limestone, vitamin-mineral premix, solka-floc, salt and an amino acid mixture. Many studies have been conducted to determine the basal EPL, and it has been proven that this value is constant in pigs (Rojas and Stein, 2012; Rodríguez et al., 2013; Kim et al., 2014; Maison et al., 2015). The standardized total tract digestibility (STTD) of P is calculated by correcting the ATTD of P for basal EPL.

The basal EPL can be measured in pigs fed a P-free diet according to the following equation: Basal endogenous phosphorus losses (mg/kg of DMI) =  $(P_f/F_i) \times 1,000 \times 1,000$ , where  $F_i$  is the total feed (g of DM) intake in the collection period. The daily basal EPL in pigs fed P-containing diets are calculated by multiplying the calculated basal EPL per kilogram of DMI by the DMI of each pig.

The STTD of P can be calculated using the following equation (Almeida and Stein, 2010):  $STTD (\%) = [P_i - (P_f - \text{Basal EPL})/P_i] \times 100$ .

Many studies have been conducted to measure the STTD of P in inorganic as well as plant P sources (Petersen and Stein, 2006; Petersen et al., 2011). Almeida and Stein (2010) used a P-free diet to determine the amount of basal EPL of pigs successfully for the first time. Symptoms of diarrhea and muscular spasms prevented the use of P-free diets before that time. In addition, it is difficult to find an appropriate protein ingredient to be included in a P-free diet which has low content of P but good balance of amino acids. Gelatin is now commonly recommended as a good source of protein to be included in the P-free diet fed to pigs (Sulabo and Stein, 2013; Kim et al., 2014).

Many studies have used this method since the discovery that gelatin could be utilized in P-free diet to measure the STTD of P in pigs (Table 1). Almeida and Stein (2012) reported that values for the STTD of P in corn and corn co-products fed to growing pigs ranged from 40.9% to 77.1%, and concluded that the P in DDGS and high protein distiller's dried grains (HP-DDG) was adequately digested by pigs, probably as a result of a relatively low concentration of phytate-bound P in DDGS and HP-DDG. Kim et al. (2012) reported that values for the STTD of P in whey powder, whey permeate and low-ash whey permeate fed to weanling pigs were 91.2%, 93.1%, and 91.8%, respectively, and all of these 3 ingredients had good P digestibility. Rojas and Stein (2012) found that value for the STTD of P in fermented soybean was greater than that in conventional soybean when fed to growing pigs. Sulabo and Stein (2013) reported that values for the STTD of P among sources of meat and bone meal ranged from 54.8% to 84.4%, which were similar to the range reported as RBV of P in meat and bone meal sources. She et al. (2015) evaluated the STTD of P in 17 kinds of Chinese plant ingredients and reported that differences exist in the STTD of P among feed

**Table 1**  
Digestibility of phosphorus (P) in feed ingredients fed to growing pigs.

P sources	ATTD of P, %		STTD of P, %		TTTD of P, %	
	No phytase	With phytase	No phytase	With phytase	No phytase	With phytase
<b>Plant sources</b>						
<b>Corn and corn co-products</b>						
Corn <sup>1,2,3</sup>	19.9–36.4	42.5–57.8	26.4–42.5	50.2–64.4	40.5	–
DDGS <sup>1,2,3</sup>	68.0–72.2	71.0–78.5	72.0–76.5	75.5–82.8	–	–
Corn gluten feed <sup>2,4</sup>	80.7	83.1	84.6	87.1	–	–
Corn germ meal <sup>2,4</sup>	49.0	64.4	53.2	68.3	–	–
Corn gluten meal <sup>2,4</sup>	70.6	77.6	75.2	87.4	–	–
<b>Wheat and wheat co-products</b>						
Wheat <sup>4</sup>	51.5	–	56.9	–	–	–
Wheat bran <sup>4</sup>	57.4	–	62.8	–	–	–
Wheat feed flour <sup>4</sup>	48.9	–	58.0	–	–	–
Wheat red dog <sup>4</sup>	54.3	–	60.2	–	–	–
Wheat shorts <sup>4</sup>	53.9	–	61.4	–	–	–
<b>Rice co-products</b>						
Broken rice <sup>5</sup>	46.1	63.7	53.5	71.3	–	–
Brown rice <sup>5</sup>	31.6	58.5	38.0	63.7	–	–
Defatted rice bran <sup>5</sup>	32.0	41.2	35.4	43.1	–	–
Full fat rice bran <sup>5</sup>	27.1	42.9	28.9	44.6	–	–
Rice mill feed <sup>5</sup>	31.7	48.6	36.6	53.2	–	–
<b>Oilseed meals</b>						
Bakery meal <sup>2</sup>	54.9	67.5	58.6	71.2	–	–
Canola meal <sup>6,7,8</sup>	41.52–47.32	64.08–68.05	45.3–51.2	63.6–72.1	34.3	61.4
Copra meal <sup>9</sup>	60.6	80.8	70.6	90.3	–	–
Cottonseed meal <sup>7</sup>	41.8	56.0	45.6	60.0	–	–
Palm kernel meal <sup>9</sup>	48.9	64.1	57.9	73.5	–	–
Peanut meal <sup>4</sup>	38.2	–	48.2	–	–	–
Soybean meal <sup>4,6,7</sup>	41.6–56.3	66.2–72.5	46.1–62.0	71.4–78.0	36.0–40.9	70.8
Sunflower meal <sup>7</sup>	33.0	55.4	37.4	59.8	–	–
<b>Inorganic sources</b>						
Dicalcium phosphate <sup>10</sup>	81.5	–	88.4	–	–	–
Monocalcium phosphate <sup>10</sup>	88.0	–	94.9	–	–	–
Monosodium phosphate <sup>10</sup>	91.9	–	98.2	–	–	–
<b>Animal sources</b>						
Meat and bone meal <sup>11</sup>	52.1–80.1	–	54.8–84.4	–	–	–
Whey powder <sup>12</sup>	84.3	–	91.2	–	–	–
Whey permeate <sup>12</sup>	86.1	–	93.1	–	–	–

