# Invited Speakers

**S1.1**
**Approaches and Challenges for Evaluating Phosphorus Sources for Poultry**
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Phosphorus (P) is an element with special relevance for sustainable food production. All animal species have a specific requirement for P. Excretion of P may negatively affect the environment, and the global raw phosphate stores are limited. Therefore, responsible handling of P sources is necessary along the entire food chain. An optimised use in poultry feeding needs to consider the great differences in P availability between P containing raw materials and the efficacy of phytase supplements. However, the confusion about what P availability is and how it should be measured is great. This presentation gives an overview of the most relevant approaches, along with their respective advantages and disadvantages. If the scientific community agrees that the confusion is a disadvantage that hinders an efficient use of research results then coordinated activities are needed for developing a comprehensive system of available P. This has to link a requirement module with a feed evaluation module, which is best to achieve on quantitative P retention measurements. Suggestions for how to proceed are given.

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**Keywords**: phosphorus; evaluation; availability; retention; bones

## Introduction

All animals need to absorb phosphate from the intestine to cover their requirement. The amount needed varies largely and depends on animal factors such as species, age and targeted level of performance. This topic will be dealt with in other presentations of the symposium and can therefore be neglected in this contribution. The concentration of total phosphorus (P) in the diet not only depends on these animal factors but also upon the raw materials that are used in mixing the diets, in particular the sources and binding forms of P, and the presence of intrinsic or added phytase.

Avoiding deficiencies is a major objective in optimising the dietary P concentration, but other issues have also become relevant. Not only may phosphate be accumulated in the soil and leached if the concentration becomes too high, with negative consequences for surface waters such as lake eutrophication. It is also a fact that global raw phosphate resources, which are essential for feed phosphate production, are limited. Prices for feed phosphates sharply increased in 2008, which may indicate limitations in the feed phosphate production process. Europe has hardly any raw phosphate deposits. The ban of animal by-products from feeding, although loosened in the meantime, led to phosphate being irreversibly withdrawn from the nutrient cycle (Rodehutscord *et al.*, 2002a). In consequence, responsible handling of P sources is an important issue of sustainable agricultural production in general and livestock production in particular.

The more precise the supply of dietary P should be adjusted to the specific requirement of available P, the better the knowledge about the availability of P from feed ingredients has to be. Also, phytase products need to be given a "replacement value" or an equivalency for a certain amount of available P. While these statements in their general form do probably not provoke disagreement, different definitions of availability exist and different methods to measure availability are used. This sometimes causes confusion when P availability is discussed and limits comparability of data. It also encumbers the compiling of feeding tables using data from different countries or laboratories.

This presentation gives an overview of techniques that are used in measuring P availability, and their advantages and disadvantages. The need for a harmonisation of methods is discussed and an example of a possible standard method is given.

## Differentiation by chemical analyses

It is well known that phytate is the predominant source of P in plant-based raw materials. Since birds produce hardly any endogenous phytase, their ability to utilise P from phytate is very limited. The analytical differentiation of total P and phytate P and the calculation of non-phytate P (NPP), implemented by committees such as NRC (1994) and GfE (1999), therefore already was a substantial improvement in the accuracy of P evaluation for poultry. It implied the assumptions that phytate P is completely unavailable and NPP is available at a constant proportion of 70% irrespective of its source. In the meantime studies have shown that both assumptions are only rough approximations. Phytate P can be utilised to a certain extent, for example if raw materials contain intrinsic phytase (Oloffs *et al.*, 2000). Also, the utilisation of NPP is not constant but varies in dependence on source (e.g. De Groote and Huyghebaert, 1997; Rama Rao and Ramasubba Reddy, 2001; Wendt and Rodehutscord, 2004).

Available P and NPP do not mean the same, although in the literature theses terms are often used synonymously. The following definition of available P is suggested here.

Available P is the proportion of dietary total P that, at marginal levels of P supply, can be utilised to cover the P requirement of an animal. It is a quality criterion and describes the potential of a diet or a raw material.

This definition *per se* is not linked to a certain method of determination. Different approaches are used by different laboratories.

**Differentiation by involving animal studies**

The measurement of P availability needs animal studies. Reliable *in vitro* approaches may be available in future for estimating P availability, but their development and validation also depend on animal studies.

Different animal approaches are in use. Irrespective of the approach, it is of crucial importance that, if the potential of a P source is to be studied, the dietary P level is below the requirement. P intake above the requirement makes the animal excrete a higher proportion, which has nothing to do with the quality of the P source under test. Hence, the adjustment of the P level in the diet needs special consideration, because the necessary available P concentration in the diet for growing poultry continuously declines with ageing of birds. The "upper tolerable level" of P in availability studies is therefore not a constant but depends on species and age. It is the lower the older the birds are (Rodehutscord, 2006).

