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REVIEW

Phytate and phosphorus utilization by broiler chickens and laying hens fed maize-based diets

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Abstract Maize grain is primarily used as an energy source for poultry and other animals. Maize has relatively high phytate-P content and very low intrinsic phytase activity. Given that feed phosphates are produced from finite rock phosphate resources, a reduction in the use of feed phosphates in maize-based diets by increasing the utilization of plant P sources by animals is necessary to make poultry meat and egg production more sustainable. The utilization of P by poultry is affected by two intrinsic characteristics of maize: the concentration of inositol phosphates and the activity of the intrinsic phytase of the grain in the digestive tract. The objective of this review is to present data on the variation that exists in composition of maize relevant for P use and to address factors that influence P utilization in maize-based diets of poultry. Broiler chickens and laying hens have the potential to degrade phytate in the gastrointestinal tract, but this is depressed by high dietary Ca and P concentrations. Published values of phytate degradation in broilers are overall higher than those in laying hens. Differences also exist between broiler chickens and growing turkeys and Pekin ducks. The exogenous supplementation of microbial phytases and the introduction of transgenic high phytase maize in poultry diets are efficient not only for the improvement of phytate-P digestibility, production performance, egg quality and bone mineralization, but also for the reduction of P excreta to control environmental impact.

Keywords broiler, ducks, high phytase maize, laying hens, low phytate maize, phytase, turkeys

1 Introduction

Maize grain is one of the most important feedstuffs used in the poultry industry throughout the world. Maize is primarily used as an energy source for animals because it has a high concentration of starch that is almost completely digestible[1] and a low concentration of non-starch polysaccharides. Maize also contains minerals such as P and Ca, which contributes to provide the animal with these essential nutrients.

Animals have a P requirement that is determined by skeletal growth and other physiologic processes such as energy metabolism or nucleic acid formation. Diets for non-ruminant animals are often supplemented with mineral feed phosphates because the amount of bioavailable P provided by maize and other plant-based feed ingredients is presumed to be insufficient. However, because feed phosphates are produced from finite rock phosphate resources, a reduction in the use of feed phosphates by increasing the animals' utilization of plant P sources is necessary to make poultry meat and egg production more sustainable.

The utilization of P by poultry is affected by two intrinsic characteristics of maize: The concentration of inositol phosphates (InsP) and the activity of the grain enzyme phytase in the digestive tract. Additionally, P utilization depends on what other feed ingredients and feed additives are used together with maize in the complete diet. The objective of this review is to present data on the variation in the composition of maize relevant for P utilization by poultry and to address factors that influence this P utilization in maize-based diets. Given that conditions are different for growing broiler chickens and laying hens, each category is addressed in different sections. Differences between broiler chickens and growing turkeys and Pekin ducks will also be summarized.

2 Phytate, phosphorus and phytase in maize

2.1 Non-transgenic maize

In non-transgenic genotypes, the total P concentration is

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around 3 g·kg^{-1}. In a set of 27 maize samples that contained common hybrids as well as specialty maize with high oil content, the mean total P concentration was 3.2 g·kg^{-1} dry matter (DM) and about 70% of it was present in the form of *myo*-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate) ($InsP_6$)[2]. In this sample set, inositol pentaphosphates and other lower InsP isomers were only found in trace amounts. The variation of total P concentration among the 27 samples was high and values ranged from 2.6 to 4.0 g·kg^{-1} DM. The variation in total P concentration was mainly caused by variation in $InsP_6$-P (Fig. 1). The slope of the regression line in this figure indicates that with each 1 g increment in $InsP_6$-P only 0.17 g of nonphytate P was deposited in the grain.

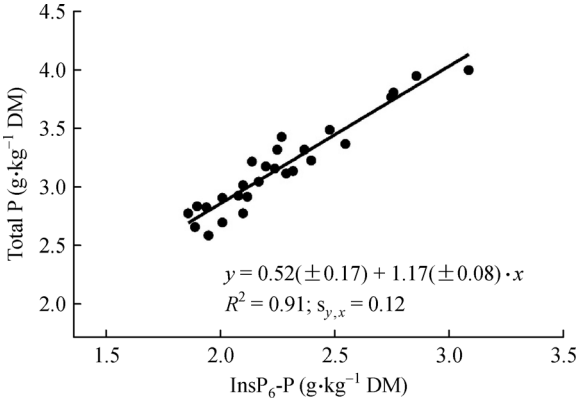

Fig. 1 Relationship between concentrations of total P and $InsP_6$-P in 27 maize samples that comprised common hybrids as well as specialty maize with high oil content. Data from Rodehutscord et al.[2].

