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Phytate degradation and phosphorus digestibility in turkeys and broiler chickens fed maize-based diets

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AA	amino acid
ADG	average daily gain (g/d)
BBM	brush border membrane
Ca	calcium
d	day
e.g.	*exempli gratia* – for example
$InsP_6$	*myo*-inositol hexakisphosphate
P	phosphorus

1 INTRODUCTION

Poultry, such as turkeys and broiler chickens, are usually fed plant seed-based diets. These plant seeds contain a substantial amount of phosphorus (P) which is an essential macro nutrient. Between 60 %-80 % of this P is bound as phytate, a salt of phytic acid (*myo*-inositol hexakisphosphate [$InsP_6$]). Phytate-bound P cannot be absorbed in the digestive tract. First, it needs to be enzymatically hydrolysed by phytases and other phosphatases. The released P can then be absorbed in the small intestine.

Maize-based diets are very common in poultry production. However, maize and maize-based diets are usually very low in intrinsic phytase activity. Chickens do have some endogenous phytase and phosphatase activity in the mucosa of their gut. However, this activity is not sufficient to hydrolyse enough P to cover their demand. Thus, current feeding recommendations are based on non-phytate P. This practice contributes to high levels of P excretion, as the phytate-P fraction represents P fed in excess of the requirement. This has potential implications for the environment, as high levels of P in the manure used as fertiliser can lead to eutrophication. Since the mid-90s of the last century, commercial phytases are available as feed additives. They are used to replace parts of the supplemented mineral P, as their inclusion leads to increased digestibility of phytate-bound P. This inclusion is done for economic reasons, as mineral P supplements are expensive. Additionally, this reduces P emissions as there is less excess P in the diets. In commercial diets, mineral P is not completely replaced by phytase supplementation. This is due to the fact that a precise prediction of phytate-P becoming available by a given phytase dose is not yet possible and thus, safety margins are included in the form of mineral P supplements. The same applies for feeding of turkeys but recommendations for P supply of turkeys are higher than those of broiler chickens. Also, less is known about endogenous capabilities for phytate degradation in turkeys compared to broiler chickens but the available data suggests lower capabilities.

The aim of this thesis was to identify if different strategies are required in turkeys and broiler chickens to promote phytate degradation and plant-based P digestibility in order

to reduce non-renewable mineral P input and consequently improve P efficiency. In order to do so, it was necessary to find out if there are fundamental differences in phytate degradation in the digestive tract of turkeys and broiler chickens. The available literature on phytate degradation and P digestibility of maize-fed chickens was reviewed and known or suspected differences to other poultry species were discussed. Turkeys and broiler chickens of the same age were studied at the same time and place receiving the same experimental diets. To assess the influence of different, age-related maturity of growing birds, half of the animals underwent the trial during their 3rd week of life and the other half during their 6th week of life. The results of the entire work are discussed in this thesis.

2 BACKGROUND

Inclusion of phosphate from non-renewable mined phosphate rock to poultry diets as a supplementary source of phosphorus (P) is a standard procedure in the poultry feed industry. The total P concentration in poultry diets might suggest it would be high enough to meet feeding recommendations without further P supplementation. However, a large proportion of this P is bound as phytate and thus, is unavailable for the animal prior to enzymatic hydrolysation by phytases and other phosphatases. Current text book knowledge states that non-ruminants, such as poultry, do not have sufficient phytase activity in their gut. Recent research showed that substantial phytate degradation can be achieved with endogenous phytase activity in chickens (Sommerfeld et al., 2019). However, this was not sufficient to achieve the same body weight as broiler chickens fed with supplemented mineral P.

