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Phosphorus digestibility and metabolizable energy concentrations of contemporary cereal grain varieties fed to growing pigs

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Meiner Familie

SUMMARY

Phosphorus digestibility and metabolizable energy concentrations of contemporary cereal grain varieties fed to growing pigs

Cereal grains are a major feedstuff in pig nutrition across the world. Due to their high starch content and their high inclusion rates, they supply the majority of energy in diets for pigs. At the same time they also provide a major part of phosphorus (P). Because most P in plant seeds is present in the form of phytate-P, which is only poorly digestible by pigs, excess excretion of P occurs primarily via faeces. To minimize P excretion an exact knowledge of digestible P (dP) content is indispensable for diet formulation. A rapid and cheap *in vitro* system for exact estimation of dP content of single batches of different feedstuffs is of great importance in practice.

Because there can be larger differences of P-digestibility between different types of cereal grains and within one type of grain, the extent of differences of P-digestibility and metabolizable energy (ME) concentrations were evaluated. Eight genotypes each of barley, rye, triticale and wheat were analyzed for apparent total tract digestibility (ATTD) of P and ME concentrations. This was done using the difference method with quantitative collection of excreta from fattening pigs.

The mean *in vivo* ATTD of P was 0.444 for barley, 0.449 for rye and 0.504 for triticale. Highest mean ATTD of P was observed for wheat (0.573). Within one grain type, significant differences for ATTD of P were found for triticale. No significant differences were found within the eight genotypes of barley, rye and triticale. Highest mean ME concentrations were found for triticale and wheat (16.1 and 16.2 MJ ME/kg dry matter (DM), respectively). Mean ME concentrations for barley (14.9 MJ ME/kg DM) and rye (14.8 MJ ME/kg DM) were significantly lower. Within one type of grain there were significant differences for barley, triticale and wheat.

For prediction of dP-values the 32 cereal grains and further 22 feed samples were analyzed for their content of hydrolyzable P (hP). The same samples were used for determination of dP content. Regression analysis showed strong correlation between dP and hP-values. By determination of hP the dP content could be predicted with a coefficient of determination (R^2) of 0.88. [(r = 0.809): dP (g/kg DM) = –0.125 ($P > 0.10$) + 1.482 ($P < 0.01$) × hP (g/kg DM)].

With precise knowledge of dP content, diet formulation can be made to match requirements closely. Supplementation with mineral P can be minimized, which has an impact on the world's reserves of rock phosphate. At the same time, P excretion via excreta can be reduced and the negative environmental effects of the P content in manure will be minimized.

KURZFASSUNG

Phosphorverdaulichkeit und Gehalte an umsetzbarer Energie aktueller Getreidegenotypen beim wachsenden Schwein

Getreide stellen in der Ernährung von Schweinen weltweit die bedeutendste Komponente dar. Durch den hohen Stärkeanteil im Getreidekorn und die hohen Getreideanteile in Rationen sind die verschiedenen Getreidearten wichtige Energie- und Phosphor-(P)-lieferanten im Schweinefutter. Da P in pflanzlichen Samen jedoch zu einem großen Teil in Form von Phytin-P vorliegt und von Monogastriern nur unzureichend genutzt werden kann, kommt es zu erhöhten Ausscheidungen des P vor allem über den Kot. Um diese Ausscheidungen zu minimieren, ist schon bei der Rationsgestaltung die genaue Kenntnis der Gehalte an verdaulichem P (vP) in den Futtermitteln unabdingbar. Eine schnelle und kostengünstige Labormethode, mit der die Gehalte an vP einzelner Futtermittelchargen genau bestimmt werden können, ist daher für die Praxis von großer Bedeutung.

Da die P-Verdaulichkeit zwischen und innerhalb verschiedener Getreidearten in großem Umfang schwanken kann, sollten systematisch die Unterschiede in der P-Verdaulichkeit und den Gehalten an umsetzbarer Energie (ME) geprüft werden. Hierfür wurden jeweils acht Genotypen von Gerste, Roggen, Triticale und Weizen sowohl auf ihre P-Verdaulichkeit als auch auf die ME-Gehalte in einem Differenzversuch an wachsenden Schweinen geprüft.

Die mittlere P-Verdaulichkeit der jeweils acht verschiedenen Genotypen lag bei Gerste bei 44,4 % (±4,40), bei Roggen bei 44,9 % (±2,12) und bei Triticale wurde eine mittlere P-Verdaulichkeit von 50,4 % (±3,83) ermittelt. Weizen zeigte mit einem mittleren Wert von 57,3 % (±2,73) die höchste P-Verdaulichkeit der vier untersuchten Getreidearten. Bei Triticale wurden Unterschiede zwischen den verschiedenen Getreidegenotypen festgestellt. Innerhalb der übrigen Getreidearten gab es keine Unterschiede. Die höchsten ME-Gehalte wurden mit 16,1 (±0,38) und 16,2 (±0,24) MJ ME/kg Trockenmasse (TM) bei Triticale und Weizen ermittelt. Die ME-Gehalte von Gerste (14,9 (±0,34) MJ ME/kg TM) und Roggen (14,8 (±0,22) MJ ME/kg TM) waren signifikant geringer. Innerhalb der Getreidearten wurden Unterschiede zwischen den verschiedenen Genotypen bei Gerste, Triticale und Weizen festgestellt.

Zur Schätzung der Gehalte an vP wurden mittels einer *in vitro*-Methode bei den 32 Getreideproben und 22 weiteren Futtermitteln die Gehalte an hydrolysiertem P (hP) ermittelt und zudem die vP-Gehalte in *in vivo*-Untersuchungen bestimmt. Durch die anschließende Regressionsanalyse der *in vivo* bestimmten vP-Gehalte und der *in vitro* bestimmten hP-Gehalte wurde folgende lineare Regressionsgleichung ermittelt ($R^2 = 0.880$): vP (g/kg TM) = -0,125 ($P > 0,10$) + 1,482 ($P < 0,01$) × hP (g/kg TM).

Durch die genaue Kenntnis der ME- und vP-Gehalte können Rationen optimal dem jeweiligen Bedarf angepasst werden. Die Supplementierung von mineralischem P kann auf ein Minimum reduziert werden, was auch im Hinblick auf die begrenzten weltweiten Reserven von Rohphosphaten von besonderer Bedeutung ist. Zudem werden die Ausscheidungen über den Kot reduziert und negative Umwelteffekte erhöhter P-Gehalte in der Gülle werden vermindert.

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ABBREVIATIONS

Abs	Absorbance
ADFom	Acid detergent fibre expressed exclusive residual ash
aNDFom	Neutral detergent fibre assayed with heat stable amylase and expressed exclusive residual ash
ATTD	Apparent total tract digestibility
BR	Basal ration
BW	Body weight
Ca	Calcium
CDS	Condensed distillers solubles
CF	Crude fibre
CP	Crude protein
d	Day
DDGS	Dried distillers grains with solubles
DE	Digestible energy
DM	Dry matter
dP	Digestible phosphorus
EE	Ether extract
GE	Gross energy
h	Hours
hP	Hydrolyzed phosphorus
$InsP_6$	*myo*-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate)
IW	Initial weight
ME	Metabolizable energy
ME_m	Metabolizable energy for maintenance

MJ	Megajoule
NE	Net energy
P	Phosphorus
pcdP	Prececal digestibility of phosphorus
Phyt.-act	Phytase activity
PTU	Phytase units
R^2	Coefficient of determination, R-square
rP	Released phosphorus
rpm	Revolutions per minute
SD	Standard deviation
SE	Standard error
tP	Total phosphorus

CHAPTER 1

General introduction

Cereal grains are a major component in rations for monogastric animals across the world. The global total grain production in 2017/18 reached 2091 mt, representing wheat as the major cereal grain (758 Mio. Tons; BMEL, 2018). In Germany, winter wheat accounts for 20.1 Mio. Tons, more than 50% of the total production of cereal grains (BLE, 2018). Due to their high starch content and the high inclusion rate, cereal grains provide the majority of energy in diets for pigs and poultry. At the same time, most phosphorus (P) in the diet is also supplied by the different grain species. As P and energy together with protein are the most expensive feed constituents, an exact knowledge of their concentration is indispensable for accurate diet formulation (Letourneau-Montminy et al., 2011).

For optimum coverage of the animals' requirements, a comprehensive chemical analysis of all valuable ingredients is mandatory. Moreover, the feed value depends on factors that influence digestibility and utilisation of nutrients and that cannot be characterized by chemical analyses alone, such as grinding intensity during feed processing, physiological status, disease load or environmental stressors. Therefore, standardised *in vivo* methods are essential for the determination of digestibility of nutrients (e.g., crude protein, amino acids) and for determination of metabolizable energy (ME) concentration.

Energy itself is not defined as a nutrient because it is not a chemical substance. It is rather considered to be a characteristic of the diet. The major energy content in a diet comes from four different dietary sources: starch, protein, fat and fibre (Patience, 2012). Each of the four distinct and unique sources has different digestibilities, and each source will be used with different levels of metabolic efficiency (Patience, 2012). There is large variation in ME concentration between the different sources. Thus, a different chemical composition leads to a different energy value of that feedstuff. As energy represents the major contribution of total feed costs in pig meat production, it is important to estimate precisely the energy value of feeds. For this purpose, different prediction equations can be used (Noblet and Henry, 1993; Susenbeth, 2005; Bulang and Rodehutscord, 2009), which are based on total tract nutrient digestibility coefficients or on the chemical composition of ration ingredients or mixed rations. To evaluate the prediction equations, regression analysis of data determined *in vivo*

and chemical analysis is needed. Depending on the different energy systems [digestible energy (DE), ME or net energy (NE)] and the different prediction equations, a diet or ingredient is given a different energy value. First, the corresponding energy value of the ingredient is dependent on the digestibility of the energy in that ingredient. The second aspect is the efficiency with which the energy in that ingredient can be used by the pig for maintenance and productive functions (Noblet, 2007). After gross energy (GE) is determined or estimated, which is the first step of all energy systems and represents the total quantity of energy contained in a feed or ingredient, the different energy systems are characterized as follows: The DE in feed ingredients is calculated from the difference between GE in the diet and GE excreted in the faeces. In most diets fed to pigs the DE varies between 70 and 90% of GE in the diet (Kil et al., 2013). To calculate the ME of a diet, energy losses in urine and combustible gases, e.g. methane, are subtracted from DE. Generally, the energy lost in methane is not taken into account when estimating ME-values for pigs, because production of methane in the hindgut of growing pigs is limited to only a few litres daily (Jentsch and Hoffmann, 1977; Christensen and Thorbek, 1987; Noblet et al., 1993; Noblet et al., 1994; Jørgensen et al., 1996). Net energy is defined as ME minus heat increment, which is the heat produced during digestion of feed, metabolism of nutrients and excretion of waste (Noblet and Henry, 1993). The NE-values are obtained based on energetic efficiency of digestible nutrients. Therefore, NE-values are believed to be more accurate in expressing the energy value for diets and feed ingredients than are DE- or ME-values (Noblet et al., 1994; Noblet, 2007). Because the nutrients are not used with similar efficiencies in the ME and NE system, the main differences in energy values correspond to the efficiency of utilisation of crude protein (CP), crude fibre (CF), starch and crude fat. Diets containing high amounts of CP or CF tend to be overestimated when expressed on an ME basis, whereas fat and starch components are underestimated in ME systems (Noblet et al., 1994). But there are also disadvantages of the NE system. It is affected more by influences of diet formulation regarding protein concentration and protein quality when compared to the ME system (Susenbeth, 2005). As NE in most systems aims to predict the energy retained in the animal's body, a determination of specific performance takes place. Different equations are needed for different types of production. Therefore ME is more universal and is independent of the metabolic fate of energy in absorbed nutrients (Susenbeth, 2005). Additionally the determination of NE-values from *in vivo* trials is much more elaborate because of the labour-intensive measurement of combustible gases needed. Published NE-values can only be used in

a particular country, whereas the provision for measurement of ME-values is internationally consistent (GfE, 2017).

The most common energy systems used in commercial pig production are ME and NE. In Germany, after thorough consideration of all aspects, the ME system is used for estimation of energy in pig diets and can be used for gestation, lactation and growth as well. In other countries, such as the Netherlands, France and Denmark, NE systems or NE-based systems are used (Noblet et al., 1994; Boisen, 2007; Blok et al., 2015). As these three countries are using different energy evaluation systems, there can be small differences between NE-values. Although absolute energy values may differ, the rank order for pig diets and feed ingredients is expected to be similar among those three NE systems (Boisen, 2007).

Because P is one of the fundamental building blocks of life and an essential element, humans, animals, plants and microorganisms are dependent on its continuous supply. It takes part in many important metabolic functions such as energy utilisation and transfer, fatty-acid transport and amino acid and protein synthesis (Cromwell, 2005; Suttle, 2010). A deficiency of P in the diet can lead to growth disorders and reduced performance of the animals. Therefore, inorganic sources of P, mostly obtained from mined rock phosphate, are often added to the diets. But global resources of rock phosphate are limited and non-renewable. The amount of reserves is dynamic and depends on current technology. Estimates of global rock phosphate stocks differ and, depending on different assumptions, the present estimation for existence of phosphate rock varies between 60 and 400 years (Steen, 1998; Cordell et al., 2009; Cordell and White, 2011). The P requirement of pigs is affected by factors such as physiological status, performance level and type of production of the animals. Additional impacts arise from the type of feedstuff, its nutrient composition and the chemical form of P in the diet, as well as environmental factors such as temperature, health status, management and housing conditions (Jongbloed and Kemme, 1990). Derived from these factors, P requirements can be calculated and are published in tables, for example by GfE (2008) and NRC (2012).

To minimize the addition of inorganic sources of P, to reduce P output and thereby diminish the concentration of P in the soil, it is necessary to know precisely the apparent total tract digestibility (ATTD) of P in feedstuffs and diets. An oversupply of inorganic P together with poor P-digestibility of the plant feedstuff leads to higher P concentration in animal manure. With exact knowledge of the ATTD of P, its overload in animal manure and the impact of environmental P pollution can be reduced. Continuous application of high-P manure

leads to high levels of P in the soil. Surface and groundwater runoff into nearby lakes and streams creates environmental problems and can contribute to eutrophication, especially in areas with a high density of animal production (Correll, 1998). Through legal provisions, for example in Germany, France, the Netherlands and Denmark (in particular the regulations concerning N and P output by the farm) supply of energy and P in feed has become an increasingly important issue (Poulsen et al., 1999; Otten, 2013). Therefore, much work has been conducted to reduce or eliminate the need for inorganic sources of P and to minimize in particular the amount of P in excreta of monogastric animals (Poulsen, 2000; Ferket et al., 2002; Knowlton et al., 2004; DLG, 2016).

In plant seeds such as cereal grains, most P is present as phytic acid (1*R*,2*S*,3*r*,4*R*,5*S*,6*s*)-cyclohexane-1,2,3,4,5,6-hexayl hexakis [dihydrogen (phosphate)]; $InsP_6$; Fig. 1) and usually present as phytate (salt form of $InsP_6$). In contrast to ruminants, where the inherent phytase activity of the rumen microbes degrade nearly all the $InsP_6$ into inorganic P (Haese et al., 2014; Haese et al., 2016), in pigs and poultry $InsP_6$ is poorly hydrolyzed (Raun et al., 1956; Eeckhout and De Paepe, 1994). The enzyme phytase, which hydrolyzes phytic acid, can increase the ATTD of P in plant feedstuffs (Düngelhoef et al., 1994; Düngelhoef and Rodehutscord, 1995; Pallauf and Rimbach, 1997).

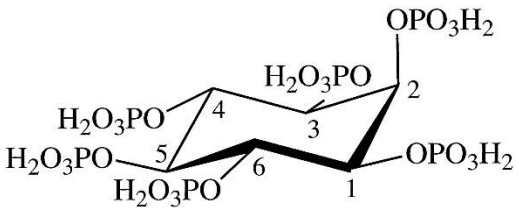

Figure 1 Energetically most favourable conformation of phytic acid (myo-inositol hexakisphosphate); the numbering of the carbon atoms is the numbering for the D-configuration (Wyss et al., 1999)

It has often been assumed that the small intestinal mucosa of monogastric animals does not generate sufficient endogenous phytase activity and that those animals depend on supplementation of microbial phytase in the feed to improve the digestibility of P (Düngelhoef et al., 1994; Selle and Ravindran, 2008). Some recent studies have shown that this theory needs to be revised for phytase utilisation in fowl. Zeller et al. (2015a) have shown that there is a high capacity in broiler microbiota to hydrolyze $InsP_6$ in the intestine. The majority of $InsP_6$ hydrolysis has already occurred in the duodenum or jejunum, but hydrolysis still continues in the ileum and caeca. The level of intestinal $InsP_6$ degradation in broilers is

dependent on calcium (Ca) and P concentration and enzyme activity (e.g., phytase) in the diet (Shastak et al., 2014; Zeller et al., 2015b).

It is well known that the content of phytic acid, as well as the activity of intrinsic phytase, varies between different plant feedstuffs and that there are differences in the ATTD of P between different types of cereal grains (Eeckhout and De Paepe, 1994), but there is a lack of data in the literature describing the variation between different genotypes within one grain species.

In the late 1990s the breeding of new low phytic acid (lpa) genotypes of major crops began (Rasmussen and Hatzack, 1998; Raboy et al., 2000; Spencer et al., 2000). In lpa genotypes the concentration of phytic acid is reduced by 30–90% without significantly affecting the concentration of total P. Meanwhile lpa genotypes of maize, barley, wheat and soybean have been investigated, covering important feedstuffs in monogastric nutrition. However, due to negative effects such as reduced yield, decreased stress tolerance and reduced efficiency, the lpa genotypes are of no great importance in agricultural production (Guttieri et al., 2006).

