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Effect of phytase on amino acid digestibility in pig: A meta-analysis

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ABSTRACT

Phytic acid represents about 60% of the phosphorus in plant-based ingredients used in pig feed. It is known to bind to proteins and amino acids and thereby interfere with digestion. The effectiveness of microbial phytase to improve the digestibility of amino acid in pig feed appears inconsistent. The objective of this study was to estimate the effect of microbial phytase on the digestibility of dietary indispensable amino acids, as a function of dietary crude protein content, amino acid content, calcium, phytic acid, neutral detergent fibre and acid detergent fibre, using a meta-analysis tool. A database derived from 34 papers published between 1994 and 2015 and describing 138 experimental treatments was compiled. Adding microbial phytase to pig feed appears to improve the digestibility of amino acids. Digestibility increased linearly for Arg (R^2 = 0.99), His 36 (R^2 = 0.98), Ile (R^2 = 0.97), Leu (R^2 = 0.97), Met (R^2 = 0.99), Thr (R^2 = 0.97), Phe (R^2 = 0.98) 37 and Val (R^2 = 0.85), and was quadratic for Lys (R^2 = 0.99), and Tyr (R^2 = 0.99). Fibre and phytase do not interact. The current study showed that microbial phytase supplementation affected positively the apparent ileal digestibility of amino acids and this effect was not modified by other dietary components such as amino acids, calcium, phytic acid and fibre. The current models allow quantifying the effect of microbial phytase on amino acids which is important to use phytase feed enzyme accurately in diet formulation.

1. Introduction

Naturally present in plant tissues, phytic acid (myo-inositol hexaphosphate) is a non-digestible compound that forms complexes with various cations and other nutrients including proteins, lipids and starch (Cosgrove, 1966) and is the principal factor limiting phosphorus availability in animal feeds. The use of microbial phytase and other enzymes to improve digestion and nutrient absorption is a common practice in monogastric livestock production (Cowieson et al., 2014). The first phytases were commercialised in 1990 and within a decade phytases were being added routinely to swine and poultry feeds to break down phytic acid, thereby increasing absorption of phosphorus and reducing its excretion into the environment (Selle and Ravindran, 2008). Phytase used in this manner may be considered as a phosphorus supplement (Selle and Ravindran, 2008; Létourneau-Montminy et al., 2012). In addition to releasing phytate-bound P, phytase counteracts other anti-nutritional properties of phytic acid, in particular its interaction with dietary proteins and amino acids. These interactions are known to decrease the digestibility of proteins and decrease the bioavailability of amino acids (Mroz et al., 1994; Johnston et al., 2004; Adedokun et al., 2015). However, the benefits of adding phytase to livestock feeds appear to be inconsistent (Adeola and Sands, 2003). Exactly how phytase improves amino acid digestibility in the animal gut is not clear. Proposed mechanisms include direct release of protein from intrinsic protein-phytate complexes,

Abbreviations: AID, apparent ileal digestibility; Ca, calcium; NDF, neutral detergent fibre; P, phosphorus; NRC, National Research Council; R^2, coefficient of determination RMSE root mean square error; RMSEP, root mean square error of prediction

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preventing formation of de *novo* binary and ternary protein-phytate complexes in the digestive tract, and decreasing inhibition of digestive proteases by phytic acid (Selle et al., 2000). In spite of numerous investigations, the factors that determine the outcome of the phytate-phytase interplay in the pig digestive tract remain unclear (Selle et al., 2012).

Meta-analysis is a statistical method of summarizing and quantifying knowledge acquired through examination of published research results (Sauvant et al., 2008). This method appeared suitable for gaining better understanding of the processes that allow phytase to increase the bioavailability of amino acid in pig feed as previously performed for P (Létourneau-Montminy et al., 2012).

The objective of this study was therefore to quantify the impact of dietary fibre, crude protein and amino acid contents and interactions of divalent cations with phytic acid on the effectiveness of microbial phytase used to improve protein digestion and amino acid digestibility in pigs.

2. Materials and methods

2.1. Data collection and coding

Studies of the effect of microbial phytase on feed digestion and utilisation in pigs were retrieved from public databases (e.g. Web of Science, CAB abstracts, Prod INRA and Science Direct) using keywords such as amino acid; phytase; apparent ileal digestibility; pigs and swine. This yielded a dataset derived from 34 publications published between 1994 and 2015 and describing 138 experimental treatments. Seven types of diets were distinguishable: corn-soybean meal; cereal-soybean meal; corn/other protein sources; cereal/other protein sources; cereals; soybean meal; and other protein sources. General information (e.g. author name; date of publication; name of journal; objective of study); qualitative data (e.g. sex; surgical procedure; inert marker used; supplier and source of phytase) and quantitative data (e.g. dietary crude protein; amino acid; Ca; P; phytic acid; acid-detergent fibre; neutral-detergent fibre; plant phytase and microbial phytase) were included. Chromic oxide (CrO_2) was the inert marker of digestibility mentioned most often (80%); whereas titanium dioxide (TiO_2) or acid insoluble ash (AIA) was mentioned in the remaining cases.