In the ripening process, cereals accumulate $InsP_6$ in globoids which are located in protein storage vacuoles. In contrast to other cereals that have the globoids mainly associated with the aleurone layer, maize has most of its $InsP_6$ located in the germ[3,4]. Consistently with this collocation, there is a loose positive relationship between the concentration of $InsP_6$-P and crude fat as well as crude protein (Fig. 2)[2], the latter two being interlinked. Given the sample pool of this study contained specialty maize bred for high oil content, maximum concentrations of 123 g·kg^{-1} DM crude fat and 112 g·kg^{-1} DM crude protein were reached. The Ca concentration overall was low (0.04 g·kg^{-1} DM) and not related to $InsP_6$-P or total P.

Non-transgenic maize grain is not commonly considered to contain intrinsic phytase activity. Phytase activity often is below the limit of detection, but assay and processing (e.g., heat) conditions affect the determined phytase activity. In a set of 27 maize hybrids, phytase activity ranged between 100 and 190 units·kg^{-1} DM when the direct incubation method[5] was used[2].

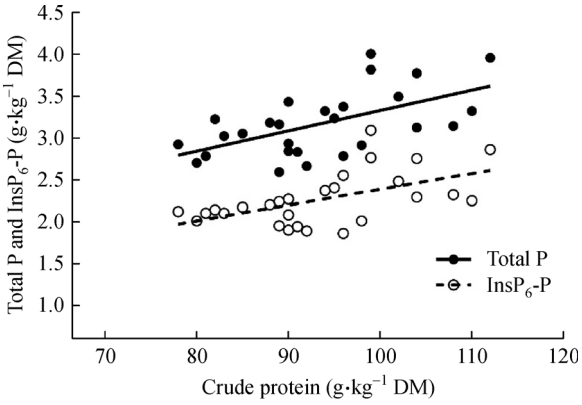

Fig. 2 Relationship between concentrations of crude protein, total P and $InsP_6$-P in the 27 maize hybrids of the GrainUp project. Data from Rodehutscord et al.[2].

2.2 Low phytate maize

Approaches to increase P utilization by animals include maize breeding for low phytate content[6]. In the study of Huff et al.[6], the phytate-P concentration was 1.0 g·kg^{-1} in the low phytate maize while it was 2.0 g·kg^{-1} in normal maize. However, total P concentrations were higher in the low phytate maize (2.7 g·kg^{-1} vs 2.3 g·kg^{-1}). Low phytate maize used in other studies also contained about 1.0 g·kg^{-1} less phytate P than normal maize, often associated with higher total P concentrations[7–10].

In a broiler chicken assay using tibia ash, relative P bioavailability ranged from 21% to 40% in normal maize and from 59% to 95% in low phytate maize[7]. Other studies using similar or other approaches in broiler chickens also showed that P utilization was higher when low phytate maize was used in comparison with normal maize hybrids[9,11,12]. Results from a laying hen study also indicated that the P utilization from low phytate maize is higher than from normal maize[8]. Comparative studies were also conducted using byproducts of maize processing. In maize gluten feed and based on bone ash responses in broilers, the relative bioavailability of P was 46% when the source was a common hybrid maize but 90% when the source was a high oil, low phytate maize[13].

2.3 High phytase maize

An alternative way to increase P utilization of animals is to develop maize cultivars with high phytase activity through transgenic technology. The overexpression of the *Aspergillus niger* gene, *phyA2*, in maize seeds was achieved by using a construct driven by the maize embryo-specific globulin-1 promoter[14]. In that study, phytase activity in transgenic inbred-line maize seeds reached about

12200 units·kg^{-1}, about a 50-fold increase compared to non-transgenic maize seeds. In another study, the phytase activity of a high phytase hybrid maize seed was up to 8047 phytase units (FTU)·kg^{-1} DM, about a 217-fold increase compared to the near-isogenic material[15]. The total P concentration was the same (3.3 g·kg^{-1}) in the high phytase hybrid maize and the near-isogenic material. However, the P utilization of roosters fed high phytase hybrid maize was greater (56% vs 38%) and the available P content was 46% higher (0.19% vs 0.13% on DM basis). No difference was observed in the metabolizable energy and amino acid availability values between the material. There was no difference in P utilization between roosters fed high phytase hybrid maize and an exogenous microbial phytase product equivalent in phytase activity of the maize-soybean meal-based diets (maize:soybean meal = 2.5:1)[15].