Another aspect of improvement of the ATTD of P in plant feedstuffs is the breeding of animals delivering higher activities of salivary phytase. In two studies by Golovan et al. (2001a; 2001b) development of transgenic mice and pigs was presented. For normal growth the transgenic pigs required almost no supplementation with inorganic P and faecal P excretion was reduced by up to 75% when compared to non-transgenic pigs. Both groups were fed a diet containing soybean meal as the sole source of P. The P-digestibility by transgenic pigs of plant feedstuffs such as barley, wheat or soybean meal can be even higher than when supplemental exogenous phytase is added to the ration. This is due to the fact that there is a greater activity of phytase in the stomach of transgenic pigs because of the continuous production of saliva, rather than a limited supply via feed (Golovan et al., 2001b).

Phosphorus digestibility can also be improved by different feed treatment procedures. Several studies have been conducted which demonstrate phytate degradation in seeds by fermentation and soaking techniques (Carlson and Poulsen, 2003; Lyberg et al., 2006; Blaabjerg et al., 2010; Missotten et al., 2010, 2010; Pedersen and Stein, 2010; Humer et al., 2013). By definition, fermented liquid feed is feed mixed with water and fermented for a certain period of time to reach steady-state conditions (Chae, 2000) characterized by low pH, high levels of lactic acid bacteria, and low enterobacterial counts. With fermentation of the complete feed or single feed ingredients, mobilisation of P from phytate caused by activation

of endogenous grain phytase can be initiated (Carlson and Poulsen, 2003; Blaabjerg et al., 2007). Different studies have reported a degradation of P bound to $InsP_6$ of 50–80% by fermentation of diets based on wheat, barley and soybean meal (Carlson and Poulsen, 2003; Lyberg et al., 2006; Blaabjerg et al., 2007). Therefore, fermentation of diets can lead to an improvement of the ATTD of P in growing pigs. The effect can be lower in diets containing higher proportions of maize or barley because the lower intrinsic phytase activity in those diets will be insufficient to degrade phytate to a greater extent (Nitrayová et al., 2009; Canibe and Jensen, 2012). Schemmer et al. (2013) reported a 17% rise in the ATTD of P when the same diet based on wheat, barley and soybean meal was fed fermented or dry (0.808 and 0.635, respectively).

Research efforts have also been directed towards establishing *in vitro* methods that allow prediction of the digestibility of P in plant feedstuffs. The procedures are based on an enzymatic assay that mimics the animal's digestion (Kehraus et al., submitted; Liu et al., 1997, 1998; Bollinger et al., 2004, 2005). The aim of *in vitro* procedures is to obtain a rapid and inexpensive estimate of ATTD of P or digestible P (dP) content of plant feedstuffs for pigs. Such an approach could help to define the dP content of individual batches of cereal grains, grain by-products, protein feeds and other feedstuffs in monogastric nutrition, especially in pigs and poultry. Previous studies using *in vitro* methods for the estimation of ATTD of P in plant feedstuffs worked with only small sample sizes and – except one study by Schlegel et al. (2014) – the experiments were conducted without a direct validation against *in vivo* data. There is a lack of data linking *in vitro* data with *in vivo* results from the same plant material. Additional research is needed in this field.

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CHAPTER 2

Scope of the thesis

Although it is well established that differences exist in energy concentration and P-digestibility between and within different types of cereal grains, there is a lack of studies clearly identifying the variation among different genotypes within one grain species.

Therefore in an collaborative research project (GrainUp), eight genotypes of barley, rye, triticale and wheat were analyzed for apparent total tract digestibility (ATTD) of phosphorus (P) and metabolizable energy (ME) concentration in an *in vivo* study with pigs. All cereal grains were selected and cultivated by the Agricultural Experiment Station of the University of Hohenheim, Stuttgart, Germany and grown in the same field under identical conditions of weather, soil and fertilisation. By using the difference method and applying quantitative collection of urine and faeces, the ATTD of P and the ME concentration were measured in young growing pigs. The scope was to demonstrate the potential differences within different genotypes of contemporary cereal grains.

Detailed chemical analysis was carried out by the State Institute for Agricultural Chemistry of the University of Hohenheim, Stuttgart, Germany. The results of the detailed analytical analysis were used to find correlations between the *in vivo* data and various chemical characteristics of the cereal grains. If possible, prediction equations should be drawn to determine the ATTD of P and the ME concentration by means of chemically analyzed variables.

The establishment of a cheap and rapid *in vitro* procedure for prediction of digestible P (dP)-values in plant feedstuffs used in diets for pigs is of great importance for practice. Because excess P in feed and manure is of major concern, especially regarding governmental regulations, the overall aim is to reduce the use of inorganic P and minimize the environmental load.

As there is a lack of studies dealing with the interplay between *in vitro* determination of hydrolyzable P (hP)-values and *in vivo* determination of dP content of the same feedstuff, the aim of a second experimental part of the present thesis was to calibrate the dP content of 54 plant feedstuffs with the results from an *in vitro* procedure. For this purpose, 65 different samples were analyzed in an enzymatic *in vitro* model to determine the hP content. This was

done using an *in vitro* method that mimicked digestion in the pig in three consecutive steps. In both experiments, identical feedstuffs were used to ensure comparability of *in vitro* and *in vivo* determination. Additional to the *in vivo* data from the 32 different cereal grain genotypes described in detail in Chapter 3, the results from another 33 *in vivo* measurements of commercial feedstuffs were used in the *in vitro* procedure and for statistical analysis.

The third and fourth chapters, as the main parts of this cumulative thesis, are manuscripts formatted according to the instructions of the journal chosen for submission.

CHAPTER 3

Phosphorus digestibility and metabolisable energy concentrations of contemporary wheat, barley, rye and triticale genotypes fed to growing pigs

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Abstract

This study was conducted to determine apparent total tract digestibility (ATTD) of phosphorus (P) and metabolisable energy (ME) concentrations for pigs of 32 different genotypes (n = 8 per grain species) of barley, rye, triticale and wheat. All genotypes were grown at the same location under the same field conditions and were fed to growing castrated crossbred pigs (initial body weight: 31.1 ± 6.95 kg) using a series of duplicate 3 × 3 Latin square designs. A basal ration, which was deficient in P, and 32 experimental rations containing 400 g/kg DM of the basal ration and 600 g/kg DM of the corresponding cereal grain were mixed. Pigs were kept in metabolism crates and the total collection method was used for separate faeces and urine collections with 7-d adaptation and 7-d collection periods. The mean ATTD of P was greater ($p < 0.05$) for wheat than for triticale, rye or barley (59.4%, 50.4%, 44.9% and 44.4%, respectively, for the mean of each grain species). Within-grain species differences ($p < 0.05$) among genotypes were obtained for ATTD of P of barley and triticale. The concentrations of ME of triticale and wheat were higher ($p < 0.05$) than that of barley and rye (16.1 and 16.2 vs. 14.9 and 14.8 MJ ME/kg DM, respectively). Differences in ME concentration among genotypes within a grain species ($p < 0.05$) were found for barley, triticale and wheat. No differences were found for rye. Compared to literature data the present study showed, in part, considerable differences in ATTD of P and ME concentration. These results should be taken into account for accurate pig ration formulation with regard to minimised P output and efficient use of ME. No significant relationships were detected between ATTD of P and phytic acid concentration or phytase activity in the grain genotypes in this study.

Keywords: Apparent total tract digestibility; Cereal grains; Metabolisable energy; Nutritive value; Phosphorus; Pigs.

Abbreviations: ADFom, acid detergent fibre expressed exclusive residual ash; aNDFom, neutral detergent fibre assayed with heat stable amylase and expressed exclusive residual ash; ATTD, apparent total tract digestibility; BW, body weight; CP, crude protein; DM, dry matter; dP, digestible phosphorus; EE, ether extract; GE, gross energy; $InsP_6$, *myo*-inositol hexakisphosphate; ME, metabolisable energy; PTU, phytase units; SD, standard deviation; SE, standard error.

1. Introduction

Due to their high starch content and their dietary inclusion level, cereal grains are the primary source of energy in pig rations. Moreover, a considerable amount of phosphorus (P) is provided by cereal grains. Most of the P in plant feedstuffs is present as phytate, which is poorly digested by pigs (Jongbloed 1987; Eeckhout and De Paepe 1994). phytate content and intrinsic phytase activity in cereal grains and other plant feedstuffs differ between and within different species and genotypes (Eeckhout and De Paepe 1994; Steiner et al. 2007). Increasing dietary P concentration above requirements results in regulatory excretion of P in faeces (Rodehutscord et al. 1999a) and poses an environmental concern especially in areas with high livestock density (Jongbloed 1987; Ferket et al. 2002; Aarnink and Verstegen 2007). As P takes part in many important metabolic functions like energy utilisation and transfer, fatty acid transport and amino acid and protein synthesis (Suttle 2010), a lack in P supply can lead to reduced growth. To avoid a loss of performance, mineral P is often added to the rations (Poulsen 2000; Knowlton et al. 2004). But for environmental and economic reasons, the amount of mineral P in livestock feeds has to be minimised.

Variation was also reported across different genotypes within-grain species in chemical characteristics and nutritive value (Fairbairn et al. 1999; Zijlstra et al. 1999; Kim et al. 2005; Rosenfelder et al. 2013). As prediction equations of the different energy systems are based on chemical and/or physical variables, the consequence of chemical variation in single feedstuffs is differences in gross energy (GE) and, thus, metabolisable energy (ME) concentrations (Patience 2012). Accordingly, an accurate assessment and knowledge of P content and digestibility as well as chemical characteristics and energy values of feedstuffs used in ration formulation is indispensable. There is a lack of systematic studies and data identifying the variation in apparent total tract digestibility (ATTD) of P and ME concentrations across genotypes within and between grain species. Interpretation of data on ATTD of P is further impeded by differences in methodologies which were applied to estimate this variable (She et al. 2018).

The objective was to investigate a wide range of current genotypes of feed grain species, which are grown and used in significant quantities in European crop and livestock production. Therefore, eight genotypes each of barley, rye, triticale and wheat were used to determine ATTD of P and ME values, thus, providing up-to-date data of contemporary cereal grains used in diet formulation for pigs. The hypothesis was that, based on precise determination of digestible P (dP) and ME values of well-characterised cereal grain genotypes, ration

formulation can be optimised which will contribute to the conservation of natural resources and the lowering of emissions from pig husbandry.

2. Materials and methods

2.1. Selection and preparation of the cereal grains

The genotypes of barley, rye, triticale and wheat (Table 1) were selected and cultivated by the Agriculture Experiment Station of the University of Hohenheim, Stuttgart, Germany. This was done in close cooperation with leading German breeding companies. The selected genotypes were part of the collaborative research project referred to as GrainUp (Rodehutscord et al. 2016). Genotypes were chosen to represent previously known differences in crude protein (CP) concentration and yield. All genotypes were grown in the 2010/2011 growing season under standardised field conditions close to the University of Hohenheim (Stuttgart, Germany; 48°42′41.5” N, 9°13′12” E; average temperature, 10.7°C; annual precipitation, 548 mm; and altitude 350 to 380 m above sea level). Standard agronomic practices were used from sowing to harvesting for all 32 genotypes.

Table 1
Genotypes of barley, rye, triticale and wheat

Barley	Rye	Triticale	Wheat
Yool	Conduct	Grenado	Skalmeje
ACK 2927	Visello	Tarzan	Tommi
Lomerit	Helltop	HYT Prime	Tobak
Campanile	Bellami	Massimo	Event
Canberra	Palazzo	Cultivo	Mulan
Anisette	Dukato	SW Talentro	Tabasco
Metaxa	Guttino	Cando	Adler
Fridericus	Dankowski Diament	Agostino	KWS Erasmus

2.2. Rations

The 32 cereal grains were individually ground through a hammer mill using a 3.0 mm screen. For determination of ATTD of P in single feedstuffs for pigs, the nutritional P supply has to be suboptimal. Therefore, the Committee for Requirement Standards of the Society of Nutrition Physiology in Germany recommended an upper tolerable level for dP of 2.0 g/kg dry matter (DM) to minimise regulatory excretion of excess P (GfE 1994). As a consequence, a basal ration low in P and supplemented with all other minerals and vitamins close to requirements was formulated (Table 2; Individual genotype data are presented in Supplementary Table S1). The basal ration was tested with identical ingredient composition 3 times within the entire experiment. The concentration of dP in the experimental rations was adjusted to a maximum of 2.0 g/ kg DM. Samples of tested feedstuffs were analysed before ration formulation and calculated based on the analysed P content and according to ATTD of P derived from previous studies and data in the literature (Düngelhoef et al. 1994; Rodehutscord et al. 1996; Steinbeck 2000). The experimental rations were formulated by blending each test ingredient into the basal ration at a rate of 600 g/kg DM. Ration preparation was made in the feed mill facilities at the Institute of Animal Science, University of Bonn, Germany. The rations were prepared in one batch for each trial and stored in dry barrels at barn temperature until fed. Experimental rations and basal ration were not pelleted to prevent any negative heat effects on intrinsic phytase activity within the pelleting process.

2.3. Animals and experimental procedure

In total, 72 male castrated crossbred pigs were obtained from the Educational and Research Center Frankenforst of the Faculty of Agriculture, University of Bonn (Königswinter, Germany), with an initial mean (± standard deviation) body weight (BW) of 31.1 ± 6.95 kg. Pigs were delivered to the experimental facilities in three subgroups of 24 pigs each. For each subgroup, a new batch of the basal ration was mixed. Groups of six pigs were allotted to duplicate 3 × 3 Latin Squares and within each Latin Square three different rations were tested. To allow a complete set of 12 Latin Squares, a barley genotype sample not related to this study was added to the 32 cereal grain genotypes and three batches of the basal ration. This design was used to minimise the effects of age or weight of the pigs within each square. Each period was divided into a 7-d adaptation period and a 7-d collection period. During adaptation to the dietary treatment, two pigs shared an indoor pen of 1.1 m ×1.7 m where separate

Table 2
Ingredients and chemical composition of the basal ration

Ingredients (g/kg)	
Wheat starch, pregelatinized	643
Beet pulp, dried	148
Potato protein	85
Whole egg powder	65
Cellulose	22
Soybean oil	17
Vitamin and mineral premix [a]	12
Calcium carbonate	8
Chemical composition (g/kg dry matter unless stated, n = 3)	
Dry matter (g/kg)	914
Ash	34.5
Crude protein	186
Ether extract	37.5
aNDFom[b]	53.2
ADFom[c]	199
Starch	525
Phosphorus	1.66
Digestible phosphorus	1.18
Calcium	6.05
Starch	525

[a] Premix provided the following, per kg: 1.5 g L-lysine HCl; 0.5 g L-tryptophan; 1.5 g Na; 0.3 g Mg; 50 mg Fe; 4 mg Cu; 50 mg Zn; 20 mg Mn; 0.15 mg I; 0.2 mg Se; 2,000 IU Vit. A; 200 IU Vit. D_3; 11 mg Vit. E; 0.1 mg Vit. K_3; 1.7 mg thiamine; 2.5 mg riboflavin; 3 mg pyridoxine; 0.01 mg biotin; 0.01 mg cyanocobalamin; 15 mg nicotinic acid; 10 mg pantothenic acid; 350 mg choline chloride
[b] aNDFom, neutral detergent fibre assayed with heat stable amylase and expressed exclusive ash;
[c] ADFom, acid detergent fibre expressed exclusive residual ash

feeding was ensured. After the adaptation period, the pigs were transferred into metabolism crates (height = 55 cm; length = 95 cm; width = 52 cm) equipped with a stainless steel trough, a slatted floor and separate collection trays for urine and faeces. Crates were oriented to allow visual contact for the pigs. Room temperature was maintained at 22 ± 2°C with a 12-h lighting programme. Pigs were fed twice daily at 08:00 and 16:00 h with approximately equal amounts at every feeding throughout the experimental period. The ration was mixed with

water directly before feeding and, after the 20-min feeding period, the pigs were offered water for at least 30 min. Feed refusal was recorded to adjust daily feed intake. Before the adaptation period, the amount of feed was adapted to BW. Based on the BW of the pigs, which was measured at the beginning and at the end of each collection period, the rations were fed in amounts corresponding to 2.0 to 2.5 times the maintenance requirement for ME (i.e. MEm [MJ/d] = 0.44 · BW0.75; (GfE 2008)). Urine was collected in a plastic container over 25 ml of 100 ml/l sulphuric acid which ensures acidification to pH 3 or less. In the experimental period, faeces and urine were collected quantitatively. The collection vessels were emptied every morning after feeding and weight was recorded. The collected faeces and a subsample of urine were immediately frozen at −18°C. The experiments were approved by the Ministry for Climate Protection, Environment, Agriculture, Nature Conservation and Consumer Protection of the State of North Rhine-Westphalia (file No. 84–02.04.2012.A144) in accordance with German animal welfare legislation (Anonymous 2006).

