Sustainable animal protein production: how to optimize dietary phosphorus utilisation

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Introduction
Introduction

- As a key component of DNA, all known metabolic energy systems, and gatekeeper of cells as phospholipids, phosphorus (P) is an essential nutrient for all life forms.

- It is a non-renewable resource with no substitute (U.S. Geological Survey, 2019).

- The sustainability of animal production and of agriculture in general depends largely on its judicious use.
Introduction

• About 60% of the phosphorus occurs in bone at a fixed ratio with calcium (Ca), while the remaining portion is found mainly in muscle,

• An adequate supply of phosphorus is therefore essential for animal growth, health, and wellbeing, and this must be provided using efficient and sustainable means that minimize the phosphorus footprint of livestock production.

Precision P formulation systems
1. Precise estimation of the content of feed ingredients

2. Precise and robust establishment of requirements

3. Implement strategies to optimize P utilization
Precise estimation of P and Ca values of feedstuffs
P and Ca values of feedstuffs

- **Total dietary Ca and P content** in feed ingredients are routinely measured by chemical analysis.

- Do not indicate what portion the animals digest or retain or how much will be excreted.

- Although these drawbacks, it is still the preferred method for Ca, mainly because of the lack of knowledge on Ca bioavailability and especially the modulating factors.

- Works are underway in pigs to provide a more accurate Ca formulation system (Lee et al., 2019).

The P system is more precise with different expression modes.
Relative bioavailability

- Availability, is an indicator of the use of a nutrient based on a predefined criterion, frequently bone mineralization (She et al. 2017; Petersen et al. 2011).

- It is the slope ratio with a reference considered 100% bioavailable, usually monocalcium phosphate.

- The main disadvantage of this method is that it is not standardized and thus the criterion and the reference may differ (Lee et al., 2019).

Availability was added in NRC (1988) and used until 2012.

In broilers, this method is the one used in most countries in Europe since 2013.
Digestibility

• Digestibility refers to the quantity of nutrient that is not found in the feces or excreta = digested or has disappeared from the digestive tract.

Unlike other nutrients, in swine the digestibility of P and Ca is estimated over the entire digestive tract as fecal digestibility (TTD) since there are no differences between fecal and ileal digestibility (González-Vega et al. 2014; Dilger and Adeola 2006; Bohlke et al. 2005).
Digestibility

Used first for P values of feedstuffs in The Netherlands (CVB, 2000) and then in France (Sauvant et al., 2004) both as apparent total tract digestibility (ATTD).

- In 2012, the NRC has proposed another method, like the one use for amino acid, the standardized total tract digestibility (STTD) that has also been adopted by CVB (2016).

- The definition of digestibility must be nuanced according to whether endogenous losses are considered.
Digestibility

\[ \text{ATTD} = (\text{Ca or P intake}) - (\text{Ca or P feces}) / (\text{Ca or P intake}) \]

Standardized total tract digestibility (STTD) considers basal endogenous losses, which represent the minimal loss of a nutrient, independent of feed composition but influenced by dry matter intake (Stein et al. 2007; She et al. 2017).

\[ \text{STTD} = (\text{Ca or P intake}) - (\text{Ca or P feces} - \text{basal endogenous losses}) / (\text{Ca or P intake}) \]
Digestibility

In broilers, the WPSA have formed a group of experts that put in place methodologies and recommend the used of prececal digestibility for P feedstuff values (Rodehutscord et al., 2013)

\[
\text{pcdP} (\%) = 100 - \left[100 \times (\text{Diet Indigestible marker} \times \text{P Digesta}) / (\text{Digesta Indigestible marker} \times \text{P Diet})\right]
\]
Digestibility

This method, like the bioavailability gives a unique P and Ca value for each feedstuff regardless of the other components of the diet,

The well-know antagonism between Ca and P in the GIT tract through

1. The formation of insoluble Ca-Phosphate complexes
2. The formation of insoluble Ca-Phytate complexes
3. The regulations of intestinal absorption with Ca and P supply vs metabolic demand

Is not considered
Modeling approach

To precisely estimate the digestibility of dietary P in a complete diet, two approaches have been used based on available literature:

1. A *mechanistic* research mathematical model that simulates the fate of dietary P forms and Ca in the GIT was developed and evaluated (Létourneau-Montminy et al., 2011).

