**oP dephosphorylation by plant phytase**

A first-order kinetics equation **(Eqn. S1)** to represent the oP dephosphorylation by plant phytase was fitted to the data by Sauvant *et al.*, (2004). The equation shows a response where initially with increasing levels of phytase there is a nearly linear increase of rate of dephosphorylation, while at very high phytase levels the curve has reached an asymptote, indicating that all the “reactive” phytate has been dephosphorylated within the given time. Sauvant *et al.*, (2004) provided two values for apparent faecal P digestibility for feed ingredients with a significant endogenous phytase activity: wheat, wheat bran, rye, barley and triticale. The first value corresponds to the feed ingredient digestibility when phytase has been denatured, e.g. by exposing the feed ingredient to extreme heating. The second value, which is higher, corresponds to the same feed material when it was processed in a way that does not affect phytase activity, cool milling for instance. The difference between the two digestibility values was assumed to be the contribution of plant phytase activity by oP dephosphorylation. With a passive transport of 0.8 NPP digestibility from the lumen of the small intestine into the blood-stream (Gunther, 1978; Jongbloed, 1987), the oP dephosphorylation by plant phytase can then be estimated.

$ Plant\_{dephos}= KmaxPlant∙(1-e^{-Rplant ∙ FTU})^{}$ **(S1)**$Plant Phytase oP dephosphorylation\left(\frac{kg}{kg}oP\right)= KmaxPlant∙(1-e^{\left(-Rplant∙FTU\right)})$$ Plant\_{dephos}= KmaxPlant∙(1-e^{-Rplant\*FTU})^{}$$Plant\_{dephos}= KmaxPlant∙(1-e^{-Rplant\*FTU})^{}$$ \left(\frac{kg}{kg}oP\right)$

where, **Plantdephos** is the amount of oP dephosphorylated per unit of oP (kg/kg oP), **KmaxPlant** is the maximum ratio of oP dephosphorylation (i.e. the total amount of “reactive” phytate), with a value of 0.337, **FTU** is the phytase activity, defined as the amount of enzyme that liberates 1µmol of inorganic P in 1 minute from 5.1 mmol solution of sodium oP at 37oC at pH 5.5,and **Rplant** is the rate of the response of oP dephosphorylation against FTU (kg/kg oP), with a value of 0.00217. The coefficient of determination (**R2**) of the fitted relationship to the data of Sauvant et al, (2004) was 0.854 and the root mean square error (**RMSE**) was 0.0391 kg/kg oP.

**oP dephosphorylation by microbial phytase**

 Microbial phytases are currently used widely in grower and finisher pig diets. There are different types of microbial phytase, but this paper quantified the effects of two main categories of phytase enzymes: 3- and 6- phytases, derived from *Aspergillus niger* and *Escherichia coli* (Adeola *et al.*, 2006). The *E. coli* phytase has a single pH optimum range (2.5 to 3.5), which is different from the two pH optimals of 2.5 and 5.5 for the fungal 3-phytase from A*. niger* (Rodriguez *et al.*, 1999).

Quantifying the effect of microbial phytase enzymes on the oP dephosphorylation required studies that used graded levels of microbial phytase up to very high FTU, “super-dosing” (Cowieson *et al.*, 2011), in order to identify the rate (**R*E.coli*** and**R*A. niger***) and maximum ratio (**Kmax.A.niger** and **Kmax.E.coli**) of dephosphorylation, by fitting an exponential equation. The studies used for this purpose should entail feed ingredients, which contained minute, preferably no plant phytase enzymes, so as to solely investigate the effect of supplementation with microbial phytase enzymes. The model considered the dephosphorylation of oP by microbial and plant phytase in the stomach as being additive, in accordance to Zimmermann *et al.*, (2001), provided that oP is not a limiting substrate.