ATTD = apparent total tract digestibility; STTD = standardized total tract digestibility; TTTD = true total tract digestibility; DDGS = distillers dried grains with soluble.

<sup>1</sup> Almeida and Stein (2010).

<sup>2</sup> Rojas et al. (2013).

<sup>3</sup> Almeida and Stein (2012).

<sup>4</sup> She et al. (2015).

<sup>5</sup> Casas and Stein (2015).

<sup>6</sup> Akinmusire and Adeola (2009).

<sup>7</sup> Rodríguez et al. (2013).

<sup>8</sup> Maison et al. (2015).

<sup>9</sup> Almaguer et al. (2014).

<sup>10</sup> Petersen and Stein (2006).

<sup>11</sup> Sulabo and Stein (2013).

<sup>12</sup> Kim et al. (2012).

ingredients and the susceptibility of the phytate in corn from phytase may be less than that from wheat and oilseed meals. Casas and Stein (2015) reported the STTD of P in brown rice, full-fat rice bran, defatted rice bran and rice mill feed fed to pigs were relatively low due to the high concentrations of phytate in these ingredients. However, the addition of microbial phytase could increase the STTD of P in most of the rice co-products. Recently, it was reported that no differences in STTD of P exist among different kinds of SBM produced from different areas in the United States (Oliveira and Stein, 2016; Sotak-Pepper et al., 2016). Results from these studies showed value for the basal EPL in pigs is constant and reliable. Furthermore, NRC (2012) reported that values for STTD of P are believed to be additive in mixed diets.

#### 2.4. True total tract digestibility of phosphorus

Total EPL from pigs can be measured by a regression analysis technique (Pettley et al., 2006). In 2001, Fan et al. (2001) used SBM

as a test ingredient and demonstrated that total EPL and true P digestibility in feed ingredients for weaning pigs can be determined by the regression method. This study concluded that although the ileal EPL was greater than total tract EPL, there were no statistical differences between true ileal and total tract P digestibility. In 2003, consistent with previous findings in weaning pigs, the same lab reported there were no differences in the true P digestibility in SBM between ileal and fecal samples in growing pigs, which further documented that the large intestine did not play a role in digestive utilization of dietary P (Ajakaiye et al., 2003).

The most important step in obtaining reliable data by this procedure is to establish a linear relationship between the apparent digestible intake and total intake of the test P in the diets. The diets are formulated by addition of graded levels of test ingredient in order to formulate diets containing graded levels of P. It is assumed that there is a linear regression between P intake and apparent digestible P. Thus, the intercept and the slope of the linear regression equation represent total EPL and true total tract digestibility

(TTTD) of P, respectively. However, there may be disadvantages of this procedure. Because it has been suggested that using regression procedure the endogenous P estimates are highly variable between individual animals, therefore, are not significantly different from zero (Dilger and Adeola, 2006). This may be because the total EPL are highly influenced by the type of the experimental diets as well as the P levels used in the regression procedure.