2. Given the large number of publications on P digestibility in pigs, meta-analysis tool was used to predict P digestibility considering dietary P forms, Ca and exogenous phytases *empirically* (Létourneau-Montminy et al., 2012; Couture et al., 2019).
Mechanistic approach
A mechanistic model simulating the fate of dietary P and Ca in the digestive tract of growing pigs has been developed using in vitro and in vivo data.
**Mechanistic approach**

A mechanistic model simulating the fate of dietary P and Ca in the digestive tract of growing pigs has been developed using in vitro and in vivo data.
Mechanistic approach

Model evaluation

ATTD P data available in literature before 2009, mostly Natuphos

\[ y = 0.98x + 0.24 \]

\[ R^2 = 0.90 \]
Mechanistic approach

Model evaluation

Phytase effect:

P: depends of PP, transit time and pH

Ca: depends of dietary PP, Ca and NPP (other cations ?)
Meta-analysis approach

- All ATTD P values published before 2009 in growing pigs
- Law of response of true digestible P (ATTD P % x total P g/kg).
- Dietary forms are PP and NPP from plant, mineral and animal.
- Mostly Natuphos phytase
**P and Ca values of feedstuffs**

### Meta-analysis approach

<table>
<thead>
<tr>
<th></th>
<th>Digested P (g/kg)</th>
<th>Coefficient</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td></td>
<td>-0.217</td>
<td>0.109</td>
<td>0.046</td>
</tr>
<tr>
<td>Body weight</td>
<td></td>
<td>0.00570</td>
<td>0.00198</td>
<td>0.004</td>
</tr>
<tr>
<td>Non-phytate P of mineral-animal origin</td>
<td></td>
<td>0.796</td>
<td>0.0156</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Non-phytate P of plant origin</td>
<td></td>
<td>0.731</td>
<td>0.0514</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Phytate P</td>
<td></td>
<td>0.208</td>
<td>0.0305</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PhytM&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>0.156</td>
<td>0.00864</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PhytM x PhytM</td>
<td></td>
<td>-0.0132</td>
<td>0.00153</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PhytP&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>0.0520</td>
<td>0.00691</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PhytM x PhytM x Phytate P</td>
<td></td>
<td>0.00298</td>
<td>0.000602</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Calcium</td>
<td></td>
<td>-0.0333</td>
<td>0.00970</td>
<td>0.001</td>
</tr>
</tbody>
</table>

- Number of experiments: 86
- Number of treatments: 377
- R²: 0.97
- RMSE: 0.161

Based on chemical analysis of Total P, PP and Ca we can assess ATTD P.
## P and Ca values of feedstuffs

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-0.0072</td>
<td>0.2003</td>
<td>0.971</td>
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<tr>
<td>Phytate P</td>
<td>0.219</td>
<td>0.083</td>
<td>0.01</td>
</tr>
<tr>
<td>Non-phytate P</td>
<td>0.762</td>
<td>0.023</td>
<td>&lt; 0.001</td>
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<tr>
<td>Non-phytate P x Non-phytate P</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Calcium</td>
<td>-0.0284</td>
<td>0.0153</td>
<td>0.066</td>
</tr>
<tr>
<td>Calcium x Non-phytate P</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>PhytM</td>
<td>0.1803</td>
<td>0.0281</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PhytM x Non-Phytate P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PhytM x PhytM</td>
<td>-0.026</td>
<td>0.0060</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PhytM x PhytM x Phytate P</td>
<td>0.007882</td>
<td>0.00214</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PhytM x PhytM x Non-phytate P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PhytM x PhytM x Calcium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PhytM x PhytM x Non-phytate P x Calcium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PhytP</td>
<td>0.064</td>
<td>0.016</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

**No CaxPP**

(Adeola et al., 2006; Narcy et al., 2010)

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Number of experiments: 86

Number of treatments: 377

$R^2$: 0.97

RMSE: 0.161
• Similar work is ongoing in broilers with the new P precaecal digestibility data

• Birds have the capacity to synthetize endogenous phytase (Applegate et al., 2003) in low Ca and P intake (Sommerfeld et al., 2019)

• Digestibility of PP maybe as high as 50-70% in broilers fed low CaP diets (Couture et al., 2019; Sommerfeld et al., 2019).

• Phytase effect is thus influence by CaP levels,

to be quantified soon
Precisely establishing P and Ca requirements
• According to the FAO (2001), a nutrient requirement is an intake level that will meet specified criteria of adequacy without risking deficit or excess.

• These criteria include an array of biological effects associated with the nutrient.