Some experiments were conducted to assess the effects of long-term feeding of hens with high phytase transgenic maize[16,17]. The laying performance and egg quality[16], relative organ weight and serum biochemical traits[17] of the hens fed diets containing high phytase transgenic maize was similar to that of hens fed diets formulated with non-transgenic maize. There was no evidence of translocation of the *phyA2* gene or its protein to the blood and visceral tissues[16], muscle tissues and reproductive organs[17], or eggs[16] of laying hens. Another experiment was conducted to investigate the effect of high phytase transgenic maize on intestinal microbiota, and the fate of transgenic DNA and protein in the digesta and tissues of broilers[18]. No adverse effects were found on the quantity and diversity of gut microorganisms, and transgenic *phyA2* DNA or protein was also confirmed to be rapidly degraded in the intestinal tract and was not transferred to the tissues of broilers.

3 Phosphorus utilization in broiler chickens fed maize-based diets

When feeding broiler chickens, maize is not used as the sole feedstuff but it is mixed with protein feeds and other feeds in various proportions. Common protein feeds, such as soybean meal, rapeseed meal and sunflower meal, are rich in phytate and most of the mixed feeds used in broiler nutrition contain 2.2–2.8 g·kg^{-1} $InsP_6$-P. The animal can metabolize this P only after it has been released from the *myo*-inositol ring and absorbed in the intestine. Dephosphorylation is a critical part of the P utilization process because it needs enzymes such as phytase and other phosphatases. This section gives an overview of $InsP_6$ dephosphorylation in broilers when fed maize-based diets and factors that affect it. Most of the recent studies focused on prececal processes, meaning that measurements were made at the end of the small intestine and refer to the sum of digestive processes occurring to that point.

3.1 Potential of gastrointestinal phytate degradation in broiler chickens

The results from several experiments have revealed that prececal disappearance of $InsP_6$ (meaning that at a minimum one phosphate group was released) in broilers that were provided maize-based diets ranged from 62% to 89%[19]. This appears to be a remarkably high range when it is considered that the diets did not contain detectable intrinsic phytase activity. It contradicts textbook statements that claim phytate P is unavailable to poultry. The origin of enzymes that enabled the $InsP_6$ disappearance to such extent was not clarified. It is likely to have been a combination of endogenous enzymes that originate from the intestinal epithelia and from the microbiota colonizing the digestive tract. Some microorganisms potentially can contribute to dephosphorylation[20–22]. Microbial diversity is much higher in the cecum than in the more anterior sections of the digestive tract[23] and very high $InsP_6$ disappearance was measured in cecal content[24]. However, consistent effects of dietary P, Ca and phytase concentrations on gastrointestinal microbiota composition and their role in $InsP_6$ dephosphorylation have not been established, and research in composition and functionality of microbiota in this field is still in an early stage[25–27].

Dephosphorylating enzymes have repeatedly been found in purified brush border membrane vesicles from the small intestine of broiler chickens and laying hens, but their quantitative relevance for $InsP_6$ degradation is hard to calculate. Phytase activity was highest in preparations from the duodenum and lower in the distal part of the ileum[28]. It appeared to be reduced with higher phosphate concentration in the intestinal lumen[29] or when diets contained an additional Ca supplement[30]. In a recent study, prececal $InsP_6$ disappearance was 42% in gnotobiotic broilers fed maize-based diets[31]. The authors of this study concluded that mucosa-derived phytases and other phosphatases can contribute substantially to $InsP_6$ degradation.

3.2 Effects of P and Ca supplements on phytate degradation in broiler chickens

It is important to note that the potential for phytate degradation in the digestive tract mentioned above only occurs under conditions of low P and Ca supply. When mineral P is added to the diet, which is very common in the poultry industry, endogenous $InsP_6$ degradation in broilers is strongly reduced, an effect even more pronounced when Ca is included in the supplement and especially when Ca is supplemented in excess of requirements. The literature in this field has recently been reviewed elsewhere[19], and the reader is referred to this reference for more detailed information.