 Little research exists, other than the studies of Adeola *et al.*, (2004) and Kies *et al.*, (2006), on the addition of microbial phytase to diets at much higher levels than industry recommended ones (500-1500 FTU/kg), due to marginal returns per unit of supplemental microbial phytase. The *in vitro* study of Adeola *et al.*, (2004) investigated supplementation with *E. coli* phytase, whilst the *in vivo* study of Kies *et al.*, (2006) investigated *A. niger* phytase supplementation; both studies super-dosed the diets with microbial phytase.

Kies *et al.*, (2006) examined the apparent P digestibility, rather than oP dephosphorylation. Expressing the effect of microbial phytase activity in terms of total P digestion fails to take into account the potentially negative effect of dietary Ca and the digestion of dephosphorylated oP separately from the digestion of plant NPP. The difference of the two digestibility values, with and without microbial phytase is the contribution of microbial phytase activity by oP dephosphorylation. The digestibility of NPP from the lumen of the small intestine into the blood stream was set at 0.8 (Gunther, 1978; Jongbloed, 1987). A nonlinear response of supplemental phytase on oP dephosphorylation was observed for both phytases, **Supplementary Figure S2**. Once oP dephosphorylation was calculated, first-order kinetics equations **S2 and S3** were fitted to the observed results for *A. niger* and *E. coli*.

$A. niger\_{dephos}= KmaxA.niger∙(1-e^{-RA.niger ∙ FTU}) $$A. niger\_{dephos}= KmaxA.niger∙(1-e^{-RA.niger\*FTU}) $ **(S2)**

$E. coli\_{dephos}= KmaxE.coli∙(1-e^{-RE.coli ∙ FTU}) $$E. coli\_{dephos}= KmaxE.coli∙(1-e^{-RE.coli\*FTU}) $ **(S3)**

where, ***A. niger*dephos** and ***E. coli*dephos** are the amounts of oP dephosphorylated per unit of oP (kg/kg oP) by ***A. niger*** and ***E. coli***, respectively, while **Kmax*A.niger*** and **Kmax*E.coli*** are the maximum ratios of oP dephosphorylation (kg/kg oP) for ***A. niger*** and ***E. coli***, respectively, with a value of **0.562** and **0.532, FTU** is the phytase activity and **R*E.coli*** and **R*A.niger*** are the rates of the response of oP dephosphorylation against FTU (kg/kg oP) with values of **0.00104** and **0.00187** for ***A. niger*** and ***E. coli***, respectively. The **R2** of the fitted relationship was 0.920 and 0.932 for *A. niger* and *E. coli*, respectively, while the **RMSE**was 0.0324 and 0.0221 kg/kg oP, respectively. The fact that the maximum ratios of oP dephosphorylation are between 0.5-0.6 probably reflects the fact that oP becomes a limiting substrate at high level of exogenous phytase inclusion.

**Dietary calcium and phytate dephosphorylation in the small intestine**

In the absence of suitable pig data, the study of Plumstead *et al.*, (2008) with broilers was used to quantify the dephosphorylation of oP by endogenous small intestine phytase enzymes using graded dietary Ca levels. It is appreciated that this assumes that the same principles of P digestion apply across pigs and chickens, despite evidence to the contrary Applegate *et al.*, (2003). The linear equation derived was:

$SI\_{dephos}= Kmax\_{SI}-\left(R\_{SI}∙Ca\right)^{} $$SI\_{dephos}= Kmax\_{SI}-\left(R\_{SI}∙Ca\right)^{} $ **(S4)**

where, **SIdephos** is the amount of oP dephosphorylated per unit of oP that enters the small intestine (kg/kg oP), **Kmax.SI** is the maximum ratio of oP dephosphorylation, with a value of **0.261**, **Ca** is the dietary Ca in (g/kg); and **RSI** isthe slope of the response of oP dephosphorylation against Ca (kg/kg oP), with a value of **0.0158**. The **R2** of the fitted relationship was 0.834 and the **RMSE** was 0.0531 kg/kg oP. Equation (4) suggests a maximum oP digestibility of 26%, which is in agreement with the study of Jongbloed et al., (1992) and the suggestion of Létourneau-Montminy and Narcy (2010) for pigs.