Plant intrinsic phytases can also contribute to phytate degradation and thus, can reduce the necessity to supplement P (Rodehutscord et al., 2022). When considering that one of the most widespread poultry feed ingredients worldwide is maize, which has very low intrinsic phytase activity (Rodehutscord et al., 2016), other phytase sources need to be considered. Exogenous microbial phytase is a well-studied feed additive and can improve P digestibility by poultry markedly (Rodehutscord et al., 2022). Within the past decades it became a standard ingredient in conventional feeds and is used to reduce P supplementation. But common inclusion rates of microbial phytase alone are not relied on to provide sufficient levels of digestible P and thus, P is still supplemented. The resulting oversupply of P, in conjunction with an oversupply of calcium (Ca), reduces the efficiency of the supplemented phytase (Tamim et al. 2004; Sommerfeld et al., 2018a). If the strategy would be changed to higher inclusion rates of phytase and omitting P supplementation, the applied phytase would be more efficient and P emissions via excreta would be reduced.

In broiler chickens, a plethora of data on phytate degradation is available. More recent feeding recommendations (FEDNA, 2018) already mention the possibility to reduce P

supplementation depending of inclusion quantity and quality of phytases and give recommendations for total P, available P, and digestible P. But there are no specific recommendations on quantity of available or digestible P based on specific phytase inclusion levels. This is likely due to the complexity of phytate degradation and P digestibility, leading to large variation, even when testing the same feed (Rodehutscord et al., 2017).

In other poultry species, less data is available on P digestibility compared to broiler chickens and data on phytate degradation is very limited. Using broiler chickens as model animal for other growing poultry, such as turkeys, would reduce the need of extensive studies in those species. However, differences in P digestibility and phytate degradation have been found between different poultry species, indicating species-specific differences in phytate degradation and P absorption (Rodehutscord and Dieckmann, 2005; Ingelmann et al., 2019; Olukosi et al., 2020). Phytate degradation was studied in both broiler chickens and turkeys and was found to be much higher in broiler chickens than turkeys (Ingelmann et al., 2019; Olukosi et al., 2020). However, in those experiments species-specific experimental diets were used which were quite different, especially in traits affecting phytate degradation, such as dietary P and Ca concentrations. Thus, the effect that can be solely ascribed to species-specific differences remains unknown.

Gut maturity and other age-related traits such as nutrient requirements and digesta passage time can also influence phytate degradation and P digestibility (Li et al., 2018). Turkeys and broiler chickens have different maturation rates (Zuidhof et al., 2014; Tůmová et al., 2020). When comparing turkeys and broiler chickens of the same age, they don't necessarily have the same maturity. Thus, it is required to compare both species at several ages to assess the influence of maturity and to obtain information on general species differences.

An understanding of species-specific similarities and differences in phytate degradation and P digestibility is required in order to appraise the feasibility of knowledge transfer from one poultry species to another. Or, if a knowledge transfer is not possible, how

different strategies for improved phytate degradation and P digestibility can be elaborated for the individual poultry species.

A comprehensive review on how phytate-P from plant-based feed can contribute substantially to P supply of poultry when phytate is degraded and how this degradation of phytate can additionally benefit digestibility of other nutrients, such as minerals and amino acids (AA), is given by Rodehutscord et al. (2022). Maize is one of the most frequently used poultry feed components worldwide. As maize generally has very low intrinsic phytase activity (Rodehutscord et al., 2016) the challenge of utilising phytate-P in maize-based diets for poultry is pronounced. The first paper included in this doctoral thesis gives an overview about those challenges associated with maize-based poultry feeds and how phytate degradation and precaecal P digestibility can be affected differently in various poultry species (chapter 5.1). A conclusion of this review was that the vast majority of studies on phytate degradation and phosphorus digestibility was conducted with broiler chickens and comparatively little information is available for turkeys, economically the second most important poultry species. Further, there were indications of fundamental differences between broiler chickens and turkeys in respect to intestinal phytate degradation and P digestibility. Thus, 2 consecutive trials were designed to study these differences and to evaluate to which extent knowledge from broiler chickens could be transferred to turkeys. Effects of phytase supplementation and dietary Ca and P level on precaecal $InsP_6$ disappearance, phytate degradation products in the digestive tract, nutrient digestibility, and growth performance were studied. To assess influence of physiological differences between turkeys and broiler chickens, endogenous mucosal phosphatase activity, digesta pH, and length of sections of the small intestine were also investigated.