The unit FTU refers to the phytase activity that liberates 1 µmol of inorganic P per minute from sodium phytate present in excess at 37 °C at pH 5.5. When chemical composition was not provided in the publication, it was calculated from NRC values (National Research Council, 1998 and National Research Council, 2012). Values were thus calculated for dietary crude protein (11% of cases), amino acids (33%), Ca (28%), P (7%), phytic acid (35%) and both acid-detergent and neutral-detergent fibre (100%). A code was assigned to each publication in the database (Sauvant et al., 2008). Most of the data referred to apparent rather than true or standardized digestibility in the ileum, and then apparent digestibility was used in the analysis.

2.2. Data investigation and statistical analysis models

All variables and possible interactions evaluated in the meta-analysis were tested as predictors of feed digestibility in terms of apparent indispensable amino acid digestibility in the pig ileum. Only the significant variables were retained in the models, namely microbial phytase (FTU/kg diet), indispensable amino acid content (g/kg diet) and neutral detergent fibre content (g/kg diet). No interaction between the independent variables was found. The digestible apparent indispensable amino acid content was calculated from the indispensable amino acid content of the diet and the product of apparent digestibility and total amino acid content.

The studies referred to three sources of microbial phytase, namely *Aspergillus Niger*, *Escherichia coli* and *Peniophora lycii*. Particular attention was paid to the meta-design (Sauvant et al., 2008), whereas the relationship between the independent variables taken two by two was identified graphically to assess the extent of variation in each experiment and to identify outliers. Co-linearity was not identified. Each dependent variable was graphed against each independent variable in order to observe the linearity (or lack thereof) of the relationship (Sauvant et al., 2008). The within-experiment and between-experiment responses of the dependent variables (coefficient of digestibility and digestible amino acid content) to covariates (dietary inclusion of microbial phytase, concentrations of crude protein, amino acids, Ca, phytic acid and neutral-detergent fibre) were examined carefully. Descriptive statistics were generated for each variable in the selected groups (Table 1). The "study" was considered as random effect as suggested by Sauvant et al. (2008) and St-Pierre (2001). The study effect should be considered random because when the database contains different individual studies, each study is a random outcome from a large population of studies and the study effect represent the sum of the effects of many factors, all with small effects on the dependent variable (St-Pierre, 2001; Sauvant et al., 2008).

Relationships between variable Y and the main explanatory variables X (microbial phytase, crude protein, amino acid, Ca, phytic acid and neutral detergent fibre concentrations) were studied using the following quadratic model:

$$\mathbf{Y_{ij} = \mu + \mu_i + \beta 1\ X_{ij} + \beta 2\ [X_{ij}]^2 + e_{ij}}$$

Where $\mathbf{Y_{ij}}$ is the dependent variable Y in experiment **i** with level **j** of microbial phytase; $\mathbf{X_{ij}}$ is the independent variable; $\boldsymbol{\mu}$ is the overall intercept across all studies; $\boldsymbol{\mu_i}$ is the effect of experiment **i** on the intercept $\boldsymbol{\mu}$ with the condition that $\Sigma\mu_i = 0$; **β1** and **β2** are respectively the linear and quadratic coefficients of the relationship and $\mathbf{e_{ij}}$ is the residual error. All statistical analyses were carried out using the MIXED procedure of SAS 9.4 software. All variables found significant ($P < 0.05$) were retained and the interactions between these variables were tested.

Table 1
Descriptive statistics in the databases.

Variables	N	Mean	S.D.	Min	Max
Independent variables					
Body weight (kg)	138	35.25	16.570	7.12	86.10
Feed composition					
Crude protein (g/kg diet)	138	177.70	34.840	101.00	273.35
Neutral-detergent fibre (g/kg diet)	132	125.80	39.740	34.57	231.91
Calcium (g/kg diet)	138	6.63	1.555	3.96	11.60
Phytic acid (g/kg diet)	138	2.26	1.003	0.09	4.90
Exogenous phytase (FTU/kg diet)	137	440.30	475.000	0.00	1894.00
Amino acid composition (g/kg diet)					
Arg	136	11.26	2.592	3.26	18.00
His	128	4.53	0.740	2.90	5.90
Ile	138	7.52	1.588	3.70	11.30
Leu	138	14.76	2.545	7.90	19.06
Lys	138	10.08	2.502	4.10	15.10
Met	128	2.94	0. 607	1.90	4.31
Phe	132	8.57	1. 495	5.50	11.50
Thr	128	6.64	1.210	4.10	9.30
Val	124	8.69	1.564	5.10	11.90
Cys	138	3.30	0.862	1.70	5.96
Tyr	125	5.57	1.314	2.60	8.13
Dependent variables					
Apparent ileal digestible amino acid (g/kg diet)					
Arg	131	9.70	2.247	4.54	16.87
His	124	3.69	0.586	2.15	4.91
Ile	138	5.93	1.264	2.83	8.64
Leu	137	11.83	1.954	6.04	16.56
Lys	131	7.95	2.211	2.83	12.88
Met	107	2.30	0.531	1.14	3.55
Phe	127	6.86	1.289	3.99	9.19
Thr	138	5.06	1.639	2.56	12.66
Val	131	6.55	1.654	1.06	11.55
Cys	83	2.20	0.545	1.39	3.30
Tyr	107	4.15	1.057	1.99	6.51
Apparent ileal digestibility (%)					
Arg	138	84.96	4. 527	71.19	93.70
His	138	80.37	5.102	63.40	90.70
Ile	137	78.87	5.339	65.82	89.90
Leu	138	80.00	5.289	63.40	89.21
Lys	138	79.24	6.247	62.10	90.70
Met	107	81.43	5.820	63.00	91.60
Phe	133	79.69	5.153	61.80	90.22
Thr	137	71.36	5.815	55.20	84.30
Val	133	75.45	5.278	64.00	87.20
Cys	84	74.61	6.049	60.90	85.40
Tyr	113	78.60	5.530	62.30	88.60