• In livestock production, a requirement is defined as the quantity necessary to maximize a production factor such as body growth or bone mineralization (Baker, 1986).
• In practice, growth alone is often a poor indicator of mineral status (Baker, 1986).

• Bone mineralization has long been the standard, but environmental issues have led several countries to review this and favor growth performance (e.g. NRC, 2012).

• In the case of Ca and P, requirement maybe define as growth according to genetic potential while ensuring optimal bone mineralization and keeping environmental risks minimal.

This means using a multicriteria approach
• Empirical approaches has been replaced by factorial in the beginning of the 90’s in swine but still used in broilers

• Factorial approach consists of quantification and the addition of the requirements for each physiological function (e.g., for maintenance and growth).

Several factorial methods estimating P and Ca requirements had been proposed such as Jondreville and Dourmad (2006), NRC (2012) and Bikker and Blok (2017) in swine.
All these model consider that P requirement follows an allometric relationship with soft tissue growth.
In pig (e.g. InraPorc; NRC, 2012) and poultry (Hauschild et al., 2015), growth models

\[
\text{Body weight} = \text{Protein} + \text{Lipid} + \text{Water} + \text{Ash}
\]

Water and Ash are estimated based on a fixed ratio with protein

Whittemore and Fawcett (1976)
Precisely establishing P and Ca requirements

Body weight = Protein + Lipid + Water + Ash

Whole-body ash

78% bone

39% Ca

18% P

22% soft tissue

17% P

Nielsen, 1973

Modifying dietary Ca and P supply will modify Body Ash
Recent data of pig body composition obtained by following pig growth using DXA scan showed clear disconnection between protein and bone mineral content deposition.
Precisely establishing P and Ca requirements

- For a more accurate representation of the metabolic fate of dietary Ca and P and ash retention, especially in pigs fed mineral deficient diets for bone mineralization, the ash and protein body components of current pig growth models need to be dissociated.
Strategies to improve P utilization
Improve mechanistic models
With the aim of representing the metabolic fate of dietary Ca and P and ash retention, a modelling approach has been performed in swine (Létourneau-Montminy et al., 2015; Lautrou et al., 2019, 2020)

Similar approach is ongoing in broilers
Létourneau-Montminy et al., 2015; Lautrou et al., 2019
Model evaluation

All data

$y = 0.978x - 2.7495$

$y = 0.9951x + 3.3995$

Ca body observed, g/pig

Ca body predicted, g/pig

P body observed, g/pig

P body predicted, g/pig
Model evaluation

Pigs at requirements

Model offers good prediction in pigs fed at requirements
Model inversion principles ([Doeschl-Wilson et al., 2006])

"Inputs become outputs"

Output = the amount of Ca and P to be absorbed
Prediction of P and Ca dietary recommendations

Input:
Initial body weight → Initial protein, lipid, water, ash

Soft tissue growth
- Water deposition
- Protein and lipid deposition

InraPorc (Van Milgen et al, 2008)

Lautrou et al., 2020
Prediction of P and Ca dietary recommendations

Input:
Initial body weight → Initial protein, lipid, water, ash

Soft tissue growth
- Water deposition
- Protein and lipid deposition

Body weight

Whole body ash growth
- Ca and P bone deposition
- Ca and P soft tissue deposition

Ca and P maintenance requirements

Outputs:
- ATTD P, STTD P
- ATTD Ca, STTD Ca, Total Ca

InraPorc (Van Milgen et al, 2008)

Predict P and Ca requirements

Lautrou et al., 2020
Prediction of P and Ca dietary recommendations

Unavoidable urinary loss:

- Ca = 2 mg/kg BW
- P = 1 mg/kg BW

Bikker and Blok, 2017

Lautrou et al., 2020
Prediction of P and Ca dietary recommendations

Input:
Initial body weight → Initial protein, lipid, water, ash

Soft tissue growth
- Water deposition
- Protein and lipid deposition

Whole body ash growth
- Ca and P bone deposition
- Ca and P soft tissue deposition

Outputs:
- ATTD P, STTD P
- ATTD Ca, STTD Ca, Total Ca

P and Ca deposition independent on protein

- Potential Ca deposition in bones, g/d
- Protein deposition, kg/d

Body weight, kg

0 50 100 150

0 0.05 0.1 0.15 0.2 0.25

0 2 4 6 8 10 12

Potential Ca deposition in bones, g/day

0 50 100 150

0 0.05 0.1 0.15 0.2 0.25

0 2 4 6 8 10 12

Protein deposition, kg/day
Prediction of P and Ca dietary recommendations

- **Default pig**: simulation of growth with InraPorc (Van Milgen et al., 2008)
- Simulation from **20 to 120 kg BW**
- Body P retention, ATTD P, STTD P, Total Ca