3 GENERAL AIM AND OVERVIEW OF INCLUDED STUDIES

The intention of this doctoral thesis was to gain a better understanding of fundamental drivers of phytate degradation in the poultry gut, in order to identify possibilities and limitations of applying knowledge about one poultry species to another.

Phytate degradation in the digestive tract of broiler chickens and turkeys fed maize-based diets has been previously studied at the University of Hohenheim and other places. The results clearly showed higher phytate degradation in broiler chickens than turkeys (Ingelmann et al., 2019). These findings were in line with later results presented by another group working on this subject (Olukosi et al., 2020). However, it remained unclear which species-specific traits were responsible for these observations and to what extent dietary differences between broiler chicken and turkeys affected the results. Further, Sommerfeld et al. (2019) hypothesised that in broiler chickens, endogenous mucosal phosphatases, including phytase, can substantially contribute to phytate degradation. This was based on up to 42 % of precaecal $InsP_6$ disappearance observed in gnotobiotic broilers fed diets with very low intrinsic phytase activity.

4 GENERAL DISCUSSION

Phytate degradation in broiler chickens is much higher than in turkeys, if both species are fed according to their respective feeding recommendations (Ingelmann et al., 2019; Olukosi et al. 2020). The aim of this thesis was to investigate the reasons for these observed differences. A second aim was to asses to what extent knowledge of the large pool of information on phytate degradation and phosphorus digestibility in chickens can be transferred to turkeys. Dietary effects such as different Ca and P levels and species-specific traits like digesta pH, jejunal mucosal phosphatase activity, and length of digestive tract sections were investigated and are discussed in this chapter. As growing animals with different maturation rates were compared, turkeys and broiler chickens were studied in 2 age groups to estimate the influence of different gut maturity.

4.1 Methodological considerations

Experimental procedures for the assessment of digestible P were standardised by WPSA (2013). However, results for digestible P can differ markedly, even when the same diet is studied (Rodehutscord et al., 2017). Thus, when comparing precaecal P digestibility and $InsP_6$ disappearance between different studies, the focus was put on similarities and differences of treatment effects, rather than absolute values of P digestibility and $InsP_6$ disappearance. In the presented experiments, conditions for sampling, sample preparation, sample analysis, and conditions during the experimental phase were identical for broiler chickens and turkeys of one age group and almost identical between age groups (diet composition was adjusted for the age but feed components from the same lots were used). Therefore, comparability of results obtained for species of one age group was given, and deemed justifiable when comparing the two age groups.

Age or age-related development of the digestive tract might influence phytate degradation (Li et al., 2018). As turkeys and broiler chickens develop at different maturation rates (Zuidhof et al., 2014; Tůmová et al., 2020), it is likely that the digestive tracts were in different developmental stages at the investigated ages. Thus, it has to be emphasised that the interpretation of the data should be limited to the investigated

timespan between the 3rd and 6th week post hatch. Identified similarities and differences between the species within this timespan should not be extrapolated as being valid for similarities and differences of the compared species in general, until proven by future studies.

In the group of Prof. Dr. Rodehutscord, a frequently applied protocol detail in broiler chicken studies on phytate degradation is to withdraw feed 2 h before the birds are sacrificed with subsequent provision of feed 1 h prior to sacrificing. This is done to standardise fill of the digestive tract. This protocol was applied in the studies described in chapters 5.2 and 5.3. However, turkeys might react differently to this treatment than broiler chickens. Thus, an inter-species standardisation might not have been achieved.

As size of mucosa samples was limited, phosphatase activity of the jejunal brush border membranes (BBM) was measured in duplicates with a single blind value per individuum. As deviations of the 2 measurements from the mean were below 5 %, results were deemed acceptable. Due to the enrichment of BBM in mucosa samples, phosphatase activity was attributed to originate from the tissue of the bird. However, presence of phosphatases originating from BBM-bound microorganisms could not be ruled out entirely.