In recent years, many studies have been done to investigate the TTTD of P in different feed ingredients fed to pigs (Table 1). Dilger and Adeola (2006) reported that true P digestibility was not different between pre-cecal and total tract collection sites, but was greater for low-phytate SBM (62.6%) compared with conventional SBM (44.5%). Endogenous P estimates were not different between the SBM varieties and averaged 4.83 mg/(kgW<sup>0.75</sup>·d). Akinmusire and Adeola (2009) found the TTTD of P in canola was greater than that in SBM for growing pigs. Zhai and Adeola (2012) reported the TTTD of P in monocalcium phosphate for 15-kg pigs was 67.5% and the total EPL were 494 mg/d. Xue and Adeola (2015) reported that, for triticale DDGS, the supplementation of 500 FTU/kg phytase in the diet could increase the ATTD of P but not the TTTD of P. Additionally, it has been demonstrated that the TTTD of P in corn and SBM for growing pigs are additive in corn–SBM diets (Zhai and Adeola, 2013a). However, more studies are needed to determine additivity of TTTD of P among different P sources in pigs.

Using the regression procedure, the ATTD and TTTD of P is calculated using the following equations (Akinmusire and Adeola, 2009):  $ATTD (\%) = 100 \times (P_I - P_O)/P_I$  and  $P_D = (TTTD \times P_I) - \text{Total EPL}$ , where  $P_I$  represents the dietary P intake,  $P_O$  is the fecal P output (mg/d),  $P_D$  represents the digested P (mg/d), EPL represents the estimate of daily total endogenous P losses (mg/d), and TTTD is the estimate of TTTD with the estimation carried out by regressing  $P_D$  against  $P_I$ .

According to these factorial estimates, the TTTD-based P requirements for different stages of production for pigs have also been evaluated (Zhai and Adeola, 2013b, 2013c). However, P digestibility varied and depended on the evaluation system employed by each study. Some studies used a basal diet, whereas others used semi-purified diets in which the test ingredients served as the sole source of P. Therefore, further studies are still needed to establish a unified and efficient procedure to determine AP.

### 2.5. Phosphorus balance

When the dietary P concentration exceeds the requirement for a specific stage of production, the urinary excretion of P is increased. Therefore, it is necessary to determine the excretion of P from urine in this case. Miller et al. (1965) reported that the retention of P was improved with an increasing dietary P concentration and the amount of retained P was influenced primarily by urinary P output. Vipperman et al. (1974) found that urinary P outputs increased with increasing dietary P input, and the urinary P output was nearly zero when dietary Ca concentration exceeded the dietary P concentration. Wu et al. (2008) reported that urinary P outputs improved with increasing dietary P input, which suggests that there is a positive correlation between urinary P output and dietary P intake. However, when the diets are below or at the concentration of P requirement, little P may actually be excreted through the urine.

### 2.6. In vitro analysis techniques

With the advantages of being rapid, convenient and economical, *in vitro* analysis techniques for determining the bioavailability of nutrients are being rapidly developed. A computer-controlled simulated digestion system has been developed to predict the energetic value of ingredients fed to poultry (Zhao et al., 2014a,

2014b). Currently, this *in vitro* procedure is used primarily to determine the available energy content for pigs. To date, limited literature has been found using *in vitro* technique to determining AP. Walk et al. (2012), who has developed an *in vitro* technique to estimate AP, reported that absorption of Ca and P may be complicated by conditions within the gastrointestinal tract, such as particle size, precipitation with anti-nutritional factors, and differential rates of delivery to the small intestine.

The challenge of *in vitro* estimation of P digestibility is that activities of the porcine digestive enzymes used to imitate intestinal fluid may not reflect the *in vivo* intestinal fluid of animals (Zhao et al., 2014a). In addition, manual conduct of *in vitro* digestion techniques such as pH regulation, digestive enzyme injection and separation of digested and undigested substance (Losada et al., 2010) may cause errors, making the technique unrepeatable. Therefore, repeatable techniques and realistic experimental conditions are needed to develop a successful *in vitro* digestion analysis technique. It appears that current technology of *in vitro* estimates of P digestibility may be less accurate than *in vivo* estimates. More work is needed of *in vitro* estimates of P digestibility.

