Animal Feed Science and Technology 238 (2018) 18–28

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

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

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ARTICLE INFO

Keywords:
Amino acids
Digestibility
Phytic acid
Phytase
Pigs
Meta-analysis

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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https://doi.org/10.1016/j.anifeedsci.2018.01.019
Received 4 July 2017; Received in revised form 8 January 2018; Accepted 23 January 2018
0377-8401/

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.