N = number of studies.
Arg = arginine, His = histidine, Ile = isoleucine, Leu = leucine, Lys = lysine, Met = methionine, Phe = phenylalanine, Thr = threonine, Val = valine, Cys = cysteine, Tyr = tyrosine.
Publications: (Mroz et al., 1994; Kemme et al., 1999; Traylor et al., 2001; Omogbenigun et al., 2003; Shim et al., 2003; Cervantes, 2004; Johnston et al., 2004; Fan et al., 2005; Liao et al., 2005a; Liao et al., 2005b; Nitrayová et al., 2006; Radcliffe et al., 2006; Nortey et al., 2007; Sands et al., 2007; Pomar et al., 2008; Woyengo et al., 2008; Nitrayová et al., 2009; Sands et al., 2009; Kiarie et al., 2010; Li et al., 2010; Cervantes et al., 2011; Sánchez-Torres et al., 2011; Yáñez et al., 2011; Zeng et al., 2011; Guggenbuhl et al., 2012; Morales et al., 2012; Olukosi et al., 2012; Almeida et al., 2013; Mok et al., 2013; Yáñez et al., 2013; Zeng et al., 2014; Adedokun et al., 2015; Kahindi et al., 2015).

2.3. Model evaluation

The leave-one-out cross-validation was used to obtain the RMSEP (root mean square error of prediction) from the value of the PRESS (predicted residual sum of squares) statistic (Causeur et al., 2003). The RMSE and the RMSEP have been used to test the accuracy of equations of predictions of the apparent ileal digestibility of amino acids and apparent digestible amino acid contents in the diet.

3. Results

The models were evaluated separately for the three types of microbial phytase included in the database (from *Aspergillus niger, Escherichia coli* and *Peniophora lycii*). The three types had similar positive effects on amino acid digestibility, they were thus combined

Table 2
Prediction of apparent ileal digestibility of indispensable amino acid (%) of pig feed as a function of microbial phytase activity, indispensable amino acid concentration and neutral detergent fibre concentration.

			P-value									
Amino acid	N	General regression equation (model)	Intercept	AA	Phytase	NDF	AA*AA	Phytase*Phytase	NDF*NDF	R^2	RMSE	RMSEP
Arginine	138	$Y = 86.1 + 0.75a + 0.30b - 8.00 \times 10^{-2}c - 1.60 \times 10^{-2}b^2$	< 0.001	< 0.001	< 0.01	< 0.001	NS	NS	NS	0.87	1.4	2.14
Histidine	138	$y = 93.75 + 1.43a + 0.29b - 0.29c - 1.70 \times 10^{-2} b^2 + 9.46 \times 10^{-4}c^2$	< 0.001	< 0.01	< 0.01	< 0.001	NS	< 0.05	< 0.001	0.91	2.8	2.17
Isoleucine	137	$y = 80.32 + 1.05a + 0.41b - 8.00 \times 10^{-2}c - 2.60 \times 10^{-2} b^2$	< 0.001	< 0.01	< 0.01	< 0.001	NS	< 0.05	NS	0.83	1.7	2.81
Leucine	138	$y = 66.54 + 3.05a + 0.43b - 7.00 \times 10^{-2}c - 0.10 a^2 - 2.80 \times 10^{-2} b^2$	< 0.001	< 0.05	< 0.01	< 0.001	< 0.05	< 0.05	NS	0.86	4.2	2.60
Lysine	138	$y = 73.90 + 1.30a + 0.44 b - 6.50 \times 10^{-2}c - 2.60 \times 10^{-2} b^2$	< 0.001	< 0.001	< 0.01	< 0.001	NS	< 0.05	NS	0.85	1.7	3.08
Methionine	107	$y = 93.65 + 2.17a + 0.27b - 0.24c - 1.80 \times 10^{-2}b^2 + 6.65 \times 10^{-4} c^2$	< 0.001	< 0.05	< 0.05	< 0.001	NS	< 0.05	< 0.001	0.94	2.6	1.99
Phenylalanine	133	$y = 80.34 + 1.04a + 0.35b - 8.00 \times 10^{-2}c - 2.00 \times 10^{-2} b^2$	< 0.001	< 0.001	< 0.01	< 0.001	NS	< 0.05	NS	0.87	1.6	2.33
Threonine	137	$y = 53.23 + 11.20a + 0.44b - 0.27c - 0.78a^2 - 2.70 \times 10^{-2}b^2 + 7.62 \times 10^{4}c^2$	< 0.001	< 0.01	< 0.01	< 0.001	< 0.05	< 0.05	< 0.01	0.84	3.3	3.33
Valine	133	$y = 79.27 + 0.40a + 0.43b - 6.40 \times 10^{-2} c - 2.90 \times 10^{-2} b^2$	< 0.001	NS	< 0.001	< 0.001	NS	< 0.05	NS	0.91	1.6	2.15
Tyrosine	113	$y = 77.80 + 1.47a + 0.44b - 6.40 \times 10^{-2} c - 2.60 \times 10^{-2} b^2$	< 0.001	< 0.001	< 0.001	< 0.001	NS	< 0.01	NS	0.93	1.5	2.23