**Dietary calcium and phytate dephosphorylation in the large intestine**

Studies, such as Sandberg *et al.*, (1993) using ileum cannulated pigs, have measured the oP complexes that exit the small and enter the large intestine, but do not distinguish between the inert Ca-oP complex and oP that enters the large intestine. They then measure the amount of oP going out of the large intestine into the faeces. The linear equation to express the dephosphorylation of the phytate which enters the large intestine was expressed as:

$LI\_{dephos}= Kmax\_{LI}-\left(R\_{LI}∙Ca\right)^{} $$LI\_{dephos}= Kmax\_{LI}-\left(R\_{LI}∙Ca\right)^{} $ **(S5)**

where, **LIdephos** is the amount of oP dephosphorylated per unit of oP that enters the LI (kg/kg oP), **Kmax.LI** is the maximum ratio of oP dephosphorylation with a value of **1.00**, **Ca** is the dietary Ca in g/kg; and **RLI** isthe slope of the response of oP dephosphorylation against Ca (kg/kg oP) with a value of **0.0756**. The **R2** of the fitted relationship was **0.792** and the **RMSE** was 0.**339** kg/kg oP.

**References**

Adeola O, Sands SJ, Simmins HP and Schulze H 2004. The efficacy of an *Escherichia coli*-derived phytase preparation. Journal of Nutrition 82, 2657-2666.

Adeola O, Olukosi AO, Jendza AJ, Dilger NR and Bedford RM 2006. Response of growing pigs to Peniophora lycii- and Escherichia coli derived phytases or varying ratios of calcium to total phosphorus. Journal of Animal Science 82, 637-644.

Applegate TJ, Angel R, Classen HL 2003. Effect of dietary calcium, 25-hydroxycholecalciferol, or bird strain on small intestinal phytase activity in broiler chickens. Poultry Science 82, 1140-1148.

Cowieson AJ, Wilcock P and Bedford RM 2011. Super-dosing effect of phytase in poultry and other monogastric. Journal of World Poultry Science 67, 225-236.

Günther KD 1978. New aspects of the mineral supply for breeding and fattening pigs. Concentrated Feed 61, 406-416.

Jongbloed AW 1987. Phosphorus in the feeding of pigs. Effect of diet on the absorption of phosphorus by growing pigs. PhD thesis. University of Wageningen, Netherlands.

Jongbloed AW and Evert H 1992. Apparent digestible phosphorus in the feeding of pigs in relation to availability, requirement and environment. 2. The requirement of digestible phosphorus for piglets, growing-finishing pigs and breeding sows. Netherlands Journal of Agriculture Science 40, 123-136.

Kies AK, Kemme PA, Sebek LBJ, van Diepen JTM and Jongbloed AW 2006. Effect of graded doses and a high dose of microbial phytase on the digestibility of various minerals in weaner pigs. Journal of Animal Science 84, 1169-1175.

Létourneau-Montminy MP and Narcy A 2010. Meta-analysis of phosphorus digestive and metabolic utilization by growing pigs: Effect of dietary phosphorus, calcium and exogenous phytase. Canadian Journal of Animal Science 90, 595–605.

Rodriguez E, Han Y and Lei GX 1999. Cloning, sequencing, and expression of an *Escherichia coli* phosphatase/phytase gene (AppA2) isolated from pig colon. Biochemical Biophysical Research Communications 257, 117-123.

Sauvant D, Perez JM and Tran G 2004. Tables of Composition and Nutritional Value of Feed. INRA. Wageningen Academic Publishers, Netherlands.

Zimmermann B, Lantzschaq JH, Mosenthina R, Biesalkib K and Drochnera D 2001. Additivity of the effect of cereal and microbial phytase on apparent phosphorus absorption in growing pigs fed diets with marginal phosphorus supply. Journal of Animal Feed Science and Technology 104, 143-152.