In availability studies the P source under test usually is added to a low-P basal diet. Raw materials must be chosen in a way that feed intake is high enough and the available P level low enough to allow for a P supplement ideally on more than one level. Interactions between the basal diet and the P source under test should be avoided by selecting the right raw materials. The availability of the test P source can then be calculated by difference or by linear regression. Very often basal diets based on maize and soybean meal are used (Kornegay *et al.*, 1996; Potter *et al.*, 1995). Phosphorus availability is low in these feeds and intrinsic phytase is hardly found. A further reduction in the basal P level is possible by using alternative sources of energy (e.g. starch) and protein (e.g. potato protein, egg albumen) with lower P concentrations, or free amino acids (Dänner and Bessei, 2002b; Rodehutscord and Dieckmann, 2005). This increases the experimental costs, but is needed in studies that use birds with a low P requirement.

The basal diet must contain all other nutrients, i.e. calcium and vitamin $D_3$, in concentrations that do not limit the utilisation of P. Wendt and Rodehutscord (2004) suggested a Ca:P ratio of 2:1 in P availability studies with ducks. Dieckmann (2004) found negative effects on P utilisation in broilers when the Ca:P ratio was higher than 2.2:1, but did not find negative effects at ratios as low as 1.5:1.

In growing poultry differences exist between species. Using the same experimental approach, it has been found that the utilisation of plant P is higher in broilers and quails than in ducks and turkeys, while the utilisation of mineral P is higher in ducks than in the other three species (Rodehutscord and Dieckmann, 2005). It is unlikely that all raw materials can be tested in all species within a reasonable period of time. But more comparative studies are needed before conclusions can be drawn as to what extent data from one species can be used in another species and to what extent safety margins must be considered. Dänner and Bessei (2002b) hypothesised that the quail is a suitable model for P availability in poultry, and the study of Rodehutscord and Dieckmann (2005) did in fact show very similar values for quails and broilers.

*Quantitative measurement of P retention.* Retention studies can be used as a measure of availability. One way to measure the retention of P is in balance studies where both feed intake and amount of excreta are quantified for a certain period of time, commonly not less than five days. Before excreta collection starts, a pre-feeding period of at least five days (Dänner and Bessei, 2002a) is needed to allow the birds to adjust in P excretion to the respective level of P intake. P retention is calculated as the difference between intake and excretion. Leske and Coon (2002) called this "retainable P". Sometimes it is called "digestible P" instead, which is less accurate. A system based on this approach, including requirements on the basis of retainable P, was introduced in The Netherlands about 10 years ago and has been in use since then (van der Klis and Kwakernaak, 2008).

A P source can be used at different levels of inclusion and the availability calculated by regression analysis. The experimental effort to quantify excretion is high, but the measurements are very precise and can be directly interpreted as P availability. Also, the number of animals needed is relatively low, and the same animals can be used for repeated measures. If the quantitative collection of excreta is not possible, percentage P retention can be measured based on spot sampled excreta if an indigestible marker is used (Leske and Coon, 2002), including all the advantages and disadvantages of the marker technique.

Quantification of P retention can also be made on the basis of comparative whole body analysis (Dieckmann, 2004). The P concentration in body homogenates is analysed after a period of feeding, and the total amount of P in the body is calculated. From this the amount of P contained in control birds homogenised at the start of the experiment is subtracted. This procedure is also very precise. It does not need metabolic cages. But the experimental effort for getting representative body homogenates is high.

*Measurement of precaecal digestibility.* The digestibility until the end of the ileum (precaecal (pc) digestibility) is an established method for measuring protein quality in poultry. It is preferred because values are unaffected by post-ileal microbial activity. It also implies that the contribution of the urine can be excluded. This can be of advantage in studies on P availability, because urinary P excretion is the major pathway if intake is above the requirement but is negligibly low below the P requirement. The intestine section that is used for collecting digesta must not be too long to ensure that P absorption is complete (Witzig *et al.*, 2006). Samples from several animals (we prefer 10 at the age of 3 weeks) are pooled into one sample. This is necessary to get sufficient material for the chemical analyses and also to reduce variation.

First comparative studies in our group have provided evidence that the precaecal digestibility can be developed as an alternative tool to measure P availability, with the advantage of being less sensitive to the P level of the diet. While the total P excretion increased non-linearly with rising intake above the P requirement, the P flow at the terminal ileum responded in a continuously linear manner (Figure 1). Transport mechanism studies with laying hens have shown evidence that an adaptation of the intestinal tissues in absorption or endogenous secretion or both to variable P intake is hardly relevant whereas the kidney is the important organ for the regulation (Huber *et al.*, 2006). This hypothesis still needs further investigation in regard to growing poultry.

In laying hens the required available P concentration in the diet is much lower than in growing poultry. This makes it difficult to study availability in hens on the basis of total excretion. Precaecal measurements are therefore more suitable to measure P availability in hens (van der Klis *et al.*, 1997; Rodehutscord *et al.*, 2002b).