N = number of studies.
Y = apparent ileal digestibility (%).
a = effect of amino acid concentration (g/ kg diet).
b = effect of added microbial phytase activity (FTU/kg).
c = effect of neutral-detergent fibre concentration (g/kg diet).
NS = effect not significant.

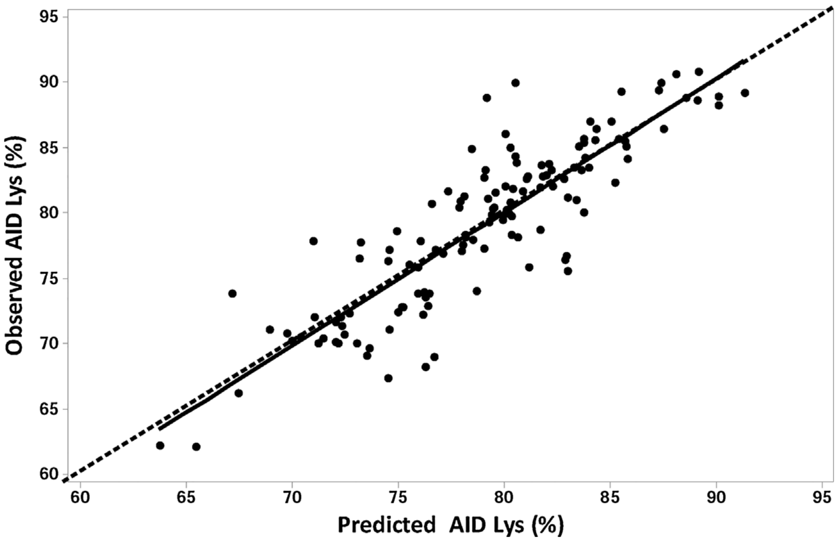

Fig. 1. Regression analysis of the relationship between observed apparent ileal digestibility of Lysine (Lys) and apparent ileal digestibility predicted by the model. Dashed line represents Y = X. Black = tested model.

in the final models. The effect of feed type (corn-soybean meal, cereal-soybean meal, corn/other protein sources, cereal/other protein sources, cereals, soybean meal, other protein sources) was also investigated in the initial steps but was not significant when feed composition was included in the model. Phytase and feed composition (indispensable amino acid and neutral detergent fibre contents) affected apparent digestibility independently of feed type. All feeds were then included in the final models.

3.1. Prediction of apparent ileal digestibility based on indispensable amino acids

The general regression equations for apparent digestibility based on the indispensable amino acids tested in this study all had an intercept different from 0 ($P < 0.05$, Table 2). The effect of amino acid concentration (a coefficient) was positive and linear for Arg, Lys, Phe, Tyr ($P < 0.001$), His and Ile ($P < 0.01$) and Met ($P < 0.05$) and both linear and quadratic for Leu ($P = 0.01$ and 0.02) and Thr ($P = 0.009$ and 0.02). The phytase effect (b coefficient) was linear and quadratic for all indispensable amino acids (linear $P < 0.01$ except for Met ($P = 0.01$); quadratic $P < 0.05$ except for Arg ($P = 0.06$)), with R^2 ranging from 0.83 for Ile to 0.94 for Met. Digestibility and neutral detergent fibre (c coefficient) were related negatively and linearly for all indispensable amino acids ($P < 0.001$). Linear and quadratic responses were obtained for His, Met and Thr (quadratic $P < 0.01$). The final models of digestibility for all indispensable amino acids thus included phytase activity and dietary composition (amino acids and neutral detergent fibre). The current models presented low RMSE (1.4–4.2) and a low RMSEP (< 3.4%). The assessment of the quality of prediction of the apparent ileal digestibility of lysine by the model showed a good accuracy (Fig. 1).