<table>
<thead>
<tr>
<th></th>
<th>NRC</th>
<th>INRA</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone mineralisation</td>
<td>85 %</td>
<td>100 %</td>
<td>85 or 100 %</td>
</tr>
<tr>
<td>Driving force</td>
<td>Protein</td>
<td>ADG</td>
<td>Potential CaBn</td>
</tr>
<tr>
<td>P requirement</td>
<td>STTD P</td>
<td>ATTD P</td>
<td>ATTD or STTD P</td>
</tr>
<tr>
<td>Ca requirement</td>
<td>Total Ca = 2.15*STTD P</td>
<td>Total Ca = 2.9*ATTD P</td>
<td>STTD Ca into Total Ca</td>
</tr>
</tbody>
</table>
Prediction of P and Ca dietary recommendations

![Graph showing body phosphorus retention vs body weight for different models.](image)

- INRA
- NRC
- Model 100%
- Model 85%
Prediction of P and Ca dietary recommendations

Body phosphorus retention, g/d

Bone mineralisation objective

Body weight, kg

INRA
NRC
Model 100%
Model 85%
Prediction of P and Ca dietary recommendations
Prediction of P and Ca dietary recommendations

![Graph showing total calcium requirements vs body weight for different models: INRA, NRC, Model 100%, Model 85%](image)

- **Total calcium requirements, g/kg**
- **Body weight, kg**

- **INRA**
- **NRC**
- **Model 100%**
- **Model 85%**
Prediction of P and Ca dietary recommendations

![Graph showing the relationship between body weight and total calcium/phosphorus ratio for different sources: Total Ca/STTD P, Total Ca/ATTD P, NRC, and INRA.](image-url)
Optimizing the use of phytic P
Optimizing the use of phytic P

- Microbial phytases has been greatly improved since their arrival in the market in the 90’s, with P equivalency that are close to double for new generations.

- For optimal action, phytic acid must be hydrolyzed upstream from the sites of absorption of P and other minerals such as Ca, Zn and Fe.

- P is absorbed mostly in the upper small intestine (Crenshaw 2001; J. Liu et al. 2000).
Considering this, the improvement has been possible by improving key factors for the efficiency of the enzyme such as:

1. Activity at stomachal pH
2. Thermostability
3. Speed of degradation in the stomach = Vmax
4. Susceptibility to protease hydrolysis
5. Higher affinity for IP₆ and IP₅
Interestingly, this higher affinity for IP₆ and IP₅, which have higher affinities for Ca, results in a ratio of Ca/P released using new generation phytases that is around 2 at 500 FTU/kg and decreases as phytase activity increases (Adeola and Cowieson 2011; Cowieson et al., 2011; Walk, 2016).

The phytase levels practiced in the field may therefore lead to an increase in the digestible Ca/P ratio.
Optimizing the use of phytic P

However, it should be noted that phytase acts on soluble phytate so phytate solubility is another important point to consider.

Cations have an inhibitory power related to their affinities for phytic acid but also the insolubility of the complexes they formed.
Phytate solubility

According to Maenz et al. (1999) in vitro the ranking is

\[
\text{pH 6: } \text{Fe}^{2+} > \text{Zn}^{2+} = \text{Fe}^{3+} > \text{Mn}^{2+} >> \text{Ca}^{2+} > \text{Mg}^{2+}
\]

\[
\text{pH 5: } \text{Fe}^{3+} > \text{Fe}^{2+} > \text{Zn}^{2+} >> \text{Mn}^{2+} > \text{Ca}^{2+} >> \text{Mg}^{2+}
\]

Reducing the pH to 4 strongly reduces the power of all minerals tested.

Phytase does not act on cations in the stomach when it hydrolyzed PP
Phytate solubility

In fact, Phytic acid can act as a Ca-binding agent under the pH conditions in the DSI (Graf, 1983). So phytase avoid the insolubilization of mineral Ca to PP.