2.4. Chemical analyses

Samples of the tested feed ingredients were collected before ration formulation and samples of the rations were collected during ration formulation. Chemical analyses of all grain samples within the research project GrainUp were done at the University of Hohenheim (Stuttgart, Germany). For chemical analyses, all grain samples were ground through a sieve with a pore size of 0.5 mm (Siebtechnik GmbH, Mülheim- Ruhr, Germany and Retsch GmbH, Haan, Germany) if not otherwise specified. Hulled genotypes of barley were not dehulled before grinding. A vibrating cup mill (Type 6-TOPF, Siebtechnik GmbH, Mülheim-Ruhr, Germany) was used to grind samples for inositol phosphate analysis and phytase activity determination. Ground samples were stored in a freezer prior to analysis of inositol phosphates and phytase.

The following chemical analyses were conducted for grain samples and rations: DM (method 3.1), ash (method 8.1), CP (method 4.1.1), ether extract (EE; method 5.1.1b), starch (method 7.2.1), neutral detergent fibre assayed with heat-stable amylase and expressed exclusive residual ash (aNDFom; 6.5.1), acid detergent fibre expressed exclusive residual ash (ADFom; 6.5.2) and the minerals Ca and P (methods 10 and 11) according to official methods (VDLUFA 2012). Phytase activity in the grains was determined according to Greiner and Egli (2003) (method 2: direct incubation). Activity was expressed in Units (U), whereby the unit was defined as 1 μmol of phosphate liberated from 100 μmol potassium phytate per minute at 45°C, pH 5.0. Phytic acid (*myo*inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate); $InsP_6$)

was measured by high-performance ion exchange chromatography (Dionex ICS-3000, Idstein, Germany, using a CarboPac® PA 200 column) with post-column derivatisation, following extraction with 0.2 M ethylenediaminetetraacetic acid (EDTA) and 0.1 M sodium fluoride at pH 10 as described by Zeller et al. (2015). Gross energy (GE) was determined using a bomb calorimeter (C 200, Ika-Werke GmbH & Co. KG, Staufen, Germany). Experimental rations and faeces of each pig and each period were analysed in duplicate. For GE determination in urine, homogenised subsamples of 5 ml were weighed in polyethylene bags and frozen at −80°C for 24 h. Afterwards the samples were freeze-dried and triplicate analyses of GE were done using the adiabatic bomb calorimeter. Urine energy was determined by subtracting the energy contained in the polyethylene bag from the combined energy in urine plus polyethylene bag.

The faeces were thawed and homogenised with an electric hand mixer and a representative subsample from each pig and experimental period was collected for subsequent chemical analysis. Faeces samples were freeze-dried (P18K-E-6, Piatkowski Forschungsgeräte, München, Germany) in duplicate. All samples were milled through a 1 mm mesh sieve using an ultra-centrifugal mill (ZM 200, Retsch, Haan, Germany) and then analysed for DM, ash, P and GE as described before.

2.5. Calculations and statistical analysis

The ATTD of P was calculated according to GfE (1994):

$$\text{ATTD of } P_{Ration} = (\text{P intake - P output})/\text{P intake}$$

where P intake is the total P intake (g) during the collection period of 7 d and P output is the total faecal P output (g) during the same period.

The digestibility of P of the corresponding cereal grain was determined by the difference method according to GfE (1994):

$$\text{ATTD of } P_{Feedstuff} = [\text{ATTD of } P_{Ration} - \text{ATTD of } P_{BR} \bullet (1\text{-}a)]/a$$

where ATTD of P_{Ration} and ATTD of P_{BR} is the ATTD of P from test ration and basal ration and a = analysed P content of feedstuff (g/kg DM) • inclusion level of feedstuff in test ration (kg/kg DM)/analysed P content in test ration (g/kg DM).

The GE consumed was calculated by multiplying the GE value of the corresponding ration fed by feed intake over the collection period lasting 7 d. The ME values were calculated by

subtracting faecal and urinary energy from GE intake without considering potential methane energy losses because they were assumed to be quantitatively insignificant. The ME value of the corresponding cereal grain was determined by the difference method applying the same procedure as outlined above for ATTD of P (Equation 2).

Data were analysed using the MIXED procedure of SAS® (version 9.2; SAS,Inst., Inc., Cary, NC, USA). The fixed effect genotype (n = 8) and experimental period (n = 3) were included in the model and analysed separately for each grain species. Pig was considered the random effect in the model. The MIXED procedure of SAS was also used for the analysis of differences among grain species. All results are reported as least squares means. The significance level was $p < 0.05$.

3. Results

3.1. Chemical composition

Barley had the highest P content of the four cereal grain species followed by triticale, wheat and rye (Table 3). The analysed contents of P in the rations were close to predicted values based on the analysed contents in the cereal grains (Table 4). All rations had a calculated concentration of less than 2.0 g dP/kg DM. The analysed P content in the basal ration ranged from 1.53 to 1.77 g/kg DM. The mean total P concentration of the rations ranged between 2.79 g/kg DM in wheat to 3.04 g/kg DM in barley. These small differences between genotypes and between different cereal grain species were also obvious in other chemical variables. This is apparent in ash, CP, EE, ADFom, starch, Ca and GE where small differences were found across samples. A wider range was quantified for aNDFom, $InsP_6$ and phytase activity. Rye had the lowest $InsP_6$ content and highest phytase activity whereas barley had the highest $InsP_6$ content and lowest phytase activity.

3.2. Phosphorus digestibility

In general, pigs remained healthy throughout the experiment. Only one pig had to be replaced by another pig at the beginning of the adaptation period for barley genotype Campanile because of an umbilical hernia.

Urinary P losses were recorded, though they were negligibly low (data not shown). The mean urinary P excretion of the pigs was 8.05 mg/d for the experimental rations and 0.417 mg/d for the basal ration. Wheat had the highest ($p < 0.05$) average ATTD of P, followed by triticale, whereas the lowest ATTD of P was found in rye and barley (Table 5). The highest

Table 3
Chemical composition of cereal grains

	Dry matter	Ash	CP[a]	EE[b]	aNDFom[c]	ADFom[d]	Starch	P[e]	Ca[f]	$InsP_6$[g]	Phyt.-act.[i]	GE[h]
	g/kg	g/kg dry matter									U/kg DM	MJ/kg DM
Barley (n = 8)												
mean	880	23.7	124	28.4	194	52.2	624	4.26	0.58	2.63	740	18.8
SD[j]	1.83	1.12	6.28	2.72	12.2	5.88	11.5	0.26	0.05	0.20	207	0.31
Rye (n = 8)												
mean	880	16.5	116	19.2	152	29.6	644	3.55	0.51	1.42	4333	18.4
SD	2.60	0.35	5.64	1.09	14.3	2.86	6.65	0.12	0.03	0.18	231	0.06
Triticale (n = 8)												
mean	882	18.1	126	19.0	140	29.2	702	4.05	0.48	1.85	2090	18.4
SD	3.42	0.67	6.32	1.34	27.1	2.64	13.1	0.25	0.10	0.13	326	0.06
Wheat (n = 8)												
mean	876	15.8	134	21.5	119	31.4	717	3.66	0.42	1.98	1900	18.6
SD	0.99	0.76	8.55	1.94	7.75	4.28	9.44	0.23	0.02	0.16	346	0.08

[a] CP, crude protein; [b] EE, ether extract; [c] aNDFom, neutral detergent fibre assayed with heat stable amylase and expressed exclusive ash; [d] ADFom, acid detergent fibre expressed exclusive residual ash; [e] P, phosphorus; [f] Ca, calcium; [g] $InsP_6$, *myo*-inositol hexakisphosphate; [h] GE, gross energy; [i] Phyt.-act., phytase activity; [j] SD, standard deviation.

Table 4
Chemical composition of rations (g/kg dry matter)

	Dry matter	Ash	CP[a]	EE[b]	aNDFom[c]	ADFom[d]	Starch	P[e]	Ca[f]	GE[g]
	g/kg	g/kg dry matter								MJ/kg DM
Barley (n = 8)										
mean	900	27.3	144	38.2	259	52.9	569	3.04	2.80	19.2
SD[h]	3.16	0.67	12.2	2.15	55.1	4.57	29.2	0.16	0.17	0.29
Rye (n = 8)										
mean	889	24.0	145	30.6	349	37.8	632	2.79	2.85	18.6
SD	3.46	0.46	4.63	6.11	41.9	1.92	57.2	0.06	0.16	0.07
Triticale (n = 8)										
mean	898	23.5	154	33.0	289	35.5	600	2.91	2.64	19.1
SD	4.50	0.61	4.66	2.27	46.9	1.98	13.9	0.15	0.07	0.32
Wheat (n = 8)										
mean	890	22.9	135	34.9	133	40.7	634	2.87	2.74	18.9
SD	2.19	0.61	4.86	1.58	25.8	4.37	9.41	0.16	0.15	0.06

[a] CP, crude protein; [b] EE, ether extract; [c] aNDFom, neutral detergent fibre assayed with heat stable amylase and expressed exclusive ash;

[d] ADFom, acid detergent fibre expressed exclusive residual ash; [e] P, phosphorus; [f] Ca, calcium; [g] GE, gross energy; [h] SD, standard deviation.

ATTD of P of all 32 genotypes used in this study was found in the wheat genotype Tobak (63.4%). In barley, there was a difference ($p < 0.05$) between the genotype Campanile and genotypes Yool, ACK2927 and Fridericus. Differences ($p < 0.05$) were also found in triticale between genotypes Tarzan and Agostino. The ATTD of P across the eight samples of rye and wheat averaged 44.9% and 57.3%, respectively, but there were no significant differences within these two grain species among the grain genotypes tested. The calculated dP concentration of the basal ration was 1.22 g/kg DM for basal ration 1, 1.07 g/kg DM for basal ration 2 and 1.26 g/kg DM for basal ration 3 with corresponding ATTD of P of 68.7%, 70.0% and 74.8%, respectively.

3.3. Energy concentration

The ME concentration was higher ($p < 0.05$) for triticale and wheat than for barley and rye (Table 6; digestibilities of DM and OM of the cereal grains are presented in Tables S2 and S3). Triticale had the widest range of ME values. A difference was observed ($p < 0.05$) between genotype Agostino, which had the lowest ME value, and genotypes Tarzan, HYT Prime and Cultivo, which had the highest ME concentrations. In wheat genotypes, the ME concentrations for Tommi and Adler were higher ($p < 0.05$) than for Mulan. No differences were observed among the eight rye genotypes.

4. Discussion

4.1. Methodical aspects

Noblet et al. (1994) mentioned that, for the purpose of estimating the energy content of pig feeds, measurements should be carried out on pigs that have similar growth potential (genotype and sex) and BW, that are kept in constant environmental conditions and that receive approximately the same amounts of dietary energy. The ration also has to meet their nutritional requirements for undisturbed growth. The experimental design of the present study was arranged to fulfil those criteria to allow a comparison across the entire experiment. Yet, differences in BW, genotype and sex of the pigs are likely to exist between published data and the current study. In particular, the genotype of pigs from experiments conducted in the late 80s and early 90s could differ compared to animals used in the present study. Over the last decades, pigs have attained greater BW gain and improved feed conversion ratio. For example, the average feed conversion ratio (kg feed/ kg BW gain) improved from 3.3 to 2.6 within the last 35 years (Knap and Wang 2012).

Table 5
Apparent total tract digestibility of phosphorus in cereal grains (each mean is based on six observations)

Barley	ls means	SE*	Rye	ls means	SE	Triticale	ls means	SE	Wheat	ls means	SE
Yool[b]	0.393[b]	0.45	Conduct	0.443	0.65	Grenado	0.505 [ab]	0.51	Skalmeje	0.583	0.35
ACK 2927[b]	0.387[b]	0.46	Visello	0.439	0.49	Tarzan	0.563[a]	0.80	Tommi	0.586	0.74
Lomerit[ab]	0.457[ab]	0.51	Helltop	0.457	0.53	HYT Prime	0.513 [ab]	0.69	Tobak	0.634	0.38
Campanile[a]	0.508[a]	0.44	Bellami	0.412	0.68	Massimo	0.480 [ab]	0.61	Event	0.608	0.42
Canberra[ab]	0.461[ab]	0.57	Palazzo	0.457	0.58	Cultivo	0.523 [ab]	0.37	Mulan	0.595	0.51
Anisette[ab]	0.479[ab]	0.55	Dukato	0.488	0.72	SW Talentro	0.509 [ab]	0.29	Tabasco	0.564	0.61
Metaxa[ab]	0.462[ab]	0.63	Guttino	0.445	0.46	Cando	0.511 [ab]	0.65	Adler	0.625	0.34
Fridericus[b]	0.405[b]	0.44	Dankowski Diament	0.452	0.71	Agostino	0.429[b]	0.86	KWS Erasmus	0.557	0.61
Mean	0.444[C]	0.44	Mean	0.449[C]	0.21	Mean	0.504[B]	0.38	Mean	0.594[A]	0.27

[a,b] Within column, mean without common superscript differ ($P < 0.05$); [A,B,C] Within row, mean without common superscript differ ($P < 0.05$);
* SE, standard error.

Table 6
Metabolisable energy concentrations (MJ/kg dry matter) of cereal grains (each mean is based on six observations)

Barley	ls means	SE*	Rye	ls means	SE	Triticale	ls means	SE	Wheat	ls means	SE
Yool	14.8[ab]	0.36	Conduct	14.5	0.65	Grenado	16.2 [ab]	0.35	Skalmeje	16.2 [ab]	0.14
ACK 2927	14.3 [c]	0.42	Visello	14.5	0.54	Tarzan	16.4 [a]	0.36	Tommi	16.6 [a]	0.38
Lomerit	15.1 [ab]	0.34	Helltop	15.0	0.17	HYT Prime	16.4 [a]	0.33	Tobak	16.2 [ab]	0.24
Campanile	15.2 [a]	0.38	Bellami	14.8	0.56	Massimo	16.1 [ab]	0.26	Event	16.1 [ab]	0.33
Canberra	15.1 [ab]	0.35	Palazzo	14.6	0.21	Cultivo	16.3 [a]	0.20	Mulan	15.7 [b]	0.59
Anisette	15.2 [ab]	0.35	Dukato	14.9	0.58	SW Talentro	16.2 [ab]	0.23	Tabasco	16.2 [ab]	0.44
Metaxa	15.1 [ab]	0.31	Guttino	15.1	0.36	Cando	15.6 [bc]	0.31	Adler	16.4 [a]	0.16
Fridericus	14.5 [b]	0.33	Dankowski Diament	14.9	0.52	Agostino	15.4 [c]	0.22	KWS Erasmus	16.3 [ab]	0.26
Mean	14.9 [B]	0.34	Mean	14.8 [B]	0.22	Mean	16.1 [A]	0.38	Mean	16.2 [A]	0.24

[a,b,c] Within column, mean without common superscript differ ($P < 0.05$); [A,B,C] Within row, mean without common superscript differ ($P < 0.05$);

* SE, standard error.

It is also obvious from the literature that previous results regarding ATTD of P are more variable than the results in this study. In some studies, pigs were fed about 96% cereal grain in the ration which can lead to excessive P and dP supplies. She et al. (2018) have shown that, when ATTD values are estimated at a P supply covering or exceeding requirements, a correction for endogenous P losses allows the estimation of standardized TTD values (STTD); for maize grain, soybean meal and canola meal, the authors demonstrated that STTD values were additive when fed in mixtures. If the supply of dP is lower than 2 g dP/kg DM it can be assumed that no regulatory mechanisms mediated by changed absorption rates take place and no correction of values as suggested by She et al. (2018) is required. Additivity of values can thus also be assumed. In the literature, there is disagreement about the influence of BW on ATTD of P. Pallauf et al. (1992) showed an influence of BW on ATTD of P, whereas Kemme et al. (1997), Rodehutscord et al. (1999b) and Steinbeck (2000) observed no relationship, when dietary P originated mainly from plant feedstuffs. In order to exclude any influence of BW in the present study, the experimental design was arranged using small Latin squares.

The ingredients used for mixing the corresponding basal ration and also for preparing each test ration were always from the same batch. Therefore, differences in ATTD of P arising from the basal components were unlikely and the potential bias introduced by preparing three basal rations over the course of the experiment was low. The difference in ATTD of P between basal ration 1, basal ration 2 and basal ration 3 was low (68.7%, 70.0% and 74.8%, respectively). Furthermore, the inclusion level of basal ration and the corresponding cereal grain genotype in all experimental rations were consistent. All rations were provided as mash feed to prevent an inactivation of endogenous phytase by high temperatures during the pelleting process (Eeckhout and De Paepe 1994; Rodehutscord et al. 1998; Slominski et al. 2007). For the same reason, the feed was given as dry feed and mixed with water just before feeding to the pigs as soaking of the rations could lead to $InsP_6$ degradation and increase the digestibility of P (Carlson and Poulsen 2003; Blaabjerg et al. 2010).

4.2. Phosphorus digestibility

Water intake was not measured in this study. Drinking water analyses provided by the water supplier show a phosphate content below 0.01 mg/l (Stadtwerke Bonn 2016). Therefore, water intake had no influence on P intake and evaluation of ATTD of P. The low mean urinary P excretion of 8.05 mg/d demonstrates that digested P, resembling absorbed P, was almost completely utilised by the pigs. It further shows that daily P intake was below the requirements for growing pigs and therefore P digestibility was not affected by dietary P

levels. The urinary P excretion in this study is in agreement with results of Rodehutscord et al. (1998), where pigs also excreted only 8 mg/d of urinary P when fed a ration with a P supply below requirements, which is required for accurate evaluation of P supply from ration ingredients.