3.2. Prediction of digestible indispensable amino acid content

The general regression equations for digestible indispensable amino acid content are presented in Table 3; in these relations, the intercepts were not significant different from 0 except for methionine. The best predictors were indispensable amino acid content (a coefficient), use of microbial phytase (b coefficient), and neutral-detergent fibre content (c coefficient). The coefficient before the a corresponds to the increase digestible content for 1 g of total amino acid added. The relationship to fibre was linear for Arg, His, Ile, Leu, Lys, Thr, Val and Tyr (all $P < 0.001$) and Met ($P = 0.01$) and linear and quadratic for Phe (linear $P = 0.001$ and quadratic $P = 0.01$). The relationship to phytase was linear for Arg, Ile, Phe and Val ($P \leq 0.01$, $R^2 = 0.99$ for Arg, 0.97 for Ile, 0.98 for Phe and 0.85 for Val), His, Leu, Met, Thr, ($P \leq 0.05$, $R^2 = 0.98$ for His, 0.97 for Leu, 0.99 for Met and 0.97 for Thr) and both linear and quadratic for Lys (linear $P < 0.01$, quadratic $P < 0.05$, $R^2 = 0.99$) and Tyr (linear $P < 0.001$, quadratic $P < 0.05$, $R^2 = 0.99$). The final models of digestibility for all indispensable amino acids thus included phytase activity and dietary composition (amino acids and neutral detergent fibre). The current models presented low RMSE (0.2–0.7) and a low RMSEP (< 0.5%).

4. Discussion

The present work aiming using meta-analysis tool to better understand and predict the impact of microbial phytase on amino acid digestibility. This has been done by fitting multiple linear regression models that included explanatory variables (microbial phytase, crude protein, amino acid, Ca) of both the ileal digestibility coefficient and the digestible content of amino acids. Cowieson et al. (2017b) recently published a work where they estimate the effects of microbial phytase on ileal amino acids digestibility coefficient in pigs. The difference between the two articles lies in the statistical analysis approach. Cowieson et al. (2017b) used an empirical

Table 3
Prediction of apparent ileal digestible indispensable amino acid content (g/kg) in pig feed containing microbial phytase, as a function of indispensable amino acid concentration and neutral detergent fibre content.

Amino acid	N	General regression equation (model)	P-value									
			Intercept	AA	Phytase	NDF	AA*AA	Phytase*Phytase	NDF*NDF	R^2	RMSE	RMSEP
Arginine	131	$Y = 0.25 + 0.92a + 1.25 \times 10^{-2}b - 8.70 \times 10^{-3}c$	NS	< 0.001	< 0.01	< 0.001	NS	NS	NS	0.99	0.5	0.23
Histidine	124	$Y = 0.25 + 0.83a + 4.50 \times 10^{-3}b - 3.00 \times 10^{-3}c$	NS	< 0.001	< 0.05	< 0.001	NS	NS	NS	0.98	0.3	0.10
Isoleucine	138	$Y = -0.27 + 0.85a + 1.42 \times 10^{-2}b - 6.53 \times 10^{-3}c$	NS	< 0.001	< 0.01	< 0.001	NS	NS	NS	0.97	0.4	0.25
Leucine	137	$Y = 1.60 + 0.77a + 1.95 \times 10^{-2}b - 9.90 \times 10^{-3}c$	NS	< 0.001	< 0.05	< 0.001	NS	NS	NS	0.97	0.7	0.44
Lysine	131	$Y = -0.17 + 0.87a + 4.30 \times 10^{-2}b - 5.44 \times 10^{-3}c - 2.44 \times 10^{-3}b^2$	NS	< 0.001	< 0.01	< 0.001	NS	< 0.05	NS	0.99	0.5	0.28
Methionine	107	$Y = 0.24 + 0.85a + 2.50 \times 10^{-3}b - 4.18 \times 10^{-3} c + 9.95 \times 10^{-6}c^2$	< 0.05	< 0.001	< 0.05	< 0.05	NS	NS	NS	0.99	0.2	0.05
Phenylalanine	127	$Y = 0.53 + 0.92a + 3.20 \times 10^{-2}b\text{-}2.13 \times 10^{-2}c - 1.80 \times 10^{-3}b^2 + 6.50 \times 10^{-5}c^2$	NS	< 0.001	< 0.01	< 0.01	NS	NS	< 0.05	0.98	0.6	0.22
Threonine	134	$Y = 0.43 + 0.75a + 9.50 \times 10^{-3}b - 5.72 \times 10^{-3}c$	NS	< 0.001	< 0.05	< 0.001	NS	NS	NS	0.97	0.4	0.22
Valine	131	$Y = 0.40 + 0.78a + 2.60 \times 10^{-2}b - 5.55 \times 10^{-3}c - 1.26 \times 10^{-3}b^2$	NS	< 0.001	< 0.01	< 0.001	NS	NS	NS	0.85	0.4	0.17
Tyrosine	107	$Y = 0.12 + 0.85a + 2.20 \times 10^{-2}b - 1.20 \times 10^{-3}c - 1.27 \times 10^{-3}b^2$	NS	< 0.001	< 0.001	< 0.001	NS	< 0.05	NS	0.99	0.4	0.13

N = number of studies.
Y = apparent ileal digestible indispensable amino acid (g/kg diet).
a = effect of amino acid concentration (g/kg diet).
b = effect of added microbial phytase activity (FTU/kg).
c = effect of neutral-detergent fibre concentration (g/kg diet).
NS = effect not significant.

approach with many simple regression models for the analysis of factor effects on apparent ileal digestibility coefficients while the current paper use a more mechanistic approach to quantify but also rank the impact of main modulating factors by including all of them at the same time in the model.