When broilers were fed diets containing different maize genotypes, the prececal degradation of $InsP_6$ and the

prececal P digestibility were lower when maize contained more $InsP_6$ (Fig. 3)[32]. Given the location of $InsP_6$ in the germ, crude fat content was also different in that study, making it difficult to unravel the causal relationships between $InsP_6$ content of maize and gastrointestinal $InsP_6$ degradation. Consistent with these differences in prececal $InsP_6$ degradation, P retention efficiency was lower when maize with higher concentrations of $InsP_6$ was used in the diets of broilers[33].

4 Phosphorus utilization in laying hens fed maize-based diets

4.1 Potential of gastrointestinal phytate degradation in laying hens

The age of laying hens is a factor influencing gastrointestinal phytate degradation. A study was conducted to determine the availability of phytate P in 20-week-old and 47-week-old ISA Brown hens fed a maize-soybean meal diet[34]. These authors found that the availability of phytate P was higher in 47-week-old (53%) than in 20-week-old (24%) hens, and that the excreta of the older hens contained less phytate P than that of the younger hens (3.1 $mg \cdot g^{-1}$ DM vs 4.5 $mg \cdot g^{-1}$ DM), with the proportion of phytate P in the total fecal P being 15% and 24%, respectively. Consistent with these differences, the *in vitro* phytase activity in the stomach, intestinal mucosa and cecum was higher in the 47-week-old hens than in the 20-week-old hens. A more recent study also found that the P digestibility from canola meal for Hy-Line Brown laying hens at the age of 32 weeks was higher than that of pullets at 17 weeks of age (32.2% vs 22.9%)[35]. The authors suggested this difference was caused by a longer retention time of digesta in the digestive tract of the hens, the maturation of digestive functions, lower endogenous P loss (344 $mg \cdot kg^{-1}$ vs 493 $mg \cdot kg^{-1}$ DM intake) and the higher metabolic demand due to eggshell formation.

4.2 Effects of Ca supplements on phytate degradation in laying hens

The P availabilities reported from the laying hen studies cited above ranged from 23% to 53%, which was much lower than $InsP_6$ degradation in broilers (62%–89%)[19]. This difference is partly due to the higher Ca content in the feed (33–45 $g \cdot kg^{-1}$ for laying hens vs 0.8–1.0 $g \cdot kg^{-1}$ for broilers) and the higher ratio between Ca and P (5.5–7.5 for laying hens vs 1.3–1.5 for broilers). The poor availability of phytate P in laying hens may not be a consequence of digestive insufficiency, but rather of the simultaneous ingestion of phytate with high amounts of Ca from mineral phosphate sources and limestone. Given the P utilizations has been confirmed to decrease with increasing levels of Ca[36,37], excess supply of Ca should be avoided. Spatial separation of limestone from the remainder of the diet of broilers has been shown to be an effective way to enhance the solubility and digestibility of phytate P and amino acids[38]. However, the effectiveness of limestone spatial separation in laying hens has not been investigated.

4.3 Effects of intrinsic and exogenous phytase on phytate degradation in laying hens

Single feeds such as barley, field bean, maize, rapeseed press cake, rapeseed meal, rye, sunflower, triticale and wheat have been found to have different P utilization in laying hens (wheat 47.4%, barley 34.3%, triticale 34.0%, rye 30.1%, rapeseed meal 27.7%, field bean 23.6%, rapeseed press cake 22.0%, oats 19.5%, maize 19.0% and sunflower 10.0%). The correlation between intrinsic phytase activity and P utilization in the different feeds (except rye and triticale) was statistically significant with $r = 0.88$[39].

The efficacy of exogenous microbial phytase in maize-soybean meal-based diets for laying hens could be evaluated through long-term feeding experiments or short-term digestibility studies. For example, it was

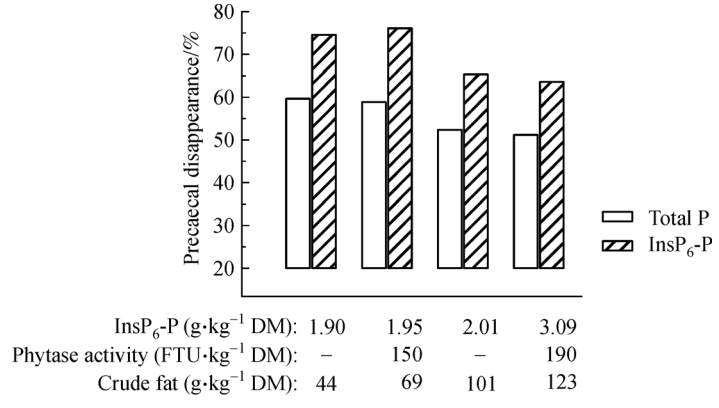

Fig. 3 Disappearance of total P and $InsP_6$-P by the end of the ileum in broiler chickens fed maize-based diets containing maize genotypes with different concentrations of $InsP_6$-P, crude fat and intrinsic phytase activity. Data from Ingelmann et al.[32].