Barley had the highest average P content of the four cereal grains followed by triticale, wheat and rye. Almost identical values were reported by Steiner et al. (2007). The values measured in the current experiment are also in agreement with values reported by NRC (2012), except for rye, where NRC (2012) reports higher values (5.5 g/kg DM). The ATTD of P in barley in this study is in agreement with results of Rodehutscord et al. (1996) and Htoo et al. (2007), whereas Berk and Schulz (1993) observed a lower ATTD of P of 39%. The low ATTD of P in barley compared to the other cereal grains can be explained by the highest $InsP_6$ content and, at the same time, lowest phytase activity. The results for ATTD of P for triticale are in agreement with results published by Berk and Schulz (1993) and Düngelhoef et al. (1994). Considerably higher values were reported by Pointillart et al. (1987) and Steinbeck (2000). The higher ATTD of P in the latter experiments may be related to the higher intrinsic phytase activity of triticale. Limited data are available for ATTD of P for rye. The NRC (2012) listed values similar to the results of our study but the values of this study are lower compared to DLG (1999). This may be a result of the fact that rye has the highest and, at the same time, most variable intrinsic phytase activity of all cereal grain species (Eeckhout and De Paepe 1994). These authors reported that phytase activity can vary between 4,132 and 6,127 phytase units (PTU)/kg. The mean phytase activity of the eight genotypes of the present dataset is at the lower end of this range. The literature reveals marked variation for ATTD of P for wheat. Düngelhoef et al. (1994) and Steinbeck (2000) reported results similar to our study for ATTD of P between 60% and 63%. Rodehutscord et al. (1996), in contrast, observed an average ATTD of P of 69% for four wheat cultivars, which is distinctly higher than those in the present study. Only one wheat genotype tested by Rodehutscord et al. (1996), with an ATTD of P of 61%, was in the range of this study. Lantzsch et al. (1992), Barrier-Guillot et al. (1996) and Hovenjürgen et al. (2003) also reported a wide range of ATTD of P from 40% to 68% across a wide range of wheat genotypes.

The differences in ATTD of P in this study compared to literature data indicate varying amounts of P, phytate P and intrinsic phytase activity among different cereal grains and different genotypes. This can lead to remarkable variability of ATTD of P (Ige et al. 2010; Woyengo et al. 2012). Differences in phytase activity especially contribute to the reported heterogeneity in ATTD of P in cereal grains. As the differences in phytate P, especially $InsP_6$,

and total P concentrations are not as different within-grain species as ATTD of P, the large differences in ATTD of P are likely caused by the lack of or differences in intrinsic phytase activity. It was, therefore, unexpected that no relationship was observed between ATTD of P and $InsP_6$ content in the cereal grains; however, this may at least partly be due to the homogeneity of the grain genotypes used in this experiment. Although no significant relationship was found between ATTD of P and $InsP_6$ content or phytase activity in the grain genotypes in this study, differences in phytate content and phytase activity were found when compared to other studies. Except for rye, where Eeckhout and De Paepe (1994) showed higher phytase activity than reported in this article, the mean phytase activity of the cereal grains was greater than previously reported by Berk and Schulz (1993), Düngelhoef et al. (1994), Eeckhout and De Paepe (1994) and Weremko et al. (1997). Especially for wheat, where phytase activity was much higher than literature suggested, this can be a causative effect for differences in ATTD of P. Only Greiner and Egli (2003) and Steiner et al. (2007) reported considerably higher phytase activity for wheat than those observed in this study, between 1,026 and 2,931 PTU/kg (Greiner and Egli 2003) and 2,886 PTU/kg (Steiner et al. 2007). Moreover, the mean proportion of phytate P from total P was lower than literature data (Berk and Schulz 1993; Düngelhoef et al. 1994; Eeckhout and De Paepe 1994; Barrier-Guillot et al. 1996). Although there is a tendency to higher phytase activity in contemporary genotypes of cereal grain species, the analytical method used for determination of plant phytase activity has to be specified in order to allow an unbiased comparison of results of different studies (Zimmermann et al. 2002; Greiner and Egli 2003; Steiner et al. 2007).

4.3. Energy concentration

Generally, the energy loss in methane is not taken into account when estimating ME values for pigs. Production of methane in the hindgut of growing pigs is limited to only a few litres a day. Assuming that the methane loss ranges only from 0.4% (low fibre ration) to 1.3% (high fibre ration) of GE intake (Jentsch and Hoffmann 1977; Christensen and Thorbek 1987; Noblet et al. 1993, 1994; Jørgensen et al. 1996), the energy loss in methane in the present study was never greater than 0.1 MJ/kg DM. This was calculated separately for each diet by considering the highest expected methane loss of 1.3% of GE intake. Even for barley, in this study, the cereal grain species with the highest fibre content and, therefore, the highest potential for microbial nutrient degradation and fermentation in the hindgut, the energy loss via methane was not greater than described above.

Published data for rye, not only for individual genotypes but also for the average ME concentrations show a considerable range, i.e. from 12.0 MJ ME/kg DM (Fairbairn et al. (1999) to 14.9 MJ ME/kg DM (Rundgren 1988); this study). Intermediate values (13.5 MJ ME/kg DM) were reported by May and Bell (1971). Data for triticale reported by Haydon and Hobbs (1991) were moderately lower than our values (15.4 versus 16.1 MJ ME/kg DM), whereas Rundgren (1988) obtained ME concentrations for triticale of only 15.0 MJ ME/kg DM. The wheat genotypes of the current experiment had ME concentrations which were 0.5 MJ ME/kg DM higher (16.2 MJ ME/kg DM vs. 15.7 MJ ME/kg DM) than those observed by Haydon and Hobbs (1991). The lowest ME values for wheat were recently reported by Lowell et al. (2015), who measured a ME concentration of 15.2 MJ ME/kg DM for growing pigs. Greater ME concentrations (15.6 MJ ME/kg DM) were observed on sows in the same study (Lowell et al. 2015), yet these are still in the lower range of values reported previously (Haydon and Hobbs 1991). May and Bell (1971), in contrast, reported a ME concentration for wheat of 16.4 MJ ME/kg DM, which is similar to values of the present study.

The variation in ME concentrations between and within the cereal grain species can be induced by several factors. First, potential sources of error are unaccounted feed refusals and incomplete collection of faeces and urine (van Kempen et al. 2003). It is also essential to collect the urine in an acid solution to prevent ammonia volatilisation. Second, the data can vary due to genotype within cereal grain species, growing site and conditions, precipitation level, light intensity, type and amount of fertiliser and soil type (Fuller et al. 1989; Kim et al. 2005). Third, rations in this experiment were not pelleted before feeding since thermal treatments used in the pelleting process can cause protein denaturation and starch gelatinisation (Yang et al. 2017). This, in turn, can increase ME concentration due to greater digestibility of nutrients. In the same study by Yang et al. (2017), it was also reported that pelleting increased apparent digestible energy.

The fibre fractions in cereal grains can also vary in their digestibility, e.g. differences exist between the digestibility of cellulose and hemicelluloses. As varying amounts of the different fibre fractions contribute to the ME of the cereal grains, the variation between the different cereal grains can affect the ME content. This is apparent for wheat, which represents the lowest aNDFom (cell wall) and ADFom (lignocellulose) contents and at the same time highest ME values.

5. Conclusions

The results clearly demonstrate the differences in ATTD of P and ME values in cereal grains. In particular, the ME values for barley and triticale, which are greater than in reported literature data, show that it is necessary to get regularly updated information for contemporary cereal grains. The data also point out that there can be significant variation within the same type of grain. This variation could not be well explained by physical and chemical characteristics. Formulating diets with use of current data for both ATTD of P and ME concentration may have beneficial environmental impacts. Precise ration formulation, particularly with regard to efficient use of P, provides the opportunity to lower faecal P output without compromising adequate supply.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Table S1. Chemical composition of cereal grains

	Dry matter	Ash	CP[a]	EE[b]	aNDFom[c]	ADFom[d]	Starch	P[e]	Ca[f]	$InsP_6$[g]	Phyt.-act.[i]	GE[h]
	g/kg	g/kg dry matter									U/kg DM	MJ/kg DM
Barley												
Yool	878	23.9	117	26.2	208	51.3	642	4.22	0.52	2.52	490	18.7
ACK 2927	879	23.9	122	25.0	180	52.6	637	3.97	0.54	2.74	780	18.7
Lomerit	880	23.9	118	28.4	209	56.3	619	4.07	0.61	2.68	640	18.7
Campanile	877	25.1	117	29.7	205	50.5	625	4.07	0.53	2.43	1040	18.7
Canberra	879	22.8	131	28.4	191	44.5	616	4.37	0.59	2.51	570	18.8
Anisette	882	23.8	127	27.2	181	52.9	620	4.55	0.62	3.07	600	18.7
Metaxa	888	24.9	132	28.3	183	46.2	623	4.72	0.59	2.60	1100	18.8
Fridericus	881	21.6	128	34.1	194	63.4	606	4.13	0.63	2.53	700	19.1
Rye												
Conduct	880	16.3	116	18.9	155	30.5	651	3.65	0.54	1.70	4540	18.3
Visello	884	16.2	114	20.2	152	33.6	640	3.51	0.56	1.55	4100	18.3
Helltop	875	16.4	120	20.9	134	26.3	649	3.72	0.48	1.28	4380	18.4
Bellami	881	16.5	115	19.0	170	30.7	638	3.49	0.48	1.24	4150	18.3
Palazzo	879	16.5	112	20.2	172	33.0	634	3.49	0.53	1.37	4070	18.3
Dukato	882	16.4	115	18.4	133	26.1	652	3.54	0.50	1.42	4480	18.4
Guttino	880	16.4	108	17.7	150	29.5	646	3.34	0.51	1.23	4760	18.3
Dankowski Diament	880	17.3	127	18.6	148	27.3	640	3.67	0.47	1.60	4190	18.5

Table continued

Table S1
continued.

	Dry matter g/kg	Ash	CP[a]	EE[b]	aNDFom[c]	ADFom[d]	Starch	P[e]	Ca[f]	$InsP_6$[g]	Phyt.-act.[i]	GE[h]
		g/kg dry matter									U/kg DM	MJ/kg DM
Triticale												
Grenado	887	17.9	118	17.7	109	27.1	703	3.75	0.52	1.98	1870	18.3
Tarzan	876	18.6	128	17.8	101	30.4	702	4.06	0.41	1.92	2630	18.4
HYT Prime	881	18.7	131	21.1	163	30.6	682	4.30	0.44	1.91	2340	18.4
Massimo	878	18.0	126	18.5	156	30.5	704	4.06	0.60	1.93	2020	18.4
Cultivo	883	19.2	134	19.4	162	31.4	692	4.32	0.61	1.79	2400	18.5
SW Talentro	881	17.9	133	17.7	169	27.2	697	4.24	0.49	1.82	1640	18.4
Cando	882	17.7	119	18.9	117	24.5	727	4.01	0.34	1.86	1710	18.3
Agostino	884	17.0	121	20.7	144	32.1	709	3.62	0.42	1.57	2110	18.4
Wheat												
Skalmeje	876	14.6	131	18.7	110	24.6	735	3.47	0.40	1.94	1800	18.6
Tommi	877	15.9	140	20.0	111	29.4	716	2.74	0.44	2.04	2690	18.7
Tobak	875	15.1	129	22.8	114	37.7	714	2.72	0.44	1.87	2120	18.6
Event	875	16.9	137	21.3	124	35.5	715	3.86	0.43	2.29	1700	18.6
Mulan	876	16.3	132	23.9	133	33.1	713	3.75	0.42	2.09	1800	18.6
Tabasco	878	16.0	125	22.2	126	30.8	720	3.41	0.41	1.79	2640	18.7
Adler	876	16.6	152	23.5	120	33.1	702	3.92	0.41	1.86	2040	18.7
KWS	876	15.4	129	19.4	115	27.4	723	3.31	0.45	1.91	1410	18.5
Erasmus												

[a] CP, crude protein; [b] EE, ether extract; [c] aNDFom, neutral detergent fibre assayed with heat stable amylase and expressed exclusive ash;
[d] ADFom, acid detergent fibre expressed exclusive residual ash; [e] P, phosphorus; [f] Ca, calcium; [g] $InsP_6$, *myo*-inositol hexakisphosphate; [h] GE, gross energy; [i] Phyt.-act., phytase activity; [j] SD, standard deviation.

Table S2. Apparent total tract digestibility (%) of dry matter of cereal grains (each mean is based on six observations).

Barley	LSM†	SE‡	Rye	LSM	SE	Triticale	LSM	SE	Wheat	LSM	SE
Yool	82.4	2.21	Conduct	86.5	3.24	Grenado	89.8	2.01	Skalmeje	90.4	0.93
ACK 2927	80.7	1.69	Visello	86.2	2.76	Tarzan	90.7	1.60	Tommi	92.1	2.09
Lomerit	81.9	1.71	Helltop	86.3	3.20	HYT Prime	89.8	1.74	Tobak	90.4	0.95
Campanile	83.0	1.78	Bellami	86.1	2.61	Massimo	89.1	1.24	Event	89.5	1.31
Canberra	81.4	2.37	Palazzo	86.3	1.10	Cultivo	89.8	0.53	Mulan	88.7	2.66
Anisette	82.4	2.04	Dukato	89.4	2.14	SW Talentro	89.3	1.04	Tabasco	90.9	1.18
Metaxa	82.4	1.91	Guttino	88.2	1.94	Cando	91.4	1.57	Adler	91.1	0.84
Fridericus	81.1	1.65	Dankowski Diament	88.2	1.55	Agostino	88.4	1.30	KWS Erasmus	91.4	1.15
Mean	81.9	0.77	Mean	87.2	1.26	Mean	89.8	0.93	Mean	90.6	1.06

†Least squares means; ‡SE, standard error.

Table S3. Apparent total tract digestibility (%) of organic matter of cereal grains (each mean is based on six observations).

Barley	LSM†	SE‡	Rye	LSM	SE	Triticale	LSM	SE	Wheat	LSM	SE
Yool	83.6	2.10	Conduct	87.3	3.15	Grenado	90.6	1.89	Skalmeje	91.0	0.92
ACK 2927	82.1	1.66	Visello	87.0	2.67	Tarzan	91.5	1.47	Tommi	92.8	1.81
Lomerit	83.2	1.56	Helltop	87.1	3.17	HYT Prime	90.7	1.59	Tobak	90.9	0.89
Campanile	84.1	1.67	Bellami	86.8	2.53	Massimo	90.0	1.14	Event	90.2	1.28
Canberra	82.7	2.25	Palazzo	87.1	1.01	Cultivo	90.6	0.51	Mulan	89.6	2.33
Anisette	83.7	1.92	Dukato	89.9	1.99	SW Talentro	90.1	0.99	Tabasco	91.6	0.89
Metaxa	83.8	1.80	Guttino	89.0	1.88	Cando	92.1	1.43	Adler	92.7	0.81
Fridericus	82.3	1.60	Dankowski Diament	89.0	1.43	Agostino	89.3	1.19	KWS Erasmus	92.0	1.02
Mean	83.2	0.73	Mean	87.9	1.19	Mean	90.6	0.89	Mean	91.4	1.15

†Least squares means; ‡SE, standard error.

CHAPTER 4

Validation of an *in vitro* method for estimating the content of digestible phosphorus in various plant feedstuffs for pigs

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Abstract

A study was conducted to validate an *in vitro* method for the estimation of the digestible phosphorus (dP) content in plant feedstuffs fed to growing pigs. In total, 65 different samples were examined *in vitro* and *in vivo*, both following a strictly standardised trial protocol. Male castrated crossbred pigs (initial body weight ± standard deviation; 30.3 ± 5.6 kg) were fed a basal ration with a deficient P supply or an experimental diet containing a specified amount of the basal ration mixed with the corresponding plant feedstuff. Pigs were kept in metabolic crates, and faeces were collected quantitatively with 7-day adaptation and 7-day collection periods for calculation of apparent total tract digestibility (ATTD) of P. For determination of hydrolyzed P (hP) as part of total P, an enzymatic digestion of the feedstuff was simulated using a three-step *in vitro* system including xylanase, pepsin and pancreatin. Calculation of dP was by linear regression analysis. Comparison of all 65 *in vivo* and *in vitro* values revealed a linear correlation (dP_{all} (g/kg DM) = –0.125 ($P > 0.10$) + 1.482 ($P < 0.01$) • hP (g/kg DM), R^2 = 0.880, $P < 0.001$, residual standard deviation (RSD) = 0.652). The data was extended with additional feedstuffs and corrected for outliers, i.e., when hP-values were greater than dP-values, and regression analysis was repeated without intercepts ($dP_{without\ outliers}$ (g/kg DM) = 1.469 ($P < 0.01$) • hP (g/kg DM), R^2 = 0.923, $P < 0.001$, RSD = 0.442, n = 52). The applied *in vitro* method is a rapid and suitable instrument for estimating dP-values in plant feedstuffs for pigs. With precise estimation of dP-values, diet formulation can be optimised and excretion of excess P in animal manure can be minimized.

Keywords: apparent total tract digestibility, *in vitro*, pig, phosphorus, plant feedstuffs, prediction

Abbreviations: ADFom, acid detergent fibre expressed exclusive of residual ash; aNDFom, neutral detergent fibre assayed with heat stable amylase and expressed exclusive of residual ash; Abs, absorbance; ATTD, apparent total tract digestibility; BR, basal ration; BW, body weight; CDS, condensed distillers solubles; CP, crude protein; DDGS, dried distillers grains with solubles; DM, dry matter; dP, digestible phosphorus; EE, ether extract; hP, hydrolyzed phosphorus; $InsP_6$, *myo*-inositol hexakisphosphate; IW, initial weight; ME, metabolizable energy; ME_m, metabolizable energy for maintenance; rpm, revolutions per minute; SD, standard deviation; tP, total phosphorus.