Although many hypotheses have been advanced to explain the negative effect of phytic acid on feed digestibility, much uncertainty still shrouds the exact mechanism or mechanisms involved. It is generally accepted that negatively charged phytate ions form binary complexes with protein molecules, which bear a positive net charge at pH values below their isoelectric point, for example 4.7 in the case of certain proteins in soybean meal (Csonka et al., 1926). Above the isoelectric point, a cationic bridge (usually Ca^{2+}) links phytate to protein molecules bearing a net negative charge, thus forming ternary complexes.

Phytate-protein interactions appear to be key factors determining the effect of phytate on digestibility (Selle et al., 2012). These interactions can alter protein structure, thereby decreasing protein solubility, hydrolysis by proteases and hence utilisation (Humer et al., 2015). New data show that the six anionic phosphoryl groups (HPO_3^{2-}) of phytic acid have strong kosmotropic effects and can stabilise proteins by interacting with the surrounding aqueous medium (Selle et al., 2012). Phytate increases mucin secretion into the gut (Selle and Ravindran, 2008), which increases endogenous amino acid flux, since the protein component of mucin remains largely undigested. Phytate also increases Na^+ influx into the small intestinal lumen, an effect counteracted by phytase. Sodium appears to be the counter ion of HCO_3^- generated to buffer hyper-secretion of HCl which is described as endogenous aggressor (Selle et al., 2012). These observations showed that phytate stimulates mucin secretion and increases endogenous amino acid flows in pigs, which decreased the apparent digestibility of amino acids. These effects of phytate have been counteracted by the incorporation of phytase. The principal objective of this meta-analysis was therefore to study the effect of phytase on pig feed digestibility and to shed light on the factors modulating this effect. A positive linear relationship was found between digestibility and indispensable amino acid content except in the cases of Thr and Leu. For these two amino acids, a quadratic relationship was found. The dependence of the apparent digestibility of amino acid on the amino acid content has been reported previously (Fan et al., 1994). The apparent ileal digestibility increases with the dietary level of amino acid until reaching a plateau (Stein et al., 2007). This can explain the linear effect obtained for most amino acids in the present meta-analysis where dietary levels of amino acids were below the level to reach the plateau. The improvement in apparent digestibility due to the presence of larger amounts of amino acids in the feed is due to the mathematical effect of the dietary level of amino acid on the apparent values. In fact, when curvilinear shape of nutrient concentration and apparent digestibility was found it is the consequence of endogenous losses (Stein et al., 2007). The quadratic relationship between the apparent ileal digestibility of Thr and Leu and the dietary levels of amino acids may be explained by higher dietary level and lower basal endogenous losses of these amino acids.

Phytase was found to improve apparent digestibility for each indispensable amino acid. The effect of phytase on ileal digestibility of amino acids at the dose of 500 FTU/kg is described in Table 4. The main mechanisms responsible for the negative effect of phytic acid on digestibility thus appear to be formation of phytate-protein complexes in feedstuffs, de *novo* formation of phytate-protein complexes during digestion, and formation of phytate- protease complexes in the digestive tract (Selle et al., 2000; Adeola and Sands, 2003). The effect of phytase was independent of the amino acid content of the feed, indicating that variations in dietary level of amino acids did not influence the phytase effect. This effect of phytase on apparent digestibility of amino acids could come from a direct positive effect on amino acid digestibility or by reducing the flow of endogenous amino acids. These results suggest that phytase equivalencies in amino acid used for feed formulation would be independent of dietary level of amino acid.

In this meta-analysis, the best improvement in digestibility was obtained for Thr. In fact, the percent improvement is about twice as high as for Met, which is consistent with previous results (Cowieson et al., 2017b) in pigs and (Cowieson et al., 2017a) in broiler chickens. This effect supports the hypotheses that a major effect of phytase is a reduction of endogenous losses of amino acids caused by the anti-nutritive effects of phytic acid (Pirgozliev et al., 2011). Secretions in the ileum of pigs and chickens are particularly rich in Thr (Lien et al., 1997; Ravindran et al., 1999a) and do not vary with diet or the method of determination (Cowieson et al., 2009). According to one report (Montagne et al., 2004), 28–35% of the amino acid residues in porcine mucin are Thr, which represents 11% of the total basal endogenous losses. In broilers, increasing the phytate content of the diet from 8.5 to 11.5 and 14.5 g/kg generally increased endogenous amino acid flux in the ileum (Cowieson and Ravindran, 2007). A positive correlation ($r = 0.782$; $P \leq 0.01$)

Table 4
Effect of microbial phytase on apparent ileal digestibility of indispensable amino acids (%) at the dose of 500 FTU/kg.

Amino acid	N	Apparent ileal digestibility (%)		Response to phytase (%)
		Nil	Phytase 500 FTU/kg	
Arginine	138	87.10	88.20	1.26
Histidine	138	82.79	83.82	1.24
Isoleucine	137	79.67	81.07	1.76
Leucine	138	82.79	84.24	1.75
Lysine	138	80.08	81.63	1.94
Methionine	107	82.81	83.71	1.09
Phenylalanine	133	81.70	82.95	1.53
Threonine	137	74.03	75.55	2.06
Valine	133	76.47	77.90	1.86
Tyrosine	113	78.75	80.30	1.97

N = number of studies.