## 3. Dietary factors that may affect phosphorus digestibility

### 3.1. Phosphorus concentration

The ATTD of P in monocalcium phosphate fed to pigs is not influenced by the dietary concentration of P if the concentration of P in the diet is at or below 0.64% (Stein et al., 2008). Therefore, the concentration of P in the diets used to measure the digestibility of P in feed ingredients is not critical, because the same value for ATTD of P will be measured regardless of the inclusion concentration of P. Phosphorus excretion in urine is constant when STTD P intakes were below the growth requirement (NRC, 2012). But when STTD P intakes are above requirements, the P excretion in urine increased linearly (Gutierrez et al., 2015). This finding suggests that the transport of P across the intestinal wall may not represent a limiting step for P regulation, and P excretion may thereby instead be regulated at the renal level. Mineral accretion in the femur increases with increasing STTD P intakes but reaches a plateau at a greater STTD P intake level than that required for maximum growth (Gutierrez et al., 2015). Therefore, dietary STTD P is absorbed and used for growth, but excess P is accumulated in bones until skeletal requirements are met and then it will be excreted in urine.

A recent study showed that increasing dietary P by increasing concentrations of the test ingredient within a certain range can reduce the estimated values of STTD of P, but does not affect the estimates of TTTD of P determined by the regression method (Liu et al., 2016).

### 3.2. Phytate

A linear relationship between non-phytate P and digestible P has been established (Létourneau-Montminy et al., 2012). Considering the linearity of the response, it is possible that P transportation across the intestinal wall is not a limiting step for absorption of P, at least in experimental conditions introduced in this review. Non-phytate P of mineral, animal and plant origins remains highly digestible for pigs regardless of the non-phytate P concentration, as evidenced by their estimated digestibility coefficients. It is likely that phytate P cannot be totally hydrolyzed in gut due to the limited solubility of phytate P, which depends on pH condition and the transit time of digesta (Létourneau-Montminy et al., 2011). Phytate P utilization depends on some modulating dietary factors. Estimates of digestibility of phytate P confirmed that a considerable proportion of dietary phytate P is available for

absorption without any exogenous phytase supply (Kempe et al., 2006). In fact, phytate P hydrolysis can be achieved through microbes such as lactic acid bacteria in the gastrointestinal tract (Sreeramulu et al., 1996; Schindler et al., 1997).

Many studies indicated that including low-phytate plant ingredients in swine diets can improve the bioavailability of P for pigs and may also increase the utilization of other nutrients (Bohlke et al., 2005; Htoo et al., 2007; Hill et al., 2009). In light of the challenges inherent in adding enzymes to complete diets, genetic reduction of phytate in feedstuffs provides an effective strategy for improving P utilization for pigs.

### 3.3. Calcium concentration

The dietary Ca concentration affects the ATTD of P in pigs (Stein et al., 2011). Increased concentrations of dietary Ca can decrease the ATTD of P in diets based on corn, potato protein isolate, and monosodium phosphate for pigs (Stein et al., 2011). The dietary Ca concentration is, therefore, very important when the ATTD of P in feed ingredients is determined. It is likely that increased dietary Ca may result in binding of P by Ca in the digestive tract of the animals, which may form a Ca-P complex that precludes P from being absorbed. However, these mechanisms have not been experimentally demonstrated, and research in this area is needed.

### 3.4. Calcium to phosphorus ratio

The greatest values for ATTD of P are obtained if the dietary Ca-to-total P ratio is around 1.1:1 (Stein et al., 2011; She et al., 2016). Serum P concentrations, bone ash concentration, and bone bending moment are decreased if the Ca-to-P ratio is increased from 1.3:1 to 2.0:1, 3.0:1, or 4.0:1 (Reinhart and Mahan, 1986), which indicates that the AP may also decrease as the dietary Ca to total P ratio increased. Pig growth performance and P utilization are increased by decreasing the Ca to total P ratio from 1.5:1 to 1.0:1 in low-P corn-SBM meal diets supplemented with microbial phytase (Liu et al., 1998). Lowering the dietary Ca to total P ratio to 1.0:1 in a low-P diet containing phytase increases the apparent P absorption in the small intestine (Liu et al., 2000). Moreover, a significant amount of P is absorbed in the cecum.

Wide Ca to total P ratios have detrimental effects on the efficacy of microbial phytase. Three possible mechanisms are proposed to explain this: 1) The extra dietary Ca may form an insoluble phytate complex which is not accessible for hydrolysis by microbial phytase (Fisher, 1992); 2) High dietary Ca concentrations can increase the pH of the intestinal contents, which in turn decrease the activity of microbial phytase (Sandberg et al., 1993); 3) The extra dietary Ca can directly suppress the activity of microbial phytase by means of competing for the active sites of the enzyme (Qian et al., 1996).