shown that 250 FTU·kg^{-1} diet hydrolyzed an amount of phytate P that was equivalent to 1.3 g P from monocalcium phosphate[40]. The phytate degradation of exogenous microbial phytase was affected by dietary Ca concentrations. The prececal phytate degradation in a diet with 40 g·kg^{-1} Ca was significantly reduced (48%–36%) compared to that in a diet with 30 g·kg^{-1} Ca[40].

In another study, with the addition of 300 FTU·kg^{-1} to the feed, the phytate-P degradation in soybean meal, maize and rice bran increased from 26%, 23% and 36% to 62%, 52% and 51%, respectively[41]. The P utilization of these three feeds increased from 37%, 29% and 36% to 53%, 45% and 43%, respectively. Although less than 500 FTU·kg^{-1} phytase in the diet of laying hens was considered sufficient to degrade phytate, supplementation up to 5000 FTU·kg^{-1} phytase in diet could further increase the phytate-P degradation[42] (Fig. 4).

The phytate degradation occurs mainly from crop to ileum, and the residual phytate-P content decreases caudally along the gastrointestinal tract of hens[42]. The phytase expressed in transgenic high phytase maize was as efficacious as the commercial microbial phytases in P-deficient diets for the improvement of phytate-P degradation (Fig. 4), laying performance, egg quality and bone mineralization[43].

5 Differences between broiler chickens, Pekin ducks and turkeys

Feed compounding for growing poultry species other than broiler chickens usually presumes that P utilization does not vary by species. This assumption is most likely the consequence of limited data available for growing turkeys and ducks. However, an increasing number of studies indicate that differences in gastrointestinal phytate degradation and P utilization exist between species.

When using a low-P basal diet and diets containing graded levels of monobasic calcium phosphate, P utilization of the basal diet was higher in broiler chickens than turkeys and Pekin ducks, but utilization of mineral P was highest in Pekin ducks, followed by turkeys and broiler chickens, respectively[44]. Other experiments confirmed that P utilization by Pekin ducks fed feeds with different phosphates is high[45]. It is remarkable that species differences are in the opposite direction, depending on whether plant P or mineral P is investigated. The crop plays some role in microbial enzyme production in broiler chickens and it may be speculated whether the absence of a fully functioning crop in ducks is the reason for lower phytate-P utilization in ducks compared to broiler chickens. If $InsP_6$ degradation is lower in Pekin ducks than