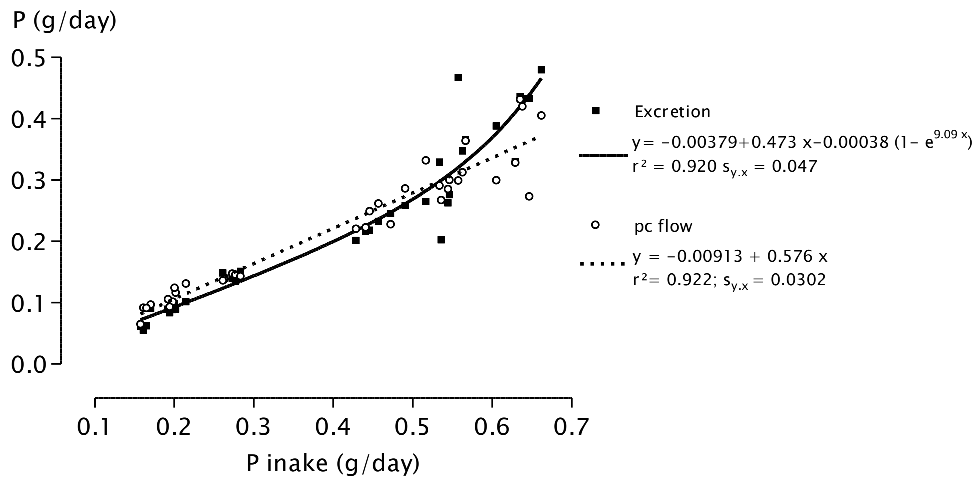


**Figure 1**: Effect of MCP supplementation on total excretion and precaecal (pc) flow of P in 3 week old broilers (unpublished)

*Measurements of bone data.* Roughly 80% of total P is retained in bones. In consequence, bone data are widely used as indicators of P availability. In poultry, tibia and toe are often used for this purpose. The bones are analysed for their ash content, sometimes also for their P and Ca content. Indirect criteria such as breaking strength, density, etc. are also used.

Experiments are usually made with different supplements including a "reference" supplement (e.g. sodium phosphate). Bones show a specific response to each supplemented P source. In the simplest case this can be described by linear regression, and the evaluation of the sources is made by a comparison of slopes ("slope-ratio-technique") (Hurwitz, 1964; Lima *et al.*, 1997; Potter *et al.*, 1995), sometimes called "relative bioavailability". The outcome of an experiment depends, therefore, upon the quality of the reference P source. Other details of the experimental procedures, particularly the duration of the study, are also relevant. Hatchlings respond very sensitive within a few days. Older birds already have a partially developed skeleton and it takes longer to provoke a response in bone measurements.

While bones can show differences in the response to different P sources, they cannot be directly taken as a measure of P availability. Recently we have tried to compare the outcome of bone measurements and retention studies by a meta-analysis of literature data. Correlations between responses in toe and tibia ash and even growth with responses in P retention from balance studies were significant (Tab. 1). But the correlations were on a low level and do not indicate that the relation is strong enough to re-calculate P availability from bone data. However, the variation in responses between individual studies was very high. Projects are needed that combine different approaches in one study and then compare the responses of birds.

Tab. 1: Correlations between responses in P retention (based on balance data) and bone responses in studies using supplements of microbial phytase or mineral P sources (unpublished meta-analysis)

| | Δ Toe ash | Δ Tibia ash | Δ Body weight gain |
|---|---|---|---|
| Δ P retention | 0.44*** | 0.73* | 0.19* |
| Δ Toe ash | - | 0.73** | 0.34*** |

***p<0.001; **p<0.05; *p<0.10.

Another question of concern in this context is to what extent the P content in individual bones is representative of the total P content in the body. If the correlation is close enough, bone data might be suitable for estimating whole body P. Hurwitz (1964) reported that the ratio between tibia P and whole body P is 1 to 19.6, but did not explain the origin of this ratio in detail. This aspect should be re-visited, using modern genotypes of different age.

*Blood phosphate concentrations.* The concentration of inorganic phosphate ($P_i$) in the blood is, in contrast to blood calcium, very variable. Especially below the P requirement the blood $P_i$ concentration responds to differences in available P intake. Hurwitz (1964) supplemented two different mineral P sources to a low-P basal diet and measured a linear response in both tibia P and blood $P_i$. The ratio of slopes for the two supplements was similar for blood $P_i$ and tibia ash. However, it is not possible to conclude that blood $P_i$ can be used to estimate P availability.

**Conclusions and suggestions**

In order to gain clarity and to enable the comparability of data the following is suggested:
1. The scientific community agrees on a definition of "availability".
2. The scientific community agrees on a system of available P which includes requirement data and raw material values, both modules closely linked together. It is the opinion of the author that this linkage can best be achieved by a system based on P retention measurements.
3. Requirement data are modelled on the basis of the factorial approach. Differences between species, growth rate and age are considered.
4. A standard protocol for animal studies on P availability and phytase efficacy is developed.
5. Research cluster on the comparability of methods and on the development of *in vitro* approaches for estimating P availability are established.
6. Working group number 2 of WPSA is the born committee to initiate, coordinate and push the mentioned activities.

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