1. Introduction

After energy and protein, phosphorus (P) is the most expensive feed resource in pig diets (Symeou et al., 2016). As inorganic P is an expensive and non-renewable resource the inclusion into the diet has to be minimized without inducing P deficiency. Moreover, excess P that is undigested or above the requirement can lead to various adverse environmental effects (Jongbloed, 1987; Golovan et al., 2001b; Ferket et al., 2002; Aarnink and Verstegen, 2007). Therefore pig rations are optimised on the basis of digestible P (dP). The dP is determined by multiplying total P content (tP) by the apparent total tract digestibility (ATTD) of the feedstuff (GfE, 20008).

To ensure the efficient use of P in the diet it is necessary to know accurately the dP content of the diet ingredients. Instead of time-consuming, labour-intensive and expensive *in vivo* measurements, a quick, accurate and relatively low-cost batch-related *in vitro* estimation of dP from single feedstuffs is of great importance. Different *in vitro* models have been developed that mimic the pig's digestive tract (Larsson et al., 1997; Liu et al., 1997, 1998; Bollinger et al., 2004, 2005; Schlegel et al., 2014). Those methods were based on a low sample size only and, as a consequence, they were not used for further research. In addition, they were insufficiently standardised and validated. Only Zhu et al. (2018) and Schlegel et al. (2014) calibrated the *in vitro* methods against *in vivo* data. Therefore, the aim of the present study was the validation of a standardised *in vitro* method (Kehraus et al., submitted). The validation was carried out on a large sample size, which was analyzed *in vivo* and *in vitro*, both following a strictly standardised experimental protocol.

2. Materials and methods

2.1. Feedstuffs

A total of 54 feedstuffs were selected, which reflected a wide range of commercial pig ration ingredients in Central Europe. Ingredients were obtained from different commercial feed mills or had been used in other studies before (alphabetical order; number of feeds per feed group in parentheses):

- Cereal grains: barley (11), maize, rye (8), sorghum, triticale (8), wheat (11),
- Grain by-products: corn cob mix, dried distillers grains with solubles (DDGS, 2), condensed distillers solubles (CDS, 2), oat husk meal, wheat bran, wheat gluten feed,
- Protein feeds: field bean,, potato protein concentrate, rapeseed expeller (2), soybean expeller, soybean meal (2).

The *in vivo* results of 32 cereal grains (Schemmer et al., 2020) and one sample each of barley, maize, potato protein concentrate, rapeseed expeller, soybean expeller and wheat (Schlegel et al., 2014) have already been published.

2.2. In vitro determination

Based on the *in vitro* method of Bollinger et al. (2004, 2005) further investigations and modifications were made by Kehraus et al. (submitted). The method was developed for estimation of dP in the diet for pigs and was used to mimic the gastric and small intestinal digestion of pigs by using endo-1,4-β-xylanase and endo-β-glucanase, pepsin/HCl and pancreatin. The modifications are described in detail by Kehraus et al. (submitted).

2.2.1. Samples and sample preparation

The samples were ground using an ultra-centrifugal mill (Type ZM200, Retsch, Haan, Germany) through 3.0 mm then 0.5 mm sieves to avoid heat damage. Initial weight (IW) of feed components with P content of <150 g/kg DM was 250.0 ± 0.2 mg. Blanks were created by performing the *in vitro* analysis without feed material. Sample material was weighed in triplicate into 43-mL polysulfone centrifuge tubes with polypropylene screw closures (Oak Ridge, Nalge Nunc, Neerijse, Belgium).

2.2.2. Enzymatic digestion and spectrophotometric measurement

A 3-mL volume of a 0.04% NaN_3 solution containing 5.3 g/L Natugrain® TS (containing ≥5600 units/g thermo stable endo-β-xylanase EC 3.2.1.8 and ≥2500 units/g thermo stable endo-β-glucanase EC 3.2.1.4; BASF, Ludwigshafen, Germany) was pipetted into each centrifuge tube, which were then sealed, gently mixed and incubated in a shaking water bath (120 rpm) at 39 °C for 1 h. After the incubation, 1 mL of a 0.85 N HCl solution with 7 g/L pepsin (from porcine stomach mucosa; P7012, EC 232-629-3, Sigma-Aldrich, Steinheim, Germany) were added. Samples were again mixed and then incubated for 2 h at 39 °C at 120 rpm. Following the second incubation step, 1.3 mL of a 0.8 M $NaHCO_3$ solution with 67.2 g/L pancreatin (from porcine pancreas, activity equivalent to 8 × USP specifications; P7545, EC 232-468-9, Sigma-Aldrich, Steinheim, Germany) were pipetted, mixed and incubated as before. Immediately after the third incubation, tubes were placed in an ice bath and 20 mL of a 2 N HCl solution was added. The solutions were mixed and centrifuged (Sorvall® RC-6 Plus Centrifuge with SS-34 Rotor, Thermo Fisher Scientific, Schwerte, Germany) at 1000*g* for 20 min (incubation procedure adapted from Bollinger et al., 2004, 2005). An aliquot (depending

on expected dP content and ranging from 1 to 6 mL) of the supernatant was pipetted from each tube and transferred to a 50-mL volumetric flask. Standards of 0, 1, 2, 6 and 10 mg/kg of a phosphorus-standard solution (439.4 mg/L KH_2PO_4 for 100 mg P/kg) were placed in 50-mL volumetric flasks, then 2.5 mL 22% HNO_3 solution (solution I) and 15 mL colouring agent consisting of equal portions of solution I, II (2.5 g NH_4VO_3 and 20 mL 65% HNO_3 per L) and solution III (50 g/L $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$) were added to each flask, mixed, incubated at room temperature for 1 h, then filled up with distilled water. The solutions were transferred into clean centrifuge tubes and centrifuged at 3000*g* for 5 min. Samples were analyzed immediately after centrifugation using a spectrophotometer (type DU® 62 UV/VIS, Beckman Coulter, Krefeld, Germany) with flow through cuvette at 415 nm (adapted from Engelen et al., 1994)).

2.3. In vivo procedure

2.3.1. Experimental diets

A basal ration (BR) was formulated to cover the requirements for energy and all nutrients according to the standard protocol (GfE, 1994). The BR was based on pregelatinised wheat starch, dried sugar beet pulp, potato protein and whole egg powder, which have no or minimal P content or phytase activity. Coarse materials of the feedstuffs listed above were ground individually through a hammer mill using a 3.0 mm screen. A reliable determination of the ATTD of P can only be achieved if the P supply is just below the requirement. This was derived from studies identifying the ATTD of P for pigs in order to minimize regulatory faecal P excretion and to maximise the potential of P absorption (GfE, 1994). Therefore, the concentration of dP in the experimental diets was adjusted to a maximum of 2.0 g/kg DM. Samples of tested feedstuffs were analyzed before diet formulation and calculated based on the analyzed P content and according to the ATTD of P derived from literature data (Düngelhoef et al., 1994; Rodehutscord et al., 1996; Steinbeck, 2000; NRC, 2012). The experimental diets were formulated by blending each test ingredient into the BR at a maximum rate of 600 g/kg. Diet preparation was made in the feed mill facilities at the Institute of Animal Science of the University of Bonn, Germany. The diets were prepared in one batch for each trial and stored in barrels at barn temperature until fed. Experimental diets and BR were not pelleted to prevent any negative heat effects on intrinsic phytase activity during pelleting.

2.3.2. Animals and experimental procedure

The experimental procedure was the same in each trial: castrated male crossbred pigs (initial body weight (BW) ± standard deviation (SD); 30.3 ± 5.6 kg) were allocated individually to metabolic crates allowing visual contact between the pigs. The pigs were located in an environmentally controlled room (ambient temperature 22 ± 2 °C) with a 12-h lighting programme. The pigs were fed twice daily, at 08:00 and 16:00h, with approximately equal amounts at every feeding throughout the experimental period. The diet was mixed with water immediately before feeding, and after the feeding period the pigs were offered water for at least 30 min. When changing the diet, the amount of feed was adapted to the BW, which was measured at the beginning and at the end of each collection period. The rations were fed in amounts corresponding to 2.0 to 2.5 times the maintenance requirement for metabolizable energy (ME; i.e., $ME_m = 0.44 \bullet BW^{0.75}$ (GfE, 2008b) based on the BW of the pigs.

In each experimental period faeces were collected quantitatively. The collection vessels were emptied every morning after feeding, weight was recorded and faeces were immediately frozen at –18 °C. All experiments were approved by the Ministry for Climate Protection, Environment, Agriculture, Nature Conservation and Consumer Protection of the State of North Rhine-Westphalia in accordance with German animal welfare legislation (file No. 84-02.04.2012.A144).

2.4. Chemical analysis

Samples of ration ingredients were taken before diet formulation, and samples of the diets were taken during diet formulation. The faeces were thawed and homogenised with an electric hand mixer and a representative subsample from each pig and experimental period was taken for subsequent chemical analysis. Faecal samples were freeze-dried (P18K-E-6, Piatkowski Forschungsgeräte, München, Germany) in duplicate. All samples were milled through a 1-mm mesh sieve using an ultra-centrifugal mill (ZM 200, Retsch, Haan, Germany). The dry matter (DM) was determined by oven-drying at 103 °C to constant weight.

All analyses were carried out according to the German Handbook of Agricultural Research and Analytic Methods (VDLUFA, 2012) using the given method numbers: ash (method 8.1), ether extract (EE; method 5.1.1b), starch (method 7.2.1), crude protein (CP, method 4.1.1), neutral detergent fibre assayed with heat stable amylase and expressed exclusive of residual ash (aNDFom; 6.5.1), acid detergent fibre expressed exclusive of residual ash (ADFom; 6.5.2). Calcium (Ca) was measured using atomic absorption spectrometry and P was analyzed colorimetrically (method 10.6.1).

2.5. Calculations and statistical analysis

Values for the *in vivo* digestibility of P were calculated according to GfE (1994):

$$\text{ATTD of } P_{Ration} = [(P_{Intake} - P_{Output})/P_{Intake}] \quad (1)$$

where P_{Intake} is the total P_{Intake} (g) during the collection period of seven days and P_{Output} is the total faecal P_{Output} (g) during the collection period.

The digestibility of P of the corresponding cereal grain was determined by the difference method according to GfE (1994):

$$\text{ATTD of } P_{Feedstuff} = [\text{ATTD of } P_{Ration} - \text{ATTD of } P_{BR} \bullet (1 - a)]/a \quad (2)$$

where ATTD of P_{Ration} and ATTD of P_{BR} is the ATTD of P from test diet and BR and a is the analyzed P content of feedstuff (g/kg DM) • inclusion rate of feedstuff in test ration (kg/kg DM)/analyzed P content in test ration (g/kg DM).

To calculate the amount of hydrolyzed P (hP) from single feedstuffs, the averaged value of the negative control (sum Abs_{blank}) was subtracted from the averaged value of the tested component. Total P and DM concentrations were required to calculate hP content per kg DM.

$$\text{hP (g/kg DM)} = \frac{\{\text{Abs} - [(\text{sum Abs}_{blank})/3]\} \bullet \text{factor} \bullet \text{photom. measuring solution (mL)} \bullet \text{digestion solution (mL)}}{\text{supernatant (mL)} \bullet \text{initial weight (g)} \bullet \text{DM (g/kg)}} \quad (3)$$

with: Abs is absorbance; factor is sum $ppm_{(of\ P\ standards)}$ /sum $Abs_{ppm\ (of\ P\ standards)}$; photometrical measuring solution is volume of solution before photometrical measurement (here 50 mL); digestion solution is volume of solution after enzymatic treatment (here 25.3 mL).

All data were analyzed using SAS 9.3 (SAS Institute, Cary, North Carolina, USA). Simple linear regression analysis was used to examine the relationship between *in vitro*-determined hP-values and *in vivo*-determined dP-values. In the regression analysis different groups of feedstuffs were examined. If the intercept was determined to be nonsignificant in the prediction model, it was excluded from the model in a second step and an adjusted R^2-value was calculated using the NOINT option of SAS. The R^2-value and the residual standard deviation (RSD) of the estimate were used to define equation accuracy. Significance was defined at $P \leq 0.05$.

3. Results

3.1. Feed ingredients and experimental rations

The chemical composition of the 17 ration ingredients is presented in Table 7, and that of the corresponding experimental rations is shown in Table 8. The mean P content of the single

feedstuffs ranged between 1.15 g/kg DM in oat husk meal to 12.9 g/kg DM in rapeseed expeller.

This wide range observed for P concentrations is also obvious for other chemical characteristics. For example, the CP content ranged between 38.6 (oat husk meal) and 846 g/kg DM (potato protein) and aNDFom ranged between 89.3 (corn cob mix) and 792 g/kg DM (oat husk meal). Therefore, the chemical composition of the different feedstuffs reflected a large proportion of the typical ingredients in pig rations across Europe and North America.

The analyzed P content in the experimental rations was in compliance with calculated values. By addition of 400–800 g/kg DM of the BR, depending on P content and estimated ATTD of P of the corresponding feedstuff, the P content of all rations was considerably reduced compared to the respective feed ingredient. All rations had a calculated concentration of less than 2.0 g dP/kg DM as requested by GfE (1994).

3.2. P-digestibility and in vitro hydrolyzed P

The ATTD of P is presented in Table 3. The greatest coefficients of ATTD of P were obtained in CDS and DDGS and the lowest for maize (0.87, 0.77 and 0.21, respectively). Accordingly, the greatest dP contents were observed for CDS and DDGS (7.15 and 6.75 g/kg DM, respectively), whereas the lowest dP content was obtained for oat husk meal, followed by maize (0.29 and 0.69 g/kg DM, respectively). A large variation was observed for hP-values among different plant feedstuffs. The hP-values ranged between 0.47 g/kg DM for sorghum and 4.64 g/kg DM for CDS.

First, regression analysis was carried out with the sample of 53 feedstuffs, which were not used by Kehraus et al. (submitted). It showed a positive relationship between hP- and dP-values:

$$dP_{new}\,(g/kg\ DM) = -0.125\ (P > 0.10) + 1.482\ (P < 0.01) \bullet hP\ (g/kg\ DM) \quad (4)$$

$R^2 = 0.880$, $P < 0.001$, RSD = 0.652

In a second step the regression analysis included additional data from Kehraus et al. (submitted) to expand the database. The resulting equation for the total of 65 feedstuffs was:

$$dP_{all}\,(g/kg\ DM) = 0.094\ (P > 0.10) + 1.286\ (P < 0.01) \bullet hP\ (g/kg\ DM) \quad (5)$$

$R^2 = 0.808$, $P < 0.001$, RSD = 0.652

Table 7

Chemical composition of the feedstuffs (g/kg dry matter), mean and range (in parentheses)

	n	Ash	Crude protein	Ether extract	aNDFom[a]	ADFom[b]	Starch	P[c]	Ca[d]	Crude fibre
Cereal grains										
Barley	11	24.8 (21.6-31.2)	124 (110-140)	26.7 (20.0-34.1)	201 (180-256)	56.5 (44.5-78.5)	612 (524-642)	4.32 (3.96-4.93)	0.60 (0.46-0.70)	45.4 (35.2-66.4)
Maize	1	15.8	88. 8	50.1	136	34.8[g]		3.27	0.09	29.0
Rye	8	16.8 (16.2-19.2)	115 (107-127)	19.3 (17.7-20.9)	154 (134-172)	30.8 (26.3-35.5)	643 (634-651)	3.58 (3.34-3.74)	0.51 (0.47-0.56)	19.2 (16.3-24.4)
Sorghum	1	14.0	95.5	35.3	151	66.8	752	2.94	0.09	27.0
Triticale	8	18.1 (17.0-19.1)	126 (118-134)	19.0 (17.7-21.1)	140 (101-169)	29.2 (24.5-32.1)	702 (682-727)	4.04 (3.62-4.32)	0.48 (0.34-0.61)	19.5 (15.8-21.4)
Wheat	11	16.4 (14.6-19.2)	133 (103-152)	20.7 (17.5-23.9)	119 (110-132)	32.4 (24.6-39.0)	710 (652-735)	3.72 (3.31-4.45)	0.40 (0.36-0.45)	22.0 (18.6-26.4)
Grain by-products										
Corn Cob Mix	1	12.9	100	46.4	89.3	33.2	699	2.75	0.06	29.4
DDGS[e]	2	52.9 (46.7-59.1)	355 (339-371)	83.2 (69.1-97.3)	404 (399-408)	196 (195-196)	37.0[g]	8.82 (8.71-8.92)	0.59 (0.32-0.86)	79.2 (71.9-86.4)
CDS[f]	2	80.2 (59.3-101)	251 (214-288)	52.8 (40.7-64.8)	97.7[g]	45.1 (14.7-75.4)		8.29 (7.92-8.65)	1.94 (1.69-2.18)	15.5 (5.73-25.2)
Oat husk meal	1	43.1	38.6	10.1	792	417		1.15	0.42	342
Wheat bran	1	54.8	176	43.3	398	124		11.5	0.82	101
Wheat gluten feed	1	51.0	165	50.5	362	117	267	8.36	1.35	76.5
Protein feeds										
Field bean	1	31.6	332	20.2	230	112	444	4.72	0.88	
Potato protein	1	19.6	846	37.0				4.61	1.39	9.00
Rapeseed expeller	2	73.5 (68.5-78.5)	356 (345-367)	107 (103-111)	360[g]	234[g]		12.6 (11.3-14.0)	8.19 (8.01-8.37)	134 (119-148)
Soybean expeller	1	67.8	474	70.0				7.26	3.35	59.0
Soybean meal	1	73.8	494	17.1	212[a]	149	118	6.81	4.97	80.4

[a] aNDFom, neutral detergent fibre assayed with heat stable amylase and expressed exclusive ash.
[b] ADFom, acid detergent fibre expressed exclusive residual ash.
[c] P, phosphorus.
[d] Ca, calcium.
[e] DDGS, dried distillers grains with solubles.
[f] CDS, condensed distillers solubles.
[g] only 1 analysis.