### 3.5. Vitamin D

It is well established that there are interactions between P metabolism and the bioconversion and actions of vitamin D (Brautbar et al., 1979). Thus, the administration of 1, 25-dihydroxy-vitamin D<sub>3</sub> [1,25(OH)<sub>2</sub>D<sub>3</sub>] can improve intestinal P transport in pigs. Furthermore, there is potent phosphatemic action of 1,25-dihydroxy-vitamin D<sub>3</sub> in hypophosphatemic pigs as a result of P mobilization from bone. In addition, it has been shown that P depletion can stimulate the conversion of 25-hydroxyvitamin D<sub>3</sub> to 1, 25(OH)<sub>2</sub>D<sub>3</sub>. The addition of vitamin D<sub>3</sub> (2,000 IU/kg) can increase the ATTD of P for pigs (Li et al., 1998). However, in a broiler study, Edwards (2002) did not observe an effect on Ca and P retention with addition of 1,100 to 8,800 IU/kg of vitamin D<sub>3</sub> to diets deficient in Ca and P, although a significant effect of vitamin D<sub>3</sub> on bone ash was observed.

## 4. Conclusions

In this review, evolution of methodology for determining the AP content in swine feeds was summarized. These methods include RBV, ATTD, STTD, TTDD, retention of P and the *in vitro* methods. Since the concept of STTD of P was recently proposed, this article, for the first time, reviewed this method as well as the others. In addition, dietary factors that may affect AP in pigs were introduced, such as P and Ca concentrations, phytate, Ca to P ratio and vitamin D. To improve the accuracy of the measurements, the EPL from the gastrointestinal tract of pigs should be taken into consideration, and additivity of digestibility values of P from each ingredient used in mixed diets is also needed to be evaluated.

## Conflict of interests

The authors declare that they have no conflict of interests.

## References

- Adhikari PA, Heo JM, Nyachoti CM. True and standardized total tract phosphorus digestibility in canola meals from *Brassica napus* black and *Brassica juncea* yellow fed to growing pigs. *J Anim Sci* 2015;93:209–16.
- Adhikari PA, Heo JM, Nyachoti CM. High dose of phytase on apparent and standardized total tract digestibility of phosphorus and apparent total tract digestibility of calcium in canola meals from *Brassica napus* black and *Brassica juncea* yellow fed to growing pigs. *Can J Anim Sci* 2016;96:121–7.
- Ajakaiye A, Fan MZ, Archbold T, Hacker RR, Forsberg CW, Phillips JP. Determination of true digestive utilization of phosphorus and the endogenous phosphorus outputs associated with soybean meal for growing pigs. *J Anim Sci* 2003;1:2766–75.
- Akinmusire AS, Adeola O. True digestibility of phosphorus in canola and soybean meals for growing pigs: influence of microbial phytase. *J Anim Sci* 2009;87:977–83.
- Almaguer BL, Sulabo RC, Liu Y, Stein HH. Standardized total tract digestibility of phosphorus in copra meal, palm kernel expellers, palm kernel meal, and soybean meal fed to growing pigs. *J Anim Sci* 2014;92:2473–80.
- Almeida FN, Stein HH. Performance and phosphorus balance of pigs fed diets formulated on the basis of values for standardized total tract digestibility of phosphorus. *J Anim Sci* 2010;88:2968–77.
- Almeida FN, Stein HH. Effects of graded levels of microbial phytase on the standardized total tract digestibility of phosphorus in corn and corn co-products fed to pigs. *J Anim Sci* 2012;90:1262–9.
- Baker SR, Kim BG, Stein HH. Comparison of values for standardized total tract digestibility and relative bioavailability of phosphorus in dicalcium phosphate and distillers dried grains with solubles fed to growing pigs. *J Anim Sci* 2013;91:203–10.
- Bohlke RA, Thaler RC, Stein HH. Calcium, phosphorus, and amino acid digestibility in low-phytate corn, normal corn, and soybean meal by growing pigs. *J Anim Sci* 2005;83:2396–403.
- Brautbar N, Walling MW, Coburn JW. Interactions between vitamin D deficiency and phosphorus depletion in the rat. *J Clin Invest* 1979;63:335–41.
- Bühler K, Liesegang A, Bucher B, Wenk C, Broz J. Influence of benzoic acid and phytase in low-phosphorus diets on bone characteristics in growing-finishing pigs. *J Anim Sci* 2010;88:3363–71.
- Casas GA, Stein HH. Effects of microbial phytase on the apparent and standardized total tract digestibility of phosphorus in rice co-products fed to growing pigs. *J Anim Sci* 2015;93:3441–8.
- Cromwell GL. The biological availability of phosphorus in feedstuffs for pigs. *Pig News Info* 1992;13:75N–8N.
- Dilger RN, Adeola O. Estimation of true phosphorus digestibility and endogenous phosphorus loss in growing pigs fed conventional and low-phytate soybean meal. *J Anim Sci* 2006;84:627–34.
- Edwards Jr HM. Studies on the efficacy of cholecalciferol and derivatives for stimulating phytate utilization in broilers. *Poult Sci* 2002;81:1026–31.
- Fan MZ, Adeola O, Asem EK, King D. Postnatal ontogeny of kinetics of porcine jejunal brush border membrane bound alkaline phosphatase, amino peptidase N, and sucrose activities in pigs. *Comp Biochem Physiol* 2002;132A:599–607.
- Fan MZ, Archbold T, Mead K, Gao Y. Partitioning various forms of phosphorus in the distal ileal digesta and feces. *Can J Anim Sci* 2000;80:780 (Abstr.).
- Fan MZ, Archbold T, Sauer WC, Lackeyram D, Rideout T, Gao Y, et al. Novel methodology allows simultaneous measurement of true phosphorus digestibility and the gastrointestinal endogenous phosphorus outputs in studies with pigs. *J Nutr* 2001;131:2388–96.
- Fisher H. Low-calcium diets enhance phytate-phosphorus availability. *Nutr Rev* 1992;50:170–1.
- Gillis MB, Norris LC, Heuser GF. Studies on the biological value of inorganic phosphates. *J Nutr* 1954;52:115–25.