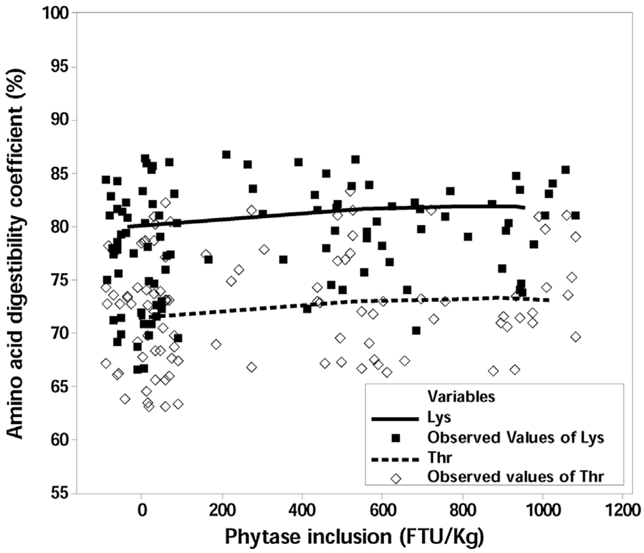

Fig. 2. Relationship between phytase inclusion and apparent ileal amino acid digestibility coefficients of Lys (Lysine) (full line) and Thr (Threonine) (dashed line).

was found between the phytase-induced reductions in endogenous amino acid flux and changes in the amino acid profile of mucin.

The effect of phytase appears maximal at 800 FTU/kg for most amino acids except Val and Met, for which a maximum is reached at 700 FTU/kg, and Phe at 900 FTU/kg. These results confirm what was found by Cowieson et al. (2017b) where dose responses for the effect of phytase on ileal amino acid digestibility was mainly beneficial from the dose of 250 FTU/Kg up 750 FTU/Kg. Phytase increased the digestibility of Thr and Lys up to a dose of 800 FTU/kg (Fig. 2) .The mean improvements found in this meta-analysis are consistent with those reported previously (Selle and Ravindran, 2008). The median response to 905 FTU/kg in cannulated pigs was 1.7% compared to 1.9% in the present study.

It should be noted that all of the studies in this analysis were conducted with cannulated pigs, in which the response to phytase does not appear as clearly as in slaughtered pigs. At 1.7%, the median response of cannulated pigs to 905 FTU/kg is somewhat smaller than the 6% in intact pigs given the same amount of phytase (Selle and Ravindran, 2008). Cannulation alters the gut micro-environment by affecting digesta pH and/or endogenous amino acid flux (Selle et al., 2000). In practice, cannulated pigs are generally meal fed, while intact pigs eat *ad libitum,* possibly explaining the different responses to phytase activity (Selle and Ravindran, 2008). In fact, Moter and Stein (2004) showed that higher feed intake increases apparent digestibility but increases also endogenous flow of amino acids, which reduces standardized digestibility. When pigs are restricted, standardized digestibility of amino acids would be already better limiting the effect of phytase. The equivalencies obtained for phytase therefore should be considered as minimal values that can be adjusted, in view of the stronger effect in intact pigs compared to cannulated pigs as discussed above.

The linear effect of phytase suggests that it should increase the digestibility of the indispensable amino acids in the feed at any level of supplementation. It is well known that phytase improves P digestion. Létourneau-Montminy et al. (2012) showed a quadratic effect of phytase on digestible P (g/kg) based on a database containing results for supplementation as high as 1.565 FTU/kg. However, a linear increase from 59.5% to 75.8% in P digestion as phytase supplementation increased from 1.000 to 20.000 FTU/kg has been observed (Zeng et al., 2014). Two mechanisms that might explain this are faster and more extensive breakdown of phytic acid and increased likelihood of phytase leaving the stomach in active form at larger doses (Kies et al., 2006). The linear effect of phytase observed in this study suggests that large doses of phytase might improve digestibility beyond the values reported for standard doses.

In the models that have emerged from the present analysis, neutral-detergent fibre has a negative effect on digestibility. In fact, this fibre was one of the best predictors, explaining 27% of the variance of apparent digestibility with respect to Lys and 66% with respect to His. Similar results have been obtained previously for standard digestibility of oilseed meal in the ileum (Messad et al., 2016).

A minimum level of dietary fibre is necessary given its important role in pig diets such as maintaining normal physiological functions and improving the functioning of the gastrointestinal tract (Wenk, 2001). As proposed by Conway (1994), fibers have three components influencing gut health, namely the diet, the mucosa and the commensal flora. The impact of the diet on gut health is important with beneficial or harmful effects (Jang et al., 2016). In fact, dietary fibre interact with mucosa and influences the composition and activity of microbiota (Williams et al., 2001), having an important beneficial effect on gut health control by providing protection against intestinal disorders, on intestinal functions and on the bacterial profile and fermentation. These effects are related to changes in the physicochemical characteristics of the digesta such as transit time, solubility, fermentability and water retention capacity of the digesta (Jha and Berrocoso, 2016).