Table 8

Chemical composition of rations (g/kg dry matter), mean and range (in parentheses)

	n	Ash	Crude protein	Ether extract	aNDFom[a]	ADFom[b]	Starch	P[c]	Ca[d]
Cereal grains									
Barley	10	27.9 (26.6-30.6)	143 (128-160)	38.6 (31.8-48.1)	267 (177-340)	54.7 (46.0-66.1)	564 (520-636)	3.05 (2.73-3.26)	2.84 (2.50-3.04)
Maize	1	22.0	116	47.6	110	41.7		2.42	2.66
Rye	8	23.9 (22.3-24.6)	142 (120-151)	31.2 (22.9-35.9)	326 (197-398)	38.0 (35.2-41.2)	624 (553-679)	2.77 (2.70-2.89)	2.83 (2.46-3.10)
Sorghum	1	20.6	135	43.1	123	65.4		2.48	1.93
Triticale	8	23.5 (22.6-24.4)	154 (148-160)	33.0 (29.9-37.1)	289 (206-361)	35.5 (32.0-38.1)	600 (582-616)	2.91 (2.62-3.11)	2.64 (2.54-2.74)
Wheat	10	23.1 (21.9-24.0)	134 (129-143)	35.5 (31.7-43.6)	158 (108-270)	40.4 (32.5-45.8)	634 (619-652)	2.88 (2.67-3.06)	2.64 (2.41-2.90)
Grain by-products									
Corn Cob Mix	1	20.3	139		92.0	37.7		2.37	1.85
DDGS[e]	2	38.3 (35.8-40.7)	210 (188-231)	56.6 (53.4-59.7)	209 (191-226)	94.0 (72.0-116)		3.42 (3.36-3.47)	4.97 (4.84-5.09)
CDS[f]	2	48.2 (42.9-53.5)	162 (151-172)	48.4 (43.8-53.0)	174 (160-187)	43.7 (38.7-48.6)		3.56 (3.52-3.59)	4.93 (4.86-5.00)
Oat husk meal	1	43.8	103	33.5	404	228		1.68	3.97
Wheat bran	1	40.0	148	44.7	216	72.9		4.00	4.90
Wheat gluten feed	1	44.0	175	62.4	251	88.0		5.86	2.40
Protein feeds									
Field bean	1	31.8	268	35.6	424	92.3		3.69	2.46
Rapeseed expeller	1	40.5	198	63.3	204	89.8		4.05	6.56
Soybean meal	1	55.1	302	41.2	212	81.8	431[g]	4.42	6.01

[a] aNDFom, neutral detergent fibre assayed with heat stable amylase and expressed exclusive ash.
[b] ADFom, acid detergent fibre expressed exclusive residual ash.
[c] P, phosphorus.
[d] Ca, calcium.
[e] DDGS, dried distillers grains with solubles.
[f] CDS, condensed distillers solubles.
[g] only 1 analysis.

Table 9

In vivo apparent total tract digestible phosphorus (P), coefficients of *in vivo* apparent total tract digestibility (ATTD) of P and hydrolyzed P from plant feedstuffs

	n	*In vivo* apparent total tract digestible P (g/kg DM)	SD [a]	*In vivo* ATTD of P (coefficients)	SD	*In vitro* hydrolyzed P (g/kg DM)	SD
Cereal grains							
Barley	11	1.92	0.26	0.44	0.05	1.28	0.20
Maize	1	0.71		0.21		0.44	
Rye	8	1.60	0.11	0.45	0.02	1.80	0.17
Sorghum	1	0.98		0.34		0.47	
Triticale	8	2.02	0.26	0.50	0.04	1.49	0.16
Wheat	11	2.21	0.21	0.60	0.05	1.42	0.26
Grain by-products							
Corn Cob Mix	1	2.10		0.76		1.32	
DDGS[b]	2	6.75	0.93	0.77	0.09	4.28	0.16
CDS[c]	2	7.15	0.81	0.86	0.04	4.67	0.70
Oat husk meal	1	0.29		0.26		0.54	
Wheat bran	1	6.46		0.56		4.16	
Wheat gluten feed	1	2.90		0.35		2.47	
Protein feeds							
Field bean	1	1.87		0.40		1.02	
Potato protein	1	1.87		0.41		0.97	
Rapeseed expeller	2	4.34	0.39	0.34	0.02	4.10	0.93
Soybean expeller	1	2.85		0.39		1.98	
Soybean meal	1	2.91		0.41		2.86	

[a] SD, standard deviation.

[b] DDGS, dried distillers grains with solubles.

[c] CDS, condensed distillers solubles.

Linear positive correlations were also identified when the data was corrected for outliers, i.e. when hP-values were greater than dP-values. In addition outliers were calculated separately. The resulting equations were:

$dP_{without\ outliers}$ (g/kg DM) = -0.020 ($P > 0.10$) + 1.480 ($P < 0.01$) • hP (g/kg DM) (6)

$R^2 = 0.917$, $P < 0.001$, RSD = 0.446, n = 52

$dP_{outliers}$ (g/kg DM) = -0.178 ($P > 0.10$) + 1.024 ($P < 0.01$) • hP (g/kg DM) (7)

$R^2 = 0.881$, $P < 0.001$, RSD = 0.444, n = 13

Because the intercepts in the equations were not significantly different from zero, in a second run the regression analysis was repeated with a straight line passing through the origin to test the model without intercepts. The equations without intercepts and their graphic representation are presented in Figures 2–5. They also show a strong linear correlation.

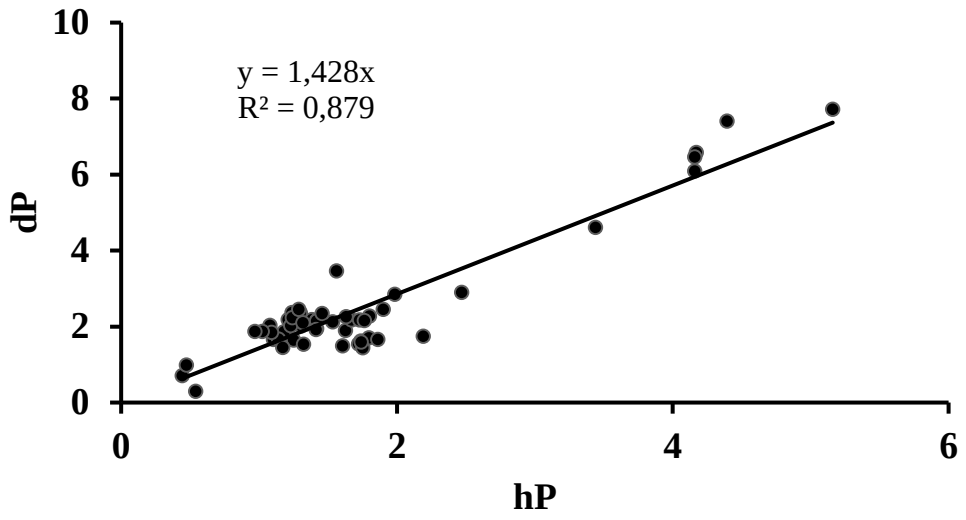

Figure 2
Relationship between hydrolyzed P (hP, g/kg DM) and digestible P (dP, g/kg DM) in plant feed ingredients (n = 54; y = 1.428 x; $R^2 = 0.879$; $P < 0.001$, RSD = 0.649)

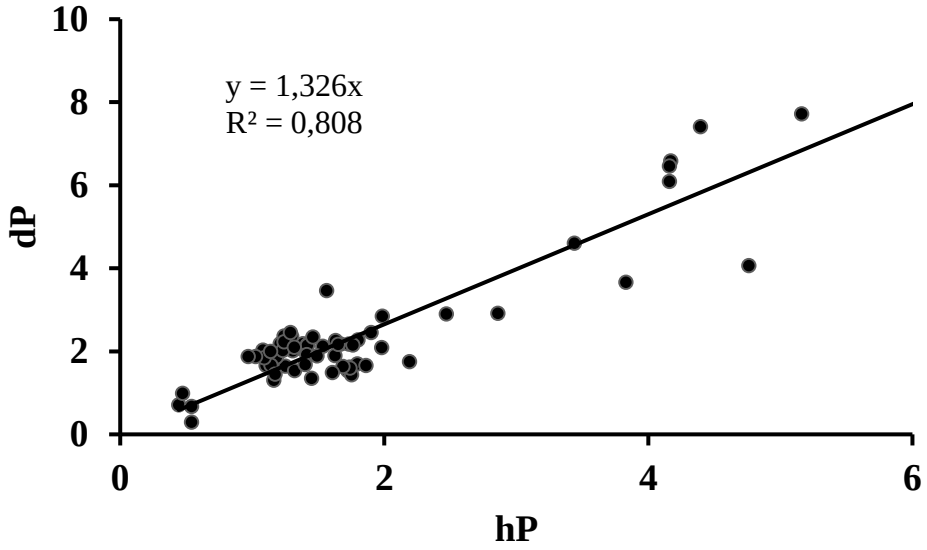

Figure 3

Relationship between hydrolyzed P (hP, g/kg DM) and digestible P (dP, g/kg DM) in plant feed ingredients (with additional data from Kehraus et al. (submitted); n = 65; y = 1.326 x; R^2 = 0.808; P < 0.001, RSD = 0.649)

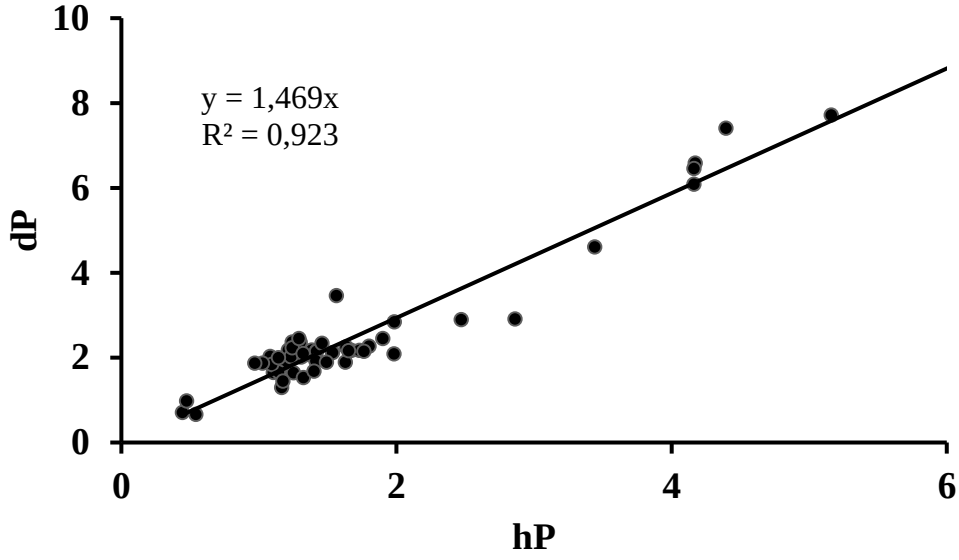

Figure 4

Relationship between hydrolyzed P (hP, g/kg DM) and digestible P (dP, g/kg DM) in plant feed ingredients (with additional data from Kehraus et al. (submitted); without outliers; n = 52; y = 1.469 x; R^2 = 0.923; P < 0.001, RSD = 0.442); samples were considered as outliers, if measured hP values are above dP values

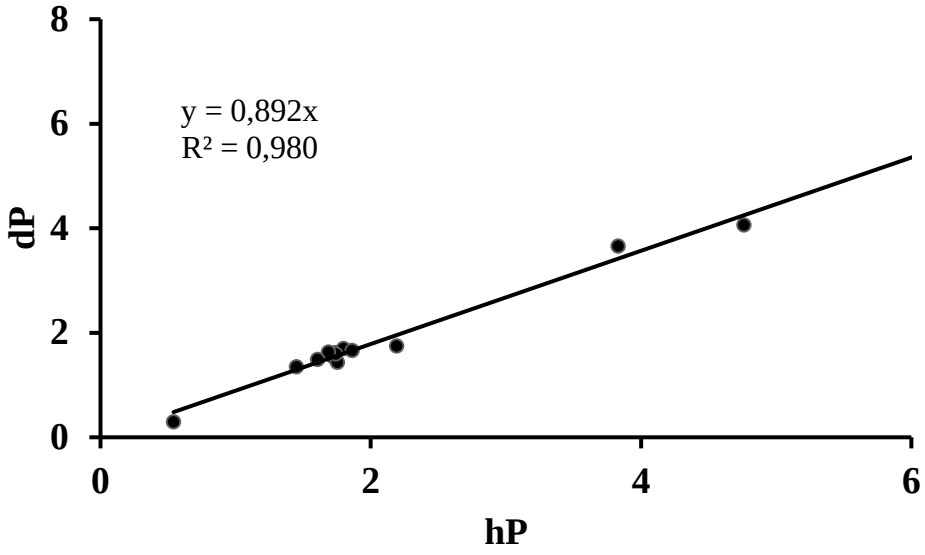

Figure 5
Relationship between hydrolyzed P (hP, g/kg DM) and digestible P (dP, g/kg DM) in plant feed ingredients (only outliers; n = 13; y = 0.892 x; R^2 = 0.980; P < 0.001, RSD = 0.433); samples were considered as outliers, if measured hP values are above dP values

4. Discussion

In general, the results for the ATTD of P were in the range of DLG (2014) and NRC (2012). The results for cereal grains match data from Jongbloed (1987), Düngelhoef et al. (1994) and Rodehutscord et al. (1996). Reported values for sorghum and soybean expeller were in agreement with NRC (2012), whereas NRC values for field beans and soybean meal were lower than those observed in the present study. It should be noted that the NRC method for measuring the ATTD of P is not the same as that applied here, but because there were no other data available, they were used as an indication. Shi et al. (2015) determined an ATTD of P for rapeseed meal of 0.35, which is in agreement with the result for rapeseed expeller, but slightly higher than the result for rapeseed meal obtained in this study. The values for CDS, DDGS and corn cob mix in this study are greater than those reported by Jongbloed (1987) and Pedersen et al. (2007). Especially for corn cob mix, the results from this study are much higher than those presented by DLG (2014) (0.76 vs. 0.55, respectively). The difference between CDS and DDGS is likely to be a result of the different ways of processing the types of distillers' grains. Before or during drying, the CDS are blendfed (Belyea et al., 2010). Depending on the varying proportions of different raw materials and the main final product there are different processing chains. The different processing steps can directly affect the enriching compounds and especially the substances influencing the ATTD of P in the final

by-product. Liu and Han (2011) have made a detailed investigation on the different P fractions during the processing of maize grain into ethanol. During the fermentation process at the beginning of ethanol production the phytate undergoes some degradation due to the action of yeast phytase, and the solubility of inorganic P is higher than that of organically bound P. They stated that more inorganic P was recovered in thin stillage than the rest of P after centrifugation. Furthermore, phytate-P partitioning into the liquid fraction and solid fraction upon centrifugation was relatively proportional to that of tP. This confirms the higher ATTD of P of CDS in comparison to DDGS.

Although there is no difference in phytase activity in high-moisture ensiled maize (e.g., corn cob mix) and dry maize grain, the availability of P can be three to four times higher in corn cob mix (Eeckhout and De Paepe, 1994). The results for fermented and liquid feed in this study show that the increased ATTD of P seems to be a result of reduced phytate-P content. Within the induced fermentation process phosphate is released from phytate and liberated as additional P, as discussed before.

The present paper is based on a large database of different feedstuffs, and identical samples were investigated *in vitro* and *in vivo* following the same strictly standardised trial protocol. This is unique and shows the high robustness of the data. Except for rye and rapeseed by-products, the developed regression equations can be applied to a wide range of feedstuffs used in current pig ration formulation.

The evaluation of relationships between calculated hP and measured dP followed the overall goal to establish a generalised equation for the prediction of dP-values in plant feedstuffs from laboratory measurements. Generalised equations are of great interest for the feed industry, as they provide the easiest handling in daily routine. However, it is also of great importance to get a precise prediction of dP-values. Therefore, regression analysis was first performed for the 53 feedstuffs that had not been used previously (Kehraus et al., submitted). To expand the database and to strengthen the regression analysis, the 12 feedstuffs from the previous study (Kehraus et al., submitted) were then added to the database and the regression analysis repeated. Although this analysis of 65 feedstuffs revealed a moderate coefficient of determination of $R^2 = 0.808$, prediction of dP-values using Equation 5 could reveal imprecise results. The inaccuracy of Equation 5 derives mainly from few outliers. Therefore, in the next step (Equation 6), rye, oat husk meal and rapeseed products were excluded from the regression analysis for the following reason: Rye, oat husk meal and rapeseed products have shown higher values for hP *in vitro* than determined dP-values *in vivo*. Because hP is defined as a part of dP, the hP-value can never exceed the *in vivo*-determined dP-value. Therefore, all

feedstuffs where hP-values were greater than *in vivo*-measured dP-values were excluded from Equation 6. The exclusion of those outliers improved the coefficient of determination by almost 14%, from 0.805 to 0.917. Equation 6 still yielded an accurate prediction of dP-values for several plant feedstuffs. In addition, regression analysis was carried out for outliers, i.e., feedstuffs that had hP-values higher than dP-values. A close relationship between hP- and dP-values was also found for outliers, with a coefficient of determination of R^2 = 0.881. The results show that the separation of feedstuffs according to hP-values that are lower or higher than measured dP-values allows a more precise prediction of dP for both groups of feedstuffs.