- Gutiérrez NA, Serão NVL, Elsbernd AJ, Hansen SL, Walk CL, Bedford MR, et al. Quantitative relationships between standardized total tract digestible phosphorus and total calcium intakes and their retention and excretion in growing pigs fed corn–soybean meal diets. *J Anim Sci* 2015;93:2174–82.
- Hill BE, Sutton AL, Richert BT. Effects of low-phytic acid corn, low-phytic acid soybean meal, and phytase on nutrient digestibility and excretion in growing pigs. *J Anim Sci* 2009;87:1518–27.
- Htoo JK, Sauer WC, Yáñez JL, Cervantes M, Zhang Y, Helm JH, et al. Effect of low-phytate barley or phytase supplementation to a barley-soybean meal diet on phosphorus retention and excretion by grower pigs. *J Anim Sci* 2007;85:2941–8.
- Kemme PA, Schlemmer U, Mroz Z, Jongbloed AW. Monitoring the stepwise phytate degradation in the upper gastrointestinal tract of pigs. *J Sci Food Agric* 2006;86:612–22.
- Ketaren PP, Batterham ES, White E, Farrell DJ, Milthorpe BK. Phosphorus studies in pigs. 1. Available phosphorus requirements of grower/finisher pigs. *Br J Nutr* 1993;70:249–68.
- Kim BG, Lee JW, Stein HH. Energy concentration and phosphorus digestibility in whey powder, whey permeate, and low-ash whey permeate fed to weanling pigs. *J Anim Sci* 2012;90:289–95.
- Kim BG, Liu Y, Stein HH. Energy concentration and phosphorus digestibility in yeast products produced from the ethanol industry, and in brewers' yeast, fish meal, and soybean meal fed to growing pigs. *J Anim Sci* 2014;92:5476–84.
- Kima JC, Simmins PH, Mullanc BP, Pluske JR. The effect of wheat phosphorus content and supplemental enzymes on digestibility and growth performance of weaner pigs. *Anim Feed Sci Technol* 2005;118:139–52.
- Létourneau-Montminy MP, Jondreville C, Sauvart D, Narcy A. Meta-analysis of phosphorus utilization by growing pigs: effect of dietary phosphorus, calcium and exogenous phytase. *Animal* 2012;6:1590–600.
- Létourneau-Montminy MP, Narcy A, Lescoat P, Magnin M, Bernier JF, Sauvart D, et al. Modeling the fate of dietary phosphorus in the digestive tract of growing pigs. *J Anim Sci* 2011;89:3596–611.
- Li DF, Che XR, Wang YQ, Hong C, Thacker PA. Effect of microbial phytase, vitamin D3, and citric acid on growth performance and phosphorus, nitrogen and calcium digestibility in growing swine. *Anim Feed Sci Technol* 1998;73:173–86.
- Liu J, Bollinger DW, Ledoux DR, Veum TL. Effects of dietary calcium: phosphorus ratios on apparent absorption of calcium and phosphorus in the small intestine, cecum, and colon of pigs. *J Anim Sci* 2000;78:106–9.
- Liu J, Bollinger DW, Ledoux DR, Veum TL. Lowering the dietary calcium to total phosphorus ratio increases phosphorus utilization in low-phosphorus corn-soybean meal diets supplemented with microbial phytase for growing-finishing pigs. *J Anim Sci* 1998;76:808–13.
- Liu JB, Cao SC, Chen L, Zhang HF. Effect of dietary phosphorus level on the determination of standardized and true total tract digestibility of phosphorus for growing pigs. *Anim Feed Sci Technol* 2016;215:117–23.
- Losada B, García Rebollar P, Álvarez C, Cachaldora P, Ibáñez MA, Méndez J, et al. The prediction of apparent metabolizable energy content of oil seeds and oil seed by-products for poultry from its chemical components, in vitro analysis or near-infrared reflectance spectroscopy. *Anim Feed Sci Technol* 2010;160:62–72.
- Maison T, Liu Y, Stein HH. Apparent and standardized total tract digestibility by growing pigs of phosphorus in canola meal from North America and 00-rapeseed meal and 00-rapeseed expellers from Europe without and with microbial phytase. *J Anim Sci* 2015;93:3494–502.