This means that Ca matrix should depend on dietary Ca, in really low-Ca diet phytase maybe limiting in Ca liberation potential.
Many studies have showed that in vivo Ca did not reduced phytase efficiency in releasing P although it may bind phosphate and reduced absorption (e.g. Driver, 2005; Adeola et al., 2006; Létourneau-Montminy et al., 2012).

When Zn is use at high doses some studies showed a decreased of P release by phytase (Augspurser et al., 2004).

As the complexes are frequently PP-Zn-Ca more attention should be paid to Ca releases by phytase especially in low Ca strategies in piglets receiving high doses of Zn.
Depletion repletion strategies
▪ Animals can enhance digestion and metabolic utilization of some minerals to overcome deficiencies (Underwood and Mertz, 1987).

▪ Dietary restrictions in Ca and P results in the regulation of their intestinal absorption, renal reabsorption, and deposition and mobilization in bone tissue, in several species (Suttle, 2010).

▪ The Ca depletion-repletion strategy is a strategy already used in dairy cows to priming them for the high Ca demand in early lactation (Goff 2006).
Depletion-repletion strategies

**Depletion**: trigger regulations by depleting bone reserves to induce an increase in P utilization efficiency without causing a deficiency strong enough to decrease growth performance.

**Repletion**: intakes are at least at requirement to allow a catch-up of the bone deficit. This allows for a reduction in dietary P intake over the overall rearing phase.

Depletion in Ca and P can be used to increase P utilization efficiency
Some work has been done

in pigs (Gonzalo et al., 2018; Létourneau-Montminy et al., 2014; Varley et al., 2011; Ryan et al., 2011; Aiyangar et al., 2010)

in broilers (Yan et al., 2005; Ashwell and Angel, 2010; Rousseau et al., 2016; Valable et al., 2018, 2020)

• to develop an effective strategy to limit P utilization and excretion without compromising animal welfare and performance

• to study the ability of this strategy to improve bone health
Depletion-repletion strategies

LLC vs CCC
↓ 40% phosphate intake
↓ 18% P excretion

C = 100% requirements P-Ca
L = 60% requirements

(Létourneau-Montminy et al., 2014) (Yan et al., 2005)
Depletion-repletion strategies

Bone mineral content body (g)

- CCCC
- CLCC
- CCLC
- LCLC
- LLLL

Feeding phases

P2  P3  P4

C = 100% requirements P-Ca
L = 60% requirements P-Ca

N.S.

(Gonzalo al., 2017, 218)
Depletion-repletion strategies

Bone mineral content body (g)

Body weight (kg)

P genes exp kidney

(Gonzalo al., 2017, 218)
Calcium regulations call for PTH which is a hypercalcemic hormone and thus allows a better use of dietary Ca, but with a hypophosphoremic action by promoting renal excretion of P.
Depletion-repletion strategies

*Modulating factors:*

- P vs Ca deficiency (Gonzalo et al., 2018)
- Acuity of the deficiency (Aiyangar et al., 2010)
- Bone region, with vertebrae and femur more sensitive than metacarpal and whole-body (Gonzalo et al., 2018; Varley et al., 2011)
- Repletion length and Ca and P supplies (Létourneau-Montminy et al., 2010)
Precision feeding
In conventional group-phase-feeding systems, all pigs receive the same feed during long periods.

Pigs are heterogenous and have different nutrients requirements and they vary among animals and change over time.
Precision feeding

Increasing nb phases reduce excess

Nutrient requirement (%)

Phase 1
Phase 2
Phase 3
Precision feeding

Diet rich in nutrient

Diet poor in nutrient

Pomar et al., 2015
Precision feeding

Automatic and Intelligent Precision Feeder (AIPF)

BW + ADG  Daily feed intake

Feed

A  B

transponder

Wires Transponder detection

Pomar et al., 2015
**Precision feeding**

In precision feeding systems, pigs are fed with diets tailored daily to its individual nutrient requirements and thus save nutrients. Feeding costs can be reduced by more than 4.6%, and nitrogen and phosphorus excretion by more than 38%.

Andretta et al., 2014
Conclusions and perspectives
Conclusions and perspectives

We can still improve P utilization by swine and poultry with strategies combining

- Precise estimation of P values of feedstuffs
- Precise estimation of requirements of group of animal and also individual
- Using efficient phytase
- Priming the animal to make them more efficient
- Develop precision feeding tools
Nevertheless, all these efforts of predicting precisely the use and the excretion of P should now be link with soils fertilisation.

Systemic approach should be develop to produce sustainable animal protein.
Merci!

Questions?

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