Application of BBM samples to the phosphatase activity assay was set to contain 160 µg of BBM protein according to the established protocol. At 21 d of age, BBM samples of treatments with high dietary P and Ca concentrations showed higher protein concentrations than BBM samples of low-P and low-Ca level treatments (P = 0.020, data not shown). This could have been caused by a higher density of P transporters in the tissue, as Huber et al. (2015) hypothesised that differences in P amount in the lumen of the small intestine might cause modulation of P transporters. In the hypothesised case of a modulation of P transporters, the application rate of BBM to the assay would have been reduced compared to the other treatments. In turn, this could have led to the lower phosphatase activity observed in those samples. However, at 42 d of age, an effect of P and Ca level on protein concentration in BBM samples was not observed, but the effect of P and Ca level on mucosal phosphatase activity was present nonetheless. Yet,

a correction for different protein concentrations of BBM samples between treatments would be desirable. Establishing such correction factors would require dedicated experiments and should be considered in future research on this matter.

4.2 Effects on phytate degradation

Phytate can be degraded by acidic hydrolysis, thermal decomposition, and enzymatic hydrolysation. Phytate degradation in the digestive tract of animals is always associated with the latter, as acid concentrations and thermal conditions associated with the former (March et al., 1998) cannot occur in healthy animals. In this thesis, the activity of phytases and phosphatases is generally defined as the amount of phosphate that can be hydrolysed per minute. Thus, if a factor reduces the substrate accessibility of phosphatases, for example by precipitating the substrate, this will lead to a decrease of phosphate hydrolysed per minute. Per definition, this will be termed as a reduction in phytase activity, even though the enzyme might not be affected directly.

4.2.1 Diet composition

Previous studies demonstrated that an increase in dietary Ca and P concentration led to a decrease of phytate degradation in chickens (Tamim et al., 2004; Sommerfeld et al., 2018a). Results of the present work show that this is also true for turkeys when dietary Ca and P concentrations were increased. Individual effects of P and Ca supplementations were not studied in the present work, but they appear likely to occur when considering the causative mechanisms that have been discussed for these effects in broiler studies.

As for P, an end-product inhibition by phosphate of the phosphatases involved in phytate degradation has been discussed (Greiner et al., 1993; Angel et al., 2002; Olukosi and Fru-Nji, 2014; Zeller et al., 2015; Sommerfeld et al., 2018a). This means that phosphate, one of the end-products of phytate dephosphorylation, either competes with the substrate for the active site, or non-competitively binds to another site of the enzyme thus reducing the catalytic capability of the enzyme. However, the latter mechanism doesn't appear to be present in supplemented microbial phytases, as they only have one active site (Menezes-Blackburn et al., 2022).

In the case of Ca, it has been shown that it can form potentially insoluble Ca-phytate chelates (Cheryan and Rackis, 1980). Maenz et al. (1999) have demonstrated that this can cause inhibition of microbial phytase activity due to substrate inaccessibility. This does not necessarily change the mode of action of the enzyme. But as phytase activity is defined as the amount of hydrolysed phosphate per time, substrate inaccessibility will reduce phytase activity per definition.

These underlying mechanisms of concentration-dependent inhibitory effects of P and Ca are likely to be the same for turkeys, broiler chickens, and *in vitro* assays. They can explain different phytate degradation rates by turkeys and broiler chickens fed diets with different dietary P and Ca concentrations, which have been reported by Ingelmann et al. (2019) and Olukosi et al. (2020). They could also explain differences in turkeys and broiler chickens fed identical diets with the same dietary P and Ca concentrations, as seen in the trials presented in this doctoral thesis. Here, ileal P and Ca concentrations were lower in turkeys than in broiler chickens, potentially caused by higher P and Ca absorption capabilities of turkeys compared to broiler chickens. Hypothetically, this could have led to lower P and Ca concentrations in the small intestine and consequently to lower inhibitory effects on small intestinal phytate degradation in turkeys compared to broilers. Further, Maenz et al. (1999) showed that the required Ca concentration to effectively inhibit phytase activity was pH dependent, with a critical point between pH 5 and pH 6. In detail, at pH 5 a concentration of 125 mM $CaCl_2$ did not result in any reduction of phytase activity compared to 0 mM $CaCl_2$ and 500 mM $CaCl_2$ led to a reduction of phytase activity of about 15 %. In contrast, at pH 6, a concentration of 25 mM $CaCl_2$ had the same reducing effect as 125 mM $CaCl_2$ at pH 5. At a concentration of 100 mM $CaCl_2$ and pH 6, phytase activity dropped to almost 0. At pH 7.5 even 5 mM $CaCl_2$ sufficed to reduce phytase activity to almost 0. The measured pH differences in turkeys and broiler chickens were small, but within that critical range. Thus, small differences could potentially have a substantial effect.