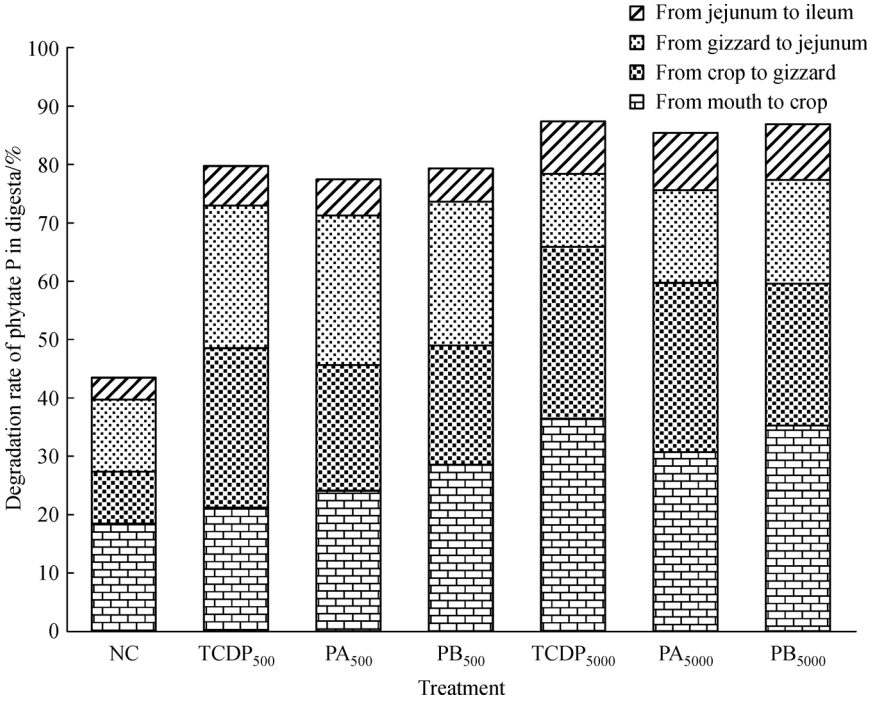

Fig. 4 Degradation rate (%) of phytate P in digesta of laying hens fed with or without added phytase from various sources. NC = negative control diet; $TCDP_{500}$ = transgenic maize-derived phytase at 500 FTU·kg^{-1} of diet; PA_{500} = commercial phytase source A (Natuphos, BASF AG, Ludwigshafen, Germany) at 500 FTU·kg^{-1} of diet; PB_{500} = commercial phytase source B (Phyzyme, Danisco Animal Nutrition, Carol Stream, IL, USA) at 500 FTU·kg^{-1} of diet; $TCDP_{5000}$ = transgenic maize-derived phytase at 5000 FTU·kg^{-1} of diet; PA_{5000} = commercial phytase source A at 5000 FTU·kg^{-1} of diet; PB_{5000} = commercial phytase source B at 5000 FTU·kg^{-1} of diet. One phytase activity unit (FTU) is defined as the quantity of enzyme that releases 1 μmol of inorganic P per minute from 1.5 mmol·L^{-1} sodium phytate at pH 5.5 at 37°C. Reprinted from Gao et al.[42], with permission from Oxford University Press.

broiler chickens, this might mean that the diminishing effects of mineral P supplements on $InsP_6$ degradation (chapter 3.2) are less pronounced in ducks than broiler chickens. Such effects may help to explain the differences in calculated digestibility of mineral P sources between poultry species.

In young turkeys, P digestibility and P retention values of dried distiller's grains with solubles (DDGS) were high in broiler chickens (94% and 92%) but lower in turkeys (76% and 71%)[46]. Differences in $InsP_6$ degradation in the digestive tract and release of phosphate are likely to have contributed to these differences. When providing low-P wheat-soybean meal-based diets to turkeys, $InsP_6$ degradation by the end of the ileum was 29% in the absence of a phytase supplement and 45% when 500 FTU·kg^{-1} of diet was added[47]. This level of $InsP_6$ degradation was remarkably lower than that reported above from similar studies with broiler chickens. When using different genotypes of maize in studies with broiler chickens and turkeys with and without a phytase supplement, prececal $InsP_6$ degradation was much lower in the turkey study than in the broiler study, irrespective of phytase addition[32]. Endogenous mucosal phytase activity was detected in the small intestine of broiler chickens[28,29] and it cannot be ruled out that this activity is different in young turkeys, leading to differences in phosphate release from $InsP_6$. Other variables such as passage rate and pH in different sections of the digestive tract can also contribute to the differences between species. However, any hypotheses derived from the thoughts on causal relationships presented here need to be tested in experiments that involve measurements of $InsP_6$ degradation, mucosal phytase activity, and microbiota composition and functionality in different sections of the gastrointestinal tract of the different species.

6 Conclusions

Common maize hybrids have a relatively high proportion of P bound as phytate P and a very low intrinsic phytase activity. The cultivation of low phytate or transgenic high phytase maize cultivars could be a way to increase P utilization by animals. Broiler chickens and laying hens have the potential of gastrointestinal phytate degradation, but this is depressed by high dietary Ca and P concentrations, and other factors. The published values of phytate degradation in broilers are normally higher than those in laying hens. Differences in P utilization and phytate degradation exist between broilers, turkeys and Pekin ducks. The exogenous supplementation of microbial phytases and the introduction of transgenic high phytase maize in poultry diets are efficient not only for the improvement of phytate-P digestibility, production performance, egg quality and bone mineralization, but also for the reduction of P excreta to control environmental pollution.

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Compliance with ethics guidelines Qiugang Ma, Markus Rodehutscord, Moritz Novotny, Lan Li, and Luqing Yang declare that they have no conflicts of interest or financial conflicts to disclose.

This article is a review and does not contain any studies with human or animal subjects performed by any of the authors.

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