A further separation of feedstuffs into the categories cereal grains, grain by-products and protein feeds revealed R^2-values of 0.218, 0.976 and 0.690, respectively. Because only for protein feeds was an improved value of R^2 observed, no further consideration was made, but continuing analysis was performed to simplify the prediction equation. As regression analysis showed very low intercept values in Equations 5–7, it was evaluated if deletion of the intercept also gave accurate results. The calculation of regression analysis with a straight line passing through the origin also revealed precise equations. The coefficients of determination were between 0.917 and 0.970. Figures 2–5 clearly demonstrate the strong positive correlation between values of hP and dP. Therefore, no intercepts are needed for an accurate prediction of dP-values from hP-values. Because the slope of Equation 7 was not significantly different from 1 (X = Y, $P < 0.10$), the measured hP-values from the outliers can be equated with dP-values without additional calculation. This means the *in vitro*-determined hP-values are equal to measured dP-values. For those feedstuffs, all of the *in vivo* available P is hydrolyzed under conditions applied *in vitro*. Further studies are warranted to evaluate whether the equation also applies when a larger sample size is used.

The high hP-values in rye, rapeseed products and oat husk meal may be due to different reasons. Rye has by far the highest phytase activity of the investigated feedstuffs (Eeckhout and De Paepe, 1994; Steiner et al., 2007). It seems plausible that rye shows the highest efficiency of phytase hydrolysis with the *in vitro* procedure and nearly all dP is rapidly hydrolyzed. Pohl (2015) described problems in the colorimetric determination because different feedstuffs can show hazes of different intensity. Therefore, in contrast to Kehraus et al. (submitted) the second centrifugation step was increased from 2000*g* to 3000*g*. This was done in order to minimize the risk of floating particles influencing the measuring process. As colorimetric determination was carried out immediately after centrifugation, this problem was avoided as far as possible. A further approach could be to filter the solution instead of the

second centrifugation step. This would have the added advantage that floating particles would be removed permanently and could not influence the spectrophotometric measurement.

Schlegel et al. (2014) measured *in vitro* P release with a method based on Liu et al. (1998) and Bollinger et al. (2004, 2005) with small modifications. They described a deviation downwards for rapeseed and soybean expeller, which had the highest crude fat contents in their study. They suspected a slower release of bound P in phospholipid form in their *in vitro* method. This does not strictly conform to the results of the present study. Rapeseed expeller showed the highest crude fat content in our study, but rye and oat husk meal represented feedstuffs with only low crude fat content. Additional work on an *in vitro* method was carried out by Zhu et al. (2018). Although the method was calibrated with *in vivo* data, their assay can only be used to predict the P-digestibility of animal protein by-products and not of plant materials. This further strengthens the importance of the database from the present study.

Comparative consideration of the present results is hampered by a lack of data in the literature dealing with large sample sizes of plant feedstuffs to predict dP-values or the ATTD of P from *in vitro* methods. Further investigation is also necessary to determine the potential of the *in vitro* procedure to predict dP-values in compound feeds.

5. Conclusions

This is the first study that links strictly standardised *in vitro* and *in vivo* data from a large sample size for validation. The estimation of dP content from linear regression equations enables a precise prediction for a wide range of plant feedstuffs. Especially for feedstuffs where currently no data for the ATTD of P or dP-values are available, the method can be an essential tool to gain an initial indication of values. The *in vitro* procedure can be used as a cheap and rapid system for batch-related analysis instead of expensive and time-consuming *in vivo* measurements that cannot be made for specific batches in a timely manner. Moreover, the present work provides a reliable database of *in vivo* ATTD of P for a range of plant feedstuffs used in rations for pigs. These values can be used for accurate ration preparation with a focus on minimizing P output into the environment.

Conflict of interest

The authors declare they have no conflicts of interest.

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CHAPTER 5

General discussion and conclusions

Environmental pollution plays an important role in society. The focus is increasingly on N and P output. The German government has recently set up a new fertiliser ordinance (Anonymous, 2017). A main subject is the material flow balance for N and P, which is directly linked to attempts to optimize nutrient supply. Feeding animals such that minimum N and P output can be achieved is indispensable in complying with the fertilizer ordinance and material flow balance requirements without the necessity to reduce animal numbers. The supply of P and N above animal requirements has not only ecological but also economic impacts on the farm. For an accurate diet formulation it is mandatory to know the exact feed value of the feedstuffs used.

Therefore, the main objective of this thesis was to characterize the apparent total tract digestibility (ATTD) of phosphorus (P) and the metabolizable energy (ME) concentration of different genotypes of barley, rye, triticale and wheat. Special focus was also given to the validation of a method to predict the digestible P (dP) content of several plant feedstuffs by a simple and rapid *in vitro* method. In a collaborative research project, the ATTD of P and ME concentration values of 32 different cereal grain genotypes were determined. It was shown that differences exist in the ATTD of P between cereal grain species. For barley and triticale, differences also occurred between genotypes of the same species. The same observation was made for ME concentrations of cereal grains, where also differences between different genotypes within grain species were found.

In vivo measurements – Phosphorus

The first priority of the *in vivo* experiment was to measure the ATTD of P. Therefore, the trial protocol followed the guidelines laid doiwn by the GfE (1994). Very similar guidelines have been followed by others (She et al., 2017), thus allowing comparison between the results of this thesis and those from other studies A detailed comparison of literature values is given in Chapter 3.

Phosphorus digestibility is measured in order to get exact data on the dP content of feedstuffs used in pig nutrition. After this, precise diet formulation considering animal requirements can be made. Because global rock phosphate stocks are limited (Cordell et al., 2009), it is of primary importance to minimize mineral P supply in the feed and to maximise

the utilisation of plant P sources. At the same time and with regard to environmental pollution, the P output via manure has to be reduced. The easiest way to decrease the P concentration in pig manure is to reduce the P concentration of the diet. Secondly, the addition of microbial phytase can increase the ATTD of P from plant feedstuffs and, as a consequence, no additional mineral P may be needed. There are three types of phytases, which can all improve phytate-P utilisation and generate additional dP from feedstuffs: intrinsic phytase from plant feedstuffs, endogenous phytase from intestinal mucosa and microbes and supplemented microbial phytase (Maenz, 2001). All experimental rations described in Chapters 3 and 4 were fed without supplementation with microbial phytase. Because in the present study experimental rations were not pelleted before feeding, intrinsic phytase was not inactivated. Temperatures above 81 °C, which may occur during pelleting, can inactivate a major part of intrinsic plant phytase activity and therefore decrease $InsP_6$ degradation and reduce P-digestibility (Jongbloed and Kemme, 1990). The influence on the ATTD of P of differences in intrinsic phytase content between different cereal grains and between different genotypes of the same grain species is less pronounced than the effect caused by microbial phytase addition; as Eeckhout and De Paepe (1991) demonstrated, plant phytases are less active than microbial phytases in the gut. The main reason is the broader pH activity range of microbial phytase, whereas intrinsic plant phytase has a small pH spectrum. Therefore, the time taken for phytate degradation by microbial phytase is extended in the gastrointestinal tract. Additionally, the effect of microbial phytase supplementation depends on the phytase activity and the specific phytase used in the ration because the pH spectrum varies between different phytases. Although the effect of microbial phytase addition has not been tested in this study, it is the most effective tool in pig nutrition to reduce P excretion from animal manure (Maenz, 2001). Additional strategies to reduce environmental P pollution are presented in Chapter 1 of this thesis.

In recent years, intensive research has been directed towards the fate of P compounds in the gastrointestinal tract of poultry. For a long time it was assumed that P-digestibility is the same for fowl and pigs. This assumption, however, has had to be revised. It must be noted that a direct comparison of fowl and pigs is not possible because different models are applied for the determination of overall P availability. A widely applied system for pigs, which was also used in this thesis, is the determination of ATTD of P, whereas for poultry the precaecal P-digestibility (pcdP) is recommended for raw-material evaluation (World's Poultry Science Association, 2013). Although the determination of P-digestibility for pigs is measured in the total tract and for poultry in the precaecal part, in the interest of comparability the term

'digestibility' is used for both species at the same time. For example, for maize the digestibility of P in poultry is much higher than in pigs (0.51–0.60 for poultry vs. 0.21 for pigs; Ingelmann et al., 2018b and Chapter 4) and turkeys (0.22–0.28; Ingelmann et al., 2018a). The higher P-digestibility shown by broilers is based on their higher potential to degrade phytate in the gastrointestinal tract (Rodehutscord and Rosenfelder, 2016). After very low $InsP_6$ reduction in the crop, it increases to about 70% of the amount consumed up to the ileum in broiler chickens (Zeller et al., 2015a) whereas it reaches only about 40% up to the ileum in pigs (Rutherfurd et al., 2014). Because plant phytase activity is very similar in the corresponding rations used in the feeding trials with pigs and fowl, and no microbial phytase was added, it can therefore be assumed that different P digestibilities depend on the different potentials for $InsP_6$ degradation in the gastrointestinal tract.

The framework of the collaborative research project GrainUp allows a direct comparison of the ATTD of P in pigs with the pcdP of broilers for the eight triticale genotypes. Both experiments were carried out without addition of microbial phytase to the experimental rations. Therefore, only intrinsic plant phytase and endogenous phytase from the animals' mucosa and microbes could have affected phytate degradation. The P-digestibility of triticale was higher in broilers than in pigs (Table 12; Witzig et al., 2018; Schemmer et al., submitted) for all eight genotypes, as has also been shown for maize grain (Ingelmann et al., 2018b). For pigs and broilers there were significant differences in the ATTD of P and pcdP between different genotypes for triticale and wheat, but no effect of intrinsic plant phytase was observed. Furthermore, total P and phytate-P concentrations had only negligible effects on ATTD of P and pcdP, respectively. This was consistent in all three studies (Schemmer et al., submitted; Ingelmann et al., 2018a; Witzig et al., 2018).

Table 10

Estimated prececal phosphorus (P) digestibility (pcdP) and apparent total tract digestibility (ATTD) of P (%) of triticale genotypes fed to broilers and pigs, respectively

	Triticale genotype[1]							
	1	2	3	4	5	6	7	8
Estimated pcdP (broilers)[2]	0.64	0.72	0.75	0.53	0.74	0.73	0.71	0.78
ATTD of P (pigs)[3]	0.51	0.54	0.49	0.48	0.52	0.51	0.54	0.40

[1] Genotypes numbered as described in Rodehutscord et al. (2016).

[2] Data from Witzig et al. (2018).

[3] Data from Schemmer et al. (submitted).

The SD of the ATTD of P within individual genotypes was greater than that between different genotypes (Chapter 3). This was the case for all four different cereal grain species. The mean SD within single genotypes ranged from 5.05 (barley) to 7.40 (wheat) and the corresponding SD between different genotypes from 2.12 (rye) to 5.07 (wheat). The high SD of ATTD of P within individual genotypes may be attributed to individual differences in net absorption of P between different pigs, which has also been described by Rodehutscord et al. (1994).

In vivo measurements – metabolizable energy

The measurement of ME concentrations of the different cereal grains was made as part of the measurement of ATTD of P. Similar to dP, a detailed protocol exists for the determination of the ME of pig feeds (GfE, 2005). Although the protocol is not the same for both measurements, the essential criteria for ME determination as required by GfE (2005) have been realised: Pigs had a body weight (BW) between 30 and 90 kg; the inclusion level of test ingredients in experimental rations was above 500 g/kg DM; each collection period lasted for more than five days; and feed was offered in two meals per day. Moreover, chemical composition (crude protein (CP), ether extract (EE) and crude fibre (CF)) of the rations was within the range receommended by GfE (2005). Account had to be taken of the deficient P level, which was necessary for the measurement of ATTD of P in this study. The P content was lower than in typical pig rations, but no negative effects on daily gain or feed conversion ratio were observed and no minimum P content was given by GfE (2005) and. As described in the trial protocol for ME measurement, the standard deviation (SD) of the digestibility of organic matter (OM) of the diets can be used to assess the accuracy of the *in vivo* measurement. The mean SD of OM digestibility of the cereal grains, as listed in Table 13, were generally low and, therefore, the accuracy of the experiment can be regarded as good.

Table 11

Mean coefficients of *in vivo* digestibility of organic matter (dOM) and mean standard deviation (SD) of experimental rations (see Chapter 3)

	Barley[1] (n=8)	Rye[1] (n=8)	Triticale[1] (n=8)	Wheat[1] (n=8)
DC_{OM}	0.83	0.88	0.91	0.91
SD	0.018	0.022	0.013	0.012

[1] The proportion of the corresponding cereal grain is 600 g/kg dry matter in the complete ration. The rest of the ration is a basal ration, which is described in detail in chapter 3.

The *in vivo* experiment clearly showed that ME concentrations of contemporary cereal grain genotypes as investigated in this study are greater than tabulated values (DLG, 2014; except for rye, where measured values were lower than values given by DLG, 2014). Differences between genotypes within grain species were observed for barley, triticale and wheat. In addition to the results determined in the *in vivo* trials, ME concentrations were also predicted by the equation of GfE (2006). The equation is based on the chemical characteristics of the cereal grains and is valid for pigs with an initial BW of 30–90 kg, which is in line with the BW of the pigs used in the present experiment. The prediction equation of GfE (2006) is as follows:

ME (MJ) = 0.0205 x digestible crude protein (g) (1)
+ 0.0398 x digestible ether extract (g)
+ 0.0173 x starch (g)
+ 0.0160 x sugar (g)
+ 0.0147 x (digestible organic matter – digestible crude protein – digestible ether extract – starch – sugar) (g)

Table 12 presents the differences between calculated ME-values via the equation of GfE (2006) and measured ME concentrations from *in vivo* measurements, which are presented in detail in Chapter 3. There were differences between measured and predicted ME-values of up to 0.88 MJ ME/kg DM for single genotypes. Differences of mean ME-values for the eight genotypes of barley, rye, triticale and wheat were between 0.34 MJ ME/kg DM for wheat and 0.58 MJ ME/kg DM for rye. The ME-values from the prediction equation of GfE (2006) can be both over- and underestimated when compared to results from *in vivo* experiments with the same plant material. The eight predicted ME-values for rye showed an overestimation for seven genotypes. On the other hand, in barley, triticale and wheat the predicted values from the equation were underestimated for most genotypes. Those differences are in the same range as those between investigated *in vivo* data and ME-values listed by DLG (2014). This can be due to the fact that the prediction equations of GfE (2006) are largely based on data from DLG (2014).

Table 12

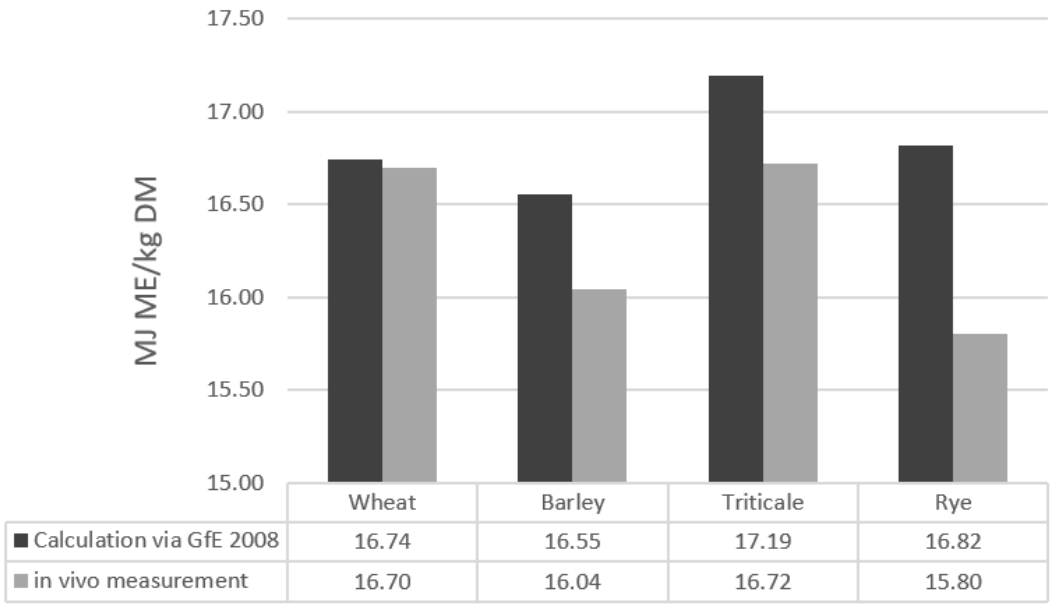
Comparison between *in vivo*-measured (chapter 3) and calculated (GfE, 2008a) ME concentrations (MJ/kg dry matter) of cereal grains

Wheat	Genotype[1]								
	1	2	3	4	5	6	7	8	mean
In vivo measurement	16.15	16.55	16.17	16.09	15.72	16.2	16.41	16.25	16.19
Calculation via GfE 2008a	15.82	15.82	15.84	15.89	15.89	15.84	15.97	15.74	15.85
Difference	0.33	0.73	0.33	0.20	-0.17	0.36	0.44	0.51	0.34
Barley	Genotype								
	1	2	3	4	5	6	7	8	mean
In vivo measurement	14.81	14.29	15.08	15.24	15.11	15.18	15.11	14.54	14.92
Calculation via GfE 2008a	14.37	14.39	14.40	14.42	14.45	14.43	14.46	14.55	14.43
Difference	0.44	-0.10	0.68	0.82	0.66	0.75	0.65	-0.01	0.49
Triticale	Genotype								
	1	2	3	4	5	6	7	8	mean
In vivo measurement	16.18	16.41	16.35	16.11	16.33	16.23	15.64	15.36	16.08
Calculation via GfE 2008a	15.52	15.55	15.55	15.57	15.57	15.58	15.60	15.60	15.57
Difference	0.66	0.86	0.80	0.54	0.76	0.65	0.04	-0.24	0.51
Rye	Genotype								
	1	2	3	4	5	6	7	8	mean
In vivo measurement	14.54	15.43	15.00	14.78	14.63	14.88	15.12	15.10	14.82
Calculation via GfE 2008a	15.42	15.40	15.46	15.37	15.36	15.41	15.36	15.43	15.40
Difference	-0.88	0.03	-0.46	-0.59	-0.73	-0.53	-0.24	-0.33	-0.58

[1] genotypes numbered as described in Rodehutscord et al. (2016)

A reason for the differences between predicted and measured values can be the plant material and the data used for the prediction equation of GfE (2006). Because the variation in the chemical composition of the cereal grains in this study and those used for the prediction equation of GfE (2006) is very low, the resulting ME-values are closely related. In contrast, differences in *in vivo* measurements are higher because of differences between the animals. As prediction equations always depend on precisely determined *in vivo* measurements, regular updated *in vivo* data are of great importance. This was also shown by the differences between listed and measured values in this experiment. In the interest of comparability the *in vivo* experiments should always follow an identical trial protocol.