High dietary P and Ca levels did not affect phytate degradation in the crop in the trials presented in this doctoral thesis. Similarly, Sommerfeld et al. (2018a) reported no effect

of dietary P or Ca level on phytate degradation in the crop. Possibly this is because pH in the crop was low enough to not show a measurable phytase activity inhibition by Ca.

Inhibitory effects of high P and Ca concentrations on phytate degradation have been shown for commercial microbial phytases. Given the nature of the mechanisms described for commercial microbial phytases, it appears very likely that endogenous mucosal phytate-degrading enzymes are affected similarly. For instance, Maenz and Classen (1998) reported an inhibitory effect of Zn on endogenous mucosal phytase activity of chickens, which was thought to be caused by formation of a mineral-phytate complex similar to the ones that were reported to inhibit microbial phytase (Maenz et al., 1999). This is supported by results from Sommerfeld et al. (2019), where gnotobiotic broilers fed diets with low phytase activity showed only 17 % precaecal $InsP_6$ disappearance when dietary P and Ca concentrations were high, whereas precaecal $InsP_6$ disappearance was 42 % when dietary P and Ca concentrations were low. In conventional broilers fed diets with low phytase activity, Tamim et al. (2004) and Sommerfeld et al. (2018a) also showed inhibitory effects on precaecal $InsP_6$ disappearance of high dietary Ca concentrations and high dietary Ca, as well as high dietary P concentrations, respectively.

Other than phytate solubility, pH can also affect phytase activity because phytases have specific pH optima where they hydrolyse the most substrate per minute. Optima of commercial microbial phytases are well studied (Menezes-Blackburn et al., 2015; Menezes-Blackburn et al., 2022) and are typically in the neutral to slightly acidic range. Endogenous mucosal phytate-degrading enzymes of chickens have also been studied in that regard. This topic is discussed in more detail in section 4.2.2.

4.2.2 Drinking water

Drinking water and drinking behaviour could potentially affect Ca intake and pH in the crop. As water intake was not measured during the experiment of this study, Ca intake via drinking water can only be estimated. The water provided to the animals during the experiments contained 50 mg Ca/l (Zweckverband Bodensee-Wasserversorgung, 2020). This is considered a medium Ca concentration. When assuming an average daily intake

of 140 ml per broiler chicken (as suggested by data provided by the breeder), average daily Ca intake via drinking water for broiler chickens would have been 7 mg/d and corresponded to 0.6 % - 1.5 % of average daily Ca intake via feed. If drinking water with a high Ca content of 140 mg/l would have been employed, the calculated average daily Ca intake via drinking water would have been 20 mg/d or 1.6 % - 4.2 % of average daily Ca intake via feed. In relation to the differences of dietary treatments, where Ca intake via feed was about 125 % higher in CaP+ compared to CaP-, Ca intake from drinking water was minimal. Within the studies presented in chapter 5.2 and 5.3 drinking water was no factor as the same water was used in all treatments. However, a general effect of Ca concentration in drinking water on phytate degradation cannot be ruled out and requires further research. For turkeys, the author could not find any reliable data on water intake.