- Miller ER, Ullrey DE, Zutaut CI, Hoefer JA, Luecke RW. Mineral balance studies with the baby pig: effects of dietary magnesium level upon calcium, phosphorus and magnesium balance. *J Nutr* 1965;86:209–12.
- Moter V, Stein HH. Effect of feed intake on endogenous losses and amino acid and energy digestibility by growing pigs. *J Anim Sci* 2004;82:3518–25.
- NRC. Nutrient requirements of swine. 11th rev. Washington, DC: Natl. Acad. Press; 2012.
- Oliveira MS, Stein HH. Digestibility of energy, amino acids, and phosphorus in a novel source of soy protein concentrate and in soybean meal fed to growing pigs. *J Anim Sci* 2016;94:3343–52.
- Petersen GI, Pedersen C, Lindemann MD, Stein HH. Relative bioavailability of phosphorus in inorganic phosphorus sources fed to growing pigs. *J Anim Sci* 2011;89:460–6.
- Petersen GI, Stein HH. Novel procedure for estimating endogenous losses and measurement of apparent and true digestibility of phosphorus by growing pigs. *J Anim Sci* 2006;84:2126–32.
- Petty LA, Cromwell GL, Lindemann MD. Estimation of endogenous phosphorus loss in growing and finishing pigs fed semi-purified diets. *J Anim Sci* 2006;84:618–26.
- Qian H, Kornegay ET, Conner Jr DE. Adverse effects of wide calcium: phosphorus ratios on supplemental phytase efficacy for weanling pigs fed two dietary phosphorus levels. *J Anim Sci* 1996;74:1288–97.
- Reinhart GA, Mahan DC. Effect of various calcium: phosphorus ratios at low and high dietary phosphorus for starter, grower and finisher swine. *J Anim Sci* 1986;63:457–66.
- Rodríguez DA, Sulabo RC, González-Vega JC, Stein HH. Energy concentration and phosphorus digestibility in canola, cottonseed, and sunflower products fed to growing pigs. *Can J Anim Sci* 2013;93:493–503.
- Rojas OJ, Liu Y, Stein HH. Phosphorus digestibility and concentration of digestible and metabolizable energy in corn, corn co-products, and bakery meal fed to growing pigs. *J Anim Sci* 2013;91:5326–35.
- Rojas OJ, Stein HH. Digestibility of phosphorus by growing pigs of fermented and conventional soybean meal without and with microbial phytase. *J Anim Sci* 2012;90:1506–12.
- Sandberg AS, Larsen T, Sandstrom B. High dietary calcium level decreases colonic phytate degradation in pigs fed a rapeseed diet. *J Nutr* 1993;123:559–66.
- Schindler B, Lantzsch HJ, Mosenhain R, Biesalski HK, Drochner W. Dose response effects of microbial phytase on P absorption in growing pigs fed P-reduced diets. In: Conference at the VII International Symposium on digestive physiology in pigs, Saint Malo, France; 1997. p. 441–5.
- She Y, Piao XS, Liu L, Li DF. Effects of dietary calcium concentrations on the apparent total tract digestibility and the balance of calcium, phosphorus, and nitrogen in growing pigs. *J Anim Sci* 2016;94(supplement 2):97–8.
- She Y, Su YB, Liu L, Huang CF, Li JT, Li P, et al. Effects of microbial phytase on coefficient of standardized total tract digestibility of phosphorus in growing pigs fed corn and corn co-products, wheat and wheat co-products and oilseed meals. *Anim Feed Sci Technol* 2015;208:132–44.
- Sotak-Pepper KM, González-Vega JC, Stein HH. Effects of production area and microbial phytase on the apparent and standardized total tract digestibility of phosphorus in soybean meal fed to growing pigs. *J Anim Sci* 2016;94:2397–402.
- Sreeramulu G, Srinivasa DS, Nand K, Joseph R. *Lactobacillus amylovorus* as a phytase producer in submerged culture. *Lett Appl Microbiol* 1996;23:385–8.