As there are different equations for the prediction of ME-values for single feedstuffs and compound feed, those of the rations were also examined with the equation for compound feed (GfE, 2008a). The comparison between estimated and measured ME-values of the experimental rations shows major differences (Figure 6). The equation by GfE (2008) for the prediction of compound feed is only for feed samples within the range of ≥150 and ≤250 g/kg DM CP, ≤60 g/kg DM EE and ≤80 g/kg DM CF and, except for the triticale rations, the crude protein content of the experimental rations was below the bounds. As described by GfE (2008a), the accuracy of the prediction can be impaired when the rations are not within the range. Deviating from the other rations, the predicted result for the wheat rations conformed with the measured result from *in vivo* studies.



	Wheat	Barley	Triticale	Rye
Calculation via GfE 2008	16.74	16.55	17.19	16.82
in vivo measurement	16.70	16.04	16.72	15.80

Figure 6

Comparison between calculated (GfE, 2008b) and *in vivo* measured ME concentrations (MJ/kg dry matter) of experimental rations

Prediction of digestible P from in vitro measurements

A second objective of this thesis was the estimation of dP-values by an *in vitro* procedure. A rapid and economically viable *in vitro* method would be highly desirable as an alternative to precise yet expensive and time-consuming *in vivo* measurements. Additionally, experiments with live animals are always in the focus of animal welfare issues. The *in vitro* method applied here is based on a three-step enzymatic digestion that mimics the animal's

digestive tract. For this purpose a large sample size of a variety of feedstuffs was measured both *in vivo* and *in vitro*. The results are presented in Chapters 3 and 4 of this thesis. The exact determination of the ATTD of P is required because there is a difference between total P (tP) in plant feedstuffs and the available P, which is determined as dP for pigs. The hydrolyzed P (hP) is a measure of P resulting from sample processing in the *in vitro* procedure and represents the minimum amount of dP for pigs. Therefore, the predicted amount of dP lies between the measured hP- and tP-values of the corresponding feedstuff. For determination of hP as part of tP, an enzymatic digestion of the feedstuff was simulated using a three-step *in vitro* system including xylanase, pepsin and pancreatin (the detailed procedure is described in Chapter 4).

The *in vitro* procedure is based on published work by Bollinger et al. (2004, 2005) and was standardised and calibrated with *in vivo* data by Kehraus et al. (submitted). Because Kehraus et al. (submitted) only used a small sample size for validation with *in vivo* data, one aim of the current thesis was the validation and establishment using a larger sample size. Kehraus et al. (submitted) used sodium phytate and monosodium phosphate (MNaP) in addition to a limited number of plant feedstuffs for method validation. Sodium phytate and MNaP were used to define the lower and upper limits of the range of values. In the present study those compounds were replaced with maize grain and condensed distillers solubles (CDS) for the assumed lower and upper limits, respectively. Values of hP for these feedstuffs were close to the limits used by Kehraus et al. (submitted). Furthermore, in contrast to sodium phytate and MNaP, maize grain and CDS were also embedded in the *in vivo* trials. Method validation was done by comparing *in vitro*-determined hP-values of all feedstuffs to *in vivo*-determined dP-values using linear regression analysis. A second adjustment in comparison to the method described by Kehraus et al. (submitted) was the reduction from six analytical (i.e., laboratory) replicates to three replicates. As there was a high repeatability in the *in vitro* procedure the reduction to three replicates leads to higher analytical sample throughput.

It is recommended that research should be carried out to reduce further the colouring of samples after the second centrifugation step at the end of the three-step incubation system. Although the gravitational acceleration (*g*) at the second centrifugation step was raised from 2000*g* to 3000*g*, a problem was still encountered with different colours of the measured solution and with floating particles, both of which can influence the absorbance measurement by the spectrophotometer. The small differences in the colour of the solution between different feedstuffs depend mostly on the intrinsic colour of the particular feedstuff. The

problem was also discussed by Pohl (2015) and Kehraus et al. (submitted). Filtration of the solution can minimize the risk of floating particles but the problem of different colouring and slight clouding remains. Nevertheless, the question arises whether the divergent results for rye, oat husk meal and rapeseed commodities could have been affected by slight clouding of the solution before spectrophotometric measurement. This should be evaluated in further analyses.

One relevant aspect that was outside the objective of this study is the analysis of compound feeds by the *in vitro* method. The present study focused on single feedstuffs, i.e., compound feed ingredients. Because to date no research has been conducted using the *in vitro* method on compound feed, further research is warranted to investigate the interactions among different ingredients in a compound feed; the use of phytase could also provide new information. In the current experiment all analyses were performed without addition of microbial phytase. Because more than 95% of German pig feed is produced with the inclusion of microbial phytase (VFT, 2012), the effect should be investigated in further experiments with the *in vitro* procedure. The potential effects of compound feed and phytase addition should be analyzed separately so that confounding is prevented.

Regression analyses between the data of *in vivo* and *in vitro* experiments were conducted. A significant relationship was found between hP- and dP-values. The resulting equations and figures displaying the relationships are presented in Chapter 4. The different equations without intercepts show a strong linear relationship. The establishment and implementation of an *in vitro* technique is of substantial value for the feed industry. As a first step, the prediction of the ATTD of P of single batches can be used for a targeted supplementation with mineral phosphates. As a future goal, a second step may be used for optimisation of phytase dosage and more cost-efficient enzyme supplementation.

Additional work in recent years on *in vitro* systems to predict dP was carried out by Schlegel et al. (2014) and Zhu et al. (2018). Samples from the batch used by Schlegel et al. (2014) also underwent studies using the *in vitro* system described in Chapter 4, yet Schlegel et al. (2014) denoted the P measured in the supernatant after the *in vitro* procedure as released P (rP) and not as hP as in our study. Although the procedures are nearly the same, the notations hP and rP as given by the authors will be used hereafter to to avoid confusion. The results of the current *in vitro* system were compared with data listed by Schlegel et al. (2014). The comparison is presented in Table 13.

Table 13

Coefficients of *in vivo* apparent total tract digestibility of P (ATTD of P), *in vitro* released P, and *in vitro* hydrolyzed P from plant feed ingredients

	In vivo ATTD of P[1]	*In vitro* released P[1]	*In vitro* hydrolyzed P[2]
Maize grain	0.212	0.159	0.133
Barley grain	0.365	0.326	0.277
Wheat grain	0.484	0.481	0.396
Potato protein concentrate	0.406	0.365	0.210
Rapeseed expeller	0.330	0.228	0.246
Soybean expeller	0.392	0.298	0.273

[1] data from Schlegel et al. (2014)
[2] data from chapter 4

Additionally, the regression analysis of ATTD of P and rP and of ATTD of P and hP are given in Figures 7 and 8, respectively. Both regressions are linear. The coefficient of determination (R^2) was higher for the regression of ATTD of P on rP than on hP (0.909 and 0.762, respectively). Except for rapeseed expeller, the values for hP were lower than for rP. The rP-values reflect dP-values more closely than do hP-values. Obviously, the *in vitro* procedure described by Schlegel et al. (2014) releases more of the maximum digestible P than the procedure described in Chapter 4, although both methods are based on a three-step *in vitro* procedure. The most obvious difference between the two methods is that Schlegel et al. (2014) used a filtration step before spectrophotometric determination of phosphate in the supernatant. The likely benefit of the filtration of the solution was described earlier in this chapter. Although Schlegel et al. (2014) also calibrated their *in vitro* data with *in vivo* data, they had only a very limited sample size (n = 6) for the calibration, so that the R^2 observed by Schlegel et al. (2014) should be inspected in additional experiments with a larger sample size that also reflects more diversity among feedstuffs.

The applicability of the study by Zhu et al. (2018) is limited to the prediction of P-digestibility of animal protein by-products because no no plant feedstuffs were used. Feedstuffs used by Zhu et al. (2018) are currently not allowed for feedstuff production in the European Union. Once again this underpins the impact of the present study as it provides data of commonly used feedstuffs and has a considerable sample size.

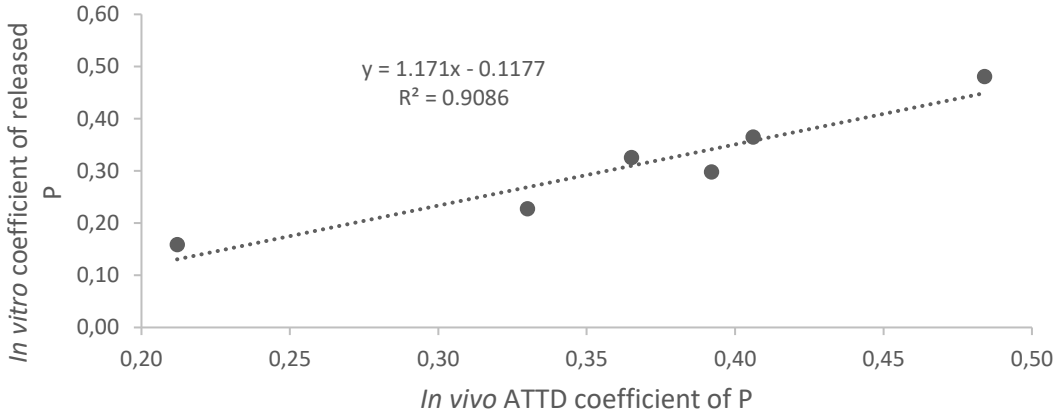


Figure 7.
Relationship between *in vitro* released P and *in vivo* apparent total tract digestibility of P in plant feed ingredients (data from Schlegel et al., (2014)

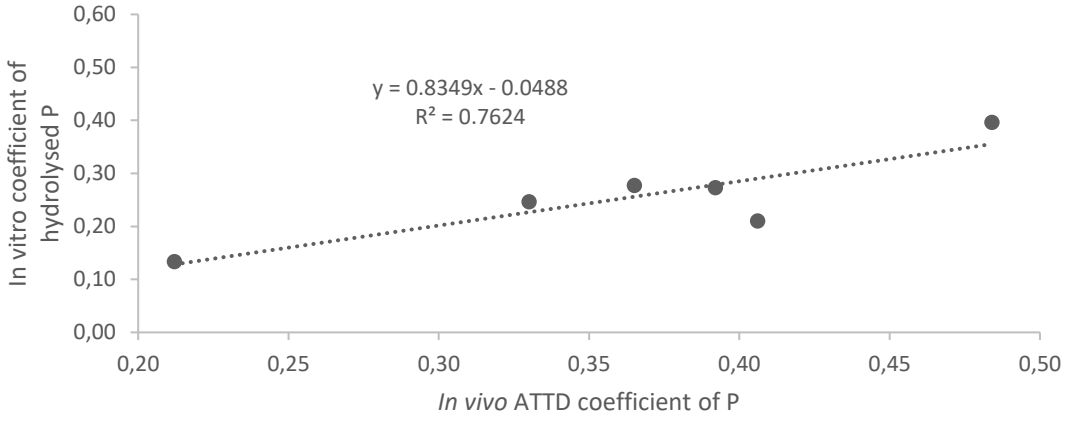


Figure 8.
Relationship between *in vitro* hydrolyzed P and *in vivo* apparent total tract digestibility of P in plant feed ingredients (data from Chapter 4)

Animals

The focus in the *in vivo* trial was set on the variability between and within different cereal grain species. The potential impact of the animal should be kept as low as possible. Because the influence of different animal genotypes was described by Hovenjürgen et al. (2002), in this experiment only pigs of the same crossbred origin were taken for all experiments. In all trials, young pigs (initial BW ± standard deviation (SD): 31.1 ± 6.95 kg) were used. In the experimental design, Latin Squares were constructed to minimize the influence of different BW of the pigs. Pallauf et al. (1992) showed that the ATTD of P can increase with increasing BW in piglets (9–25 kg BW). Steinbeck (2000) observed an effect of BW (18–44 kg BW) on ATTD of P in the basal ration but no effect of BW when ATTD of P was analyzed for wheat.

In the current study there was no influence of BW (31 – XY kg BW) on ATTD of P, both in basal and experimental rations. This was in agreement with Kemme et al. (1997) and Rodehutscord et al. (1999). A reason for the divergent observations in the literature can be an oversupply of P to the heavier pigs. Thus, to avoid interference of P supply level with ATTD of P, GfE (1994) recommended a deficiency in P supply. The required concentration of dP/kg DM of feed decreases with increasing BW and an oversupply of P results in regulatory faecal excretion, e.g., through changed absorption rates (Gutierrez et al., 2015). Additionally, the phytase activity of intrinsic plant phytase is not given in most studies and, therefore, the differences between studies and their potential influence on ATTD of P cannot be quantified.

Conclusions and outlook

The present study clearly demonstrates the relevance of *in vivo* trials to validate *in vitro* methods for quantification of dP and ME. For accurate compound feed and ration formulation and optimisation it is essential to know the particular feed value of all ingredients. Only based on exact ration formulation in terms of ME, protein and dP can an economically viable pig production which, at the same time significantly reduces N and P emissions, be achieved. This study provides new data from *in vivo* measurements of the ATTD of P and ME. Those results are the first published since the 1990s and have already been incorporated in updated tabulated feeding values for pigs (DLG, 2014). The large sample size of this project covered a wide range of contemporary feedstuffs in pig diets. Furthermore, collaborative studies with identical plant material to that used for e.g., *in vivo* studies on P-digestibility and amino acid digestibility in pigs, broilers and laying hens, provided new revealing data.

Because the focus of diet formulation, especially in the compound feed industry, is on the digestibility of nutrients, a rapid *in vitro* method is of great importance, not only for the feed industry. This is the first study that links strictly standardised *in vitro* and *in vivo* data from a large sample size for validation. The estimation of dP content from linear regression equations enable a precise prediction for a wide range of feedstuffs. Especially for feedstuffs where currently no data for ATTD of P or dP-values are available, the method can be an essential tool to gain an initial indication of values. The *in vitro* procedure can be used as a cheap and rapid system for batch-related analysis instead of expensive and time-consuming *in vivo* measurements that cannot be made for specific batches in a timely manner. The data from the present study are very helpful for further research on *in vitro* methods for the prediction of the ATTD of P because of the large sample size and database of *in vitro* as well as *in vivo* data.

Further investigation is needed to determine the potential of the *in vitro* system to predict dP values in compound feeds.

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TAGUNGSBEITRÄGE

68. Jahrestagung der Gesellschaft für Ernährungsphysiologie, 18.-20.03.2014 Göttingen, Deutschland

Schemmer, R., Spillner, C., Boge, H.M., Südekum, K.-H.:

Metabolizable energy concentrations for growing pigs of contemporary cereal grain varieties (Vortrag)

Joint Annual Meeting American Dairy Science Association and American Society of Animal Science, 08.-12.07.2013, Indianapolis, Indiana, USA

Schemmer, R., Drüing, B., Stalljohann, G., Südekum, K.-H.:

Effect of a controlled fermentation process on the content of digestible phosphorus in diets for growing pigs (Poster)

124. VDLUFA-Kongress, 18.-20.09.2012, Passau, Deutschland

Schemmer, R., Drüing, B., Südekum, K.-H.:

In vivo-Untersuchungen zum Einfluss kontrollierter Fermentationsprozesse auf den Gehalt an verdaulichem Phosphor in Rationen für wachsende Schweine (Vortrag)

123. VDLUFA-Kongress, 13.-16.09.2011, Speyer, Deutschland

Schemmer, R., Südekum, K.-H.:

In vivo- und *in vitro*-Untersuchungen zur Ermittlung der Gehalte an verdaulichem Phosphor in Futtermitteln für Schweine. (Vortrag)

VERÖFFENTLICHUNGEN

Schemmer, R., Kehraus, S. Südekum, K.-H., 2020. Validation of an in vitro method for estimating the content of digestible phosphorus in various plant feedstuffs for pigs (eingereicht)

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