It should be considered that differences in pH and buffering capacity of drinking water could affect pH of digesta and consequently phytate degradation. Also, different drinking behaviour of turkeys and broiler chickens, indicated by different moisture content of the crop (discussed in chapter 5.3), could influence crop pH. This is demonstrated by a small-scale *in vitro* trial that was conducted in addition to the studies presented in this thesis (Table 1), where water from different sources was used to moisturise feed. The author is unaware of any study that investigated the influence of drinking water pH on digesta pH in the digestive tract of poultry. It is unlikely that drinking water characteristics alone were responsible for the large inter-study variation of phytate degradation described in chapter 2. In the present comparison of turkeys and broiler chickens, different water intake behaviour of the 2 species could explain the observed differences in dry matter content of crop digesta. This might also affect crop digesta pH. This hypothesis requires experimental confirmation.

*Table 1. Influence of water source, moisture, and Ca and P level in feed (**CaP**) on feed pH.*

water bidest[1]		
40% moisture*	CaP-	6.55[c]
	CaP+	5.97[e]
60% moisture	CaP-	6.61[bc]
	CaP+	5.95[e]
water medium[2]		
40% moisture	CaP-	6.67[b]
	CaP+	5.88[f]
60% moisture	CaP-	6.65[b]
	CaP+	5.95[e]
water hard[3]		
40% moisture	CaP-	6.74[a]
	CaP+	5.96[e]
60% moisture	CaP-	6.77[a]
	CaP+	6.07[d]
SEM		0.022
P-values		
water type		< 0.001
moisture		0.005
feed		< 0.001
water type × moisture		0.287
water type × feed		< 0.001
Moisture × feed		0.318
water type × moisture × feed		0.023

[1]water is from the same source as "water medium" but bi-distilled; pH 5.28, 22°C.
[2]water is from Lake of Constance. Dissolved substances and hardness according to provider: 50 mg Ca/l; 8.5 mg Mg/l; hardness: 7.17°dH. Measured pH 7.79, 22°C.
[3]water is from Radolfzell core city water supply. Dissolved substances and hardness according to provider: 113 mg Ca/l; 30.1 mg Mg/l; hardness: 22.8°dH. Measured pH 7.45, 22°C.
*Moisture of 40 % was achieved by adding 3 ml water of the respective source to 4.5 g of the respective dried feed. It resembles the moisture content measured in the crop digesta of turkeys.
Moisture of 60 % was achieved by adding 3 ml water of the respective source to 2 g of the respective dried feed. It resembles the moisture content measured in the crop digesta of broiler chickens.

4.2.3 Exogenous and endogenous phytases and other phosphatases

When studying $InsP_6$ disappearance *in vivo*, several possible sources of phytase have to be considered. These sources can be plant intrinsic, endogenous microbial, endogenous mucosal, or exogenous microbial. Phosphatases other than phytases have to be considered additionally.

4.2.3.1 Plant intrinsic phytases

Generally, heat tolerance of plant intrinsic phytases is low, and they lose a considerable amount of their activity after feed processing steps such as extrusion and pelleting (Rodehutscord et al., 2022). They are also susceptible to low pH and proteolytic enzymes in the stomach (Phillippy, 1999). As indicated by Ingelmann et al. (2019) and the results presented in this doctoral thesis, phytate degradation in the crop is less in young turkeys than broiler chickens. This might be due to less favourable conditions for phytase activity, such as higher pH and lower water content, and shorter retention time. The latter requires sophisticated studies, as current knowledge about retention times is limited. With maize as a main feed component there is an additional challenge, as maize generally has very low intrinsic phytase activity (Rodehutscord et al., 2016). As maize is one of the most used ingredients for poultry feed worldwide, other strategies for phytate degradation are required.

4.2.3.2 Exogenous microbial phytase

Microbial phytases can be engineered to withstand high temperatures during pelleting and proteolytic enzymes in the stomach. Inclusion rate is practically only limited by cost. However, due to legislations, they cannot be used in organic farming. Via genetic engineering, they can also be produced by plants (Ma et al., 2019), or even animals (Golovan et al., 2001). In practise, this is not relevant due to legislation and customer preferences on most markets.