It has been reported that the source and the amount of the fibre in a feed both affect the endogenous amino acid levels in the ileal digesta (Sauer and Ozimek, 1986). It has been shown repeatedly that fibre increases endogenous protein synthesis and losses of endogenous amino acids in the terminal ileum of monogastric livestock (Sauer et al., 1991; Nyachoti et al., 1997; Montagne et al., 2004). Due to its physical characteristics and abrasive action, fibre accelerates sloughing of the intestinal mucosa and induces mucus production by the gut mucosa (Schulze et al., 1995; Nyachoti et al., 1997). It can also adsorb peptides, amino acids and digestive enzymes, thus slowing digestion and absorption, which can induce an increase in endogenous secretions (Nyachoti et al., 1997). Although, fibre affects negatively the apparent ileal digestibility of all amino acids, its effect is more pronounced in apparent ileal digestibility of Thr due to its high concentrations in endogenous amino acids in pig as mentioned above. Increasing the fibre content from 100 g/kg diet to 150 g/kg diet generates a decrease on apparent ileal digestibility of Thr (−5.3%) compared to (−3.1%) for His.

It would therefore come as no surprise that fibre also reduces the effectiveness of phytase. As a constituent of cell walls, it appears to protect phytic acid from hydrolysis by phytase, an effect that has been countered by combining phytase with carbohydrases to obtain greater improvement of feed digestibility in pigs (Ravindran et al., 1999b; Debicki-Garnier et al., 2014). Although the effect of neutral-detergent fibre on digestibility appeared strong in the present study, no interaction between fibre and microbial phytase was found. This seems in contradiction with the hypothesized effect of phytase on endogenous amino acid losses. However, this may be due to the absence of intra-study variability in neutral-detergent levels, which were not reported in the publications and were therefore calculated (NRC, 2012), leading to less variance than was likely present in reality.

Phytic acid and calcium levels did not have any significant effect on digestibility in the models and no interactions with microbial phytase were found. In the case of phytic acid, varying up to a maximum of 4.9 g/kg of feed maybe insufficient to show its negative effect on digestibility. It has been shown that phytase-supplemented diets containing phytic acid in the range of 2.2–4.8 g/kg were of similar digestibility in terms of amino acids (Liao et al., 2005a). The level of calcium had little or no effect on the efficacy of phytase for liberating digestible P in the digestive tract of pigs (Létourneau-Montminy et al., 2012). Dietary Ca database values vary between 3.96 g/kg and 11.60 g/kg, which cover most part of the data used in the field in growing pigs. However, within studies, dietary Ca was generally maintained fix while protein, amino acids and phytase varied precluding to study it accurately. Other than the effect of the database, this absence of prediction of the impact of ternary complexes on the effect of phytase on digestibility of amino acids can be related to the animal model. Current models were based on cannulated pigs, so it will be important to study also the impact of the form of phytic acid on intact animals for a better understanding of the mechanism of action of phytic acid on amino acids digestibility.

It is premature to conclude that the lack of interaction noted in this study indicates that ternary complexes are not involved in the effect of phytase on feed digestibility. One of the aims of this study was to verify whether phytic acid concentration determines the ability of phytase to increase digestibility. We found no such relationship, nor any interaction between crude protein or calcium and the effectiveness of phytase supplementation. Improvements in digestibility in terms of indispensable amino acids therefore may be due more to the chemical properties of phytic acid and the proteins present than to their concentrations in feeds, as suggested previously in studies of pigs (Liao et al., 2005a) and broiler chickens (Ravindran et al., 1999a). Meanwhile, a significant positive interaction ($P < 0.001$) between phytic acid concentration and microbial phytase activity has been demonstrated in terms of P absorption (Létourneau-Montminy et al., 2012). This is expected since the release of P is the direct result of phytase activity. However, in the prediction models obtained in present analysis, no difference was found between the diets, suggesting that the form or the amount of the phytic acid present does not appear to modulate the improvements in digestibility brought by the action of phytase.

As previously mentioned, the difference between current models and models of Cowieson et al. (2017b) is based on the statistical analysis approach. The empirical approach used by Cowieson et al. (2017b) allow to quantify each factor independently but, it does not integrate all modulating factors and could not be a predictive approach. Contrariwise, the current models, with a mechanistic approach, integrate all dietary and animal factors modulating the amino acid digestibility and then could be used to predict the effect of phytase on ileal digestibility of amino acids.

5. Conclusion

This study shows that supplementing pig feed with microbial phytase does increase feed digestibility in terms of amino acid utilisation. However, no factors modulating the efficacy of phytase were present in the models, in keeping with the absence of interactions between the independent variables and phytase. On the other hand, the models allow quantifying of the effect of phytase on digestibility, which is important for optimizing the use of phytase to increase the yield of livestock feeds and decrease the amount of nutrient that ends up being a burden on the environment. As most diets are formulated on a standardized ileal digestibility basis, it is important to mention that the effect of phytase on apparent ileal digestibility basis would be equivalent when standardized ileal digestibility is considered. In fact, phytase affects probably directly the dietary amino acid digestibility or indirectly by reducing specific endogenous amino acids losses but not basal amino acid losses.

Conflict of interest

There is no conflict of interest.

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