- Stein HH, Adeola O, Cromwell GL, Kim SW, Mahan DC, Miller PS. Concentration of dietary calcium supplied by calcium carbonate does not affect the apparent total tract digestibility of calcium, but decreases digestibility of phosphorus by growing pigs. *J Anim Sci* 2011;89:2139–44.
- Stein HH, Kadzere CT, Kim SW, Miller PS. Influence of dietary phosphorus concentration on the digestibility of phosphorus in monocalcium phosphate by growing pigs. *J Anim Sci* 2008;86:1861–7.
- Stein HH, Pedersen C, Wirt AR, Bohlke RA. Additivity of values for apparent and standardized ileal digestibility of amino acids in mixed diets fed to growing pigs. *J Anim Sci* 2005;83:2387–95.
- Stein HH, Sève B, Fuller MF, Moughan PJ, de Lange CF, Committee on Terminology to Report AA Bioavailability and Digestibility. Invited review: amino acid bioavailability and digestibility in pig feed ingredients: terminology and application. *J Anim Sci* 2007;85:172–80.
- Sulabo RC, Stein HH. Digestibility of phosphorus and calcium in meat and bone meal fed to growing pigs. *J Anim Sci* 2013;91:1285–94.
- Vipperman Jr PE, Peo Jr ER, Cunningham PJ. Effect of dietary calcium and phosphorus level upon calcium, phosphorus and nitrogen balance in swine. *J Anim Sci* 1974;38:758–65.
- Vitti DMSS, Da Silva Filho JC. Isotope dilution technique. In: Vitti DMSS, Kebreab E, editors. Phosphorus and calcium utilization and requirements in farm animals. London, United Kingdom: CAB International; 2010. p. 8–17.
- Walk CL, Bedford MR, McElroy AP. Influence of diet, phytase, and incubation time on calcium and phosphorus solubility in the gastric and small intestinal phase of an in vitro digestion assay. *J Anim Sci* 2012;90:3120–5.
- Widayaratne GP, Zijlstra RT. Nutritional value of wheat and corn distiller's dried grain with solubles: digestibility and digestible contents of energy, amino acids and phosphorus, nutrient excretion and growth performance of grower-finisher pigs. *Can J Anim Sci* 2007;87:103–14.
- Wu X, Ruan Z, Zhang YG, Hou YQ, Yin YL, Li TJ, et al. True digestibility of phosphorus in different resources of feed ingredients in growing pigs. *Asian-Aust J Anim Sci* 2008;21:107–19.
- Xue PC, Adeola O. Phosphorus digestibility response of growing pigs to phytase supplementation of triticale distillers' dried grains with solubles. *J Anim Sci* 2015;93:646–51.
- Zhai H, Adeola O. True total-tract digestibility of phosphorus in corn and soybean meal for fifteen-kilogram pigs are additive in corn-soybean meal diet. *J Anim Sci* 2013a;91:219–24.
- Zhai H, Adeola O. True digestible phosphorus requirement of 10- to 20-kg pigs. *J Anim Sci* 2013b;91:3716–23.
- Zhai H, Adeola O. True digestible phosphorus requirement for twenty- to forty-kilogram pigs. *J Anim Sci* 2013c;91:5307–13.
- Zhai H, Adeola O. True total-tract digestibility of phosphorus in monocalcium phosphate for 15-kg pigs. *J Anim Sci* 2012;90:98–100.
- Zhang YG, Jiang WM, Tao LH. The effect of dietary P level on the digestibility and output of P and nitrogen. *J Food Agric Environ* 2010;8:348–55.
- Zhao F, Ren LQ, Mi BM, Tan HZ, Zhao JT, Li H, et al. Developing a computer-controlled simulated digestion system to predict the concentration of metabolizable energy of feedstuffs for rooster. *J Anim Sci* 2014a;92:1537–47.
- Zhao F, Zhang L, Mi BM, Zhang HF, Hou SS, Zhang ZY. Using a computer-controlled simulated digestion system to predict the energetic value of corn for ducks. *Poult Sci* 2014b;93:1410–20.