In the digestive tract of chicken, the crop is the main site of activity for exogenous microbial phytases (Classen et al., 2016; Zeller et al., 2016). In turkeys, concentrations of $InsP_6$ in the crop was unaffected by supplemented microbial phytase in 3-week-old

birds (chapter 5.2, Table 4) and only slightly lower compared to treatments without supplemented microbial phytase in 6-week-old birds (chapter 5.3, Table 4). Thus, in turkeys the main site of exogenous microbial phytase activity is most likely in a more posterior part of the digestive tract. Detailed information on flow dynamics though the digestive tract would be beneficial for a better understanding of phytase activity and phytate degradation along the digestive tract. This applies for all phosphatases discussed in chapter 4.2.3. Sharma et al. (2023) propose using location aware microdevices for monitoring gastrointestinal dynamics. The method used by the authors showed promising results in pigs. But it is unclear if the microdevice passes though the digestive tract in similar fashion as the targeted dietary components. The current microdevice's size of approximately 1 cm might be to large for application in poultry and might be retained in the gizzard.

4.2.3.3 Endogenous microbial phytase

Compared to the targeted application of exogenous microbial phytase, knowledge about quality and quantity of endogenous microbial phytases and phosphatases is limited. In caeca, the microbial community is very divers (Witzig et al. 2015) and shows high phytase activity and phytate degradation (Dersjant-Li et al., 2015; Zeller et al., 2015). However, contribution to P digestibility via phytate degradation in the hindgut is thought to be very limited, as there is no data that indicates substantial P absorption in the hindgut.

4.2.3.4 Endogenous mucosal phytase/phosphatase

Endogenous mucosal phytase activity has been studied at least since the 1950s. Steenbock et al. (1953) detected phytase activity in the gut of chicken. However, phytase activity in the digestive tract of chicken was considered insufficient and feeding recommendations focused on supply with non-phytate P (NRC, 1994; GfE, 1999). In recent years however, it has been hypothesised that endogenous mucosal phytase activity can cause substantial precaecal $InsP_6$ disappearance in broilers fed low-P diets, based on substantial precaecal $InsP_6$ disappearance of gnotobiotic broiler chickens (Sommerfeld et al., 2019).

It is indicated that the full potential of endogenous mucosal phytase activity can only be assessed when feeding diets without mineral P supplementation, as P supplementation could impair endogenous mucosal phytase activity in two ways. First, endogenous mucosal phytase activity could also be reduced by supplemented P via end-product inhibition, the mechanism described in chapter 4.2.1. Second, Onyango et al. (2006) and Huber et al. (2015) hypothesised that dietary P supplementation could lead to a downregulation of phytase expression by the brush border membrane. This could lead to lower overall phytase activity in the small intestine. This hypothesis could partly be supported by the findings of the present studies (chapters 5.2 and 5.3). In 6-wk-old turkeys and broiler chickens, there was a significant decrease in endogenous mucosal phytase and phosphatase activity when P was supplemented in the feed, compared to un-supplemented feed ($P = 0.034$, Figure 1). Whereas in 3-wk-old turkeys and broiler chickens, endogenous mucosal phytase and phosphatase activity was only numerically lower when P was supplemented in the feed, compared to un-supplemented ($P = 0.104$, Figure 1).

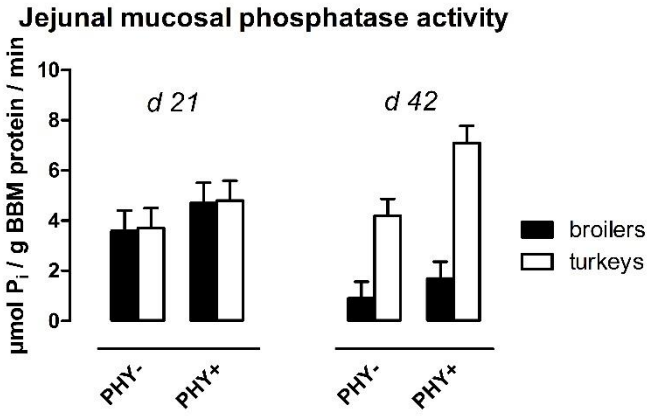

*Figure 1. Effects of species, age, and phytase (***PHY***) supplementation on endogenous mucosal phosphatase activity measured in brush border membrane enriched mucosa (***BBM***) in the jejunum of 21 d old and 42 d old turkeys and broiler chickens. Data from chapters 5.2 and 5.3.*

In the literature, dephosphorylating enzymes in the intestinal mucosa are often separated into two categories. The first category being “phytase” which requires the ability to dephosphorylate $InsP_6$ and pH optima for activity were described being in the

neutral to slightly acidic range (Davies et al., 1970; Maenz and Classen, 1998; Menezes-Blackburn et al., 2022). The second category being “alkaline phosphatases” where enzymes are capable to dephosphorylate other chemical compounds but $InsP_6$. In contrast to phytase, pH optima of those phosphatases were reported to be between pH 9.3 (Motzok, 1950) and pH 11 (Maenz and Classen, 1998) hence, the categorisation as “alkaline” phosphatases. However, Davies and Motzok (1972) reported that the pH optimum of those phosphatases was influenced by the type and concentration of substrate used for dephosphorylation. This could explain the discrepancy in pH optima as Motzok (1950) employed Na-glycerophosphate and Maenz and Classen (1998) used *para*-nitrophenolphosphate for their assays. To the author’s knowledge there is no study on pH optimum of non-phytase-phosphatase activity using degradation products of phytate, e.g. lower inositol phosphates, as substrate. However, the substrate dependency of pH optimum of mucosal phosphatases described by Davies and Motzok (1972) implies that this is worth to be studied. Consequently, the common differentiation between acidic phytases and alkaline phosphatases of the gut mucosa might be an artefact of the substrates employed for determination of non-phytase-phosphatase activity. Generally, endogenous mucosal non-phytase phosphatase activity should be measured using lower inositol phosphates as substrate, as the ability to dephosphorylate those chemical compounds is of interest. Whether or not phytase and phosphatase activity, originating from gut mucosa, can be differentiated without purification of the respective enzymes, is debatable. In chicken small intestine, Maenz and Classen (1998) found highest activities of mucosal phytase in the duodenum and a gradual decrease in activity along the small intestine with lowest activity in the ileum. Kriseldi et al. (2021a) reported lowest concentration of intestinal alkaline phosphatase (determined using an ELISA-kit) in the duodenum of broiler chickens and gradually increasing concentrations along the small intestine with highest concentration in the ileum. This suggests that the small intestine of chicken is adapted for phytate degradation, as the proximal part appears to be optimised for the first steps and the distal part for the further steps of phytate degradation. This would also imply that it is very important to consider the whole small intestine when assessing the possible

contribution of intestinal dephosphorylating enzymes to phytate degradation. Further characterisation of the mucosal enzymes, like K_m, v_{max}, and resulting degradation products, is very desirable.

It has been discussed that endogenous mucosal phytase of chickens might be in part a 5-phytase (Sommerfeld et al., 2019; Rodehutscord et al., 2022), as in gnotobiotic broiler chickens fed diets with no measurable Ins(1,2,3,4,6)P_5 concentration, nor intrinsic phytase activity, Ins(1,2,3,4,6)P_5 was found in the terminal ileum. Ingelmann et al. (2019) also found considerable concentrations of Ins(1,2,3,4,6)P_5 in the terminal ileum of broiler chickens and turkeys fed diets without phytase supplementation and with Ins(1,2,3,4,6)P_5 below the limit of quantification. In the experiments presented in this doctoral thesis, Ins(1,2,3,4,6)P_5 in the terminal ileum was found at the same concentration in 3-week old broiler chickens and turkeys fed diets without phytase supplementation and with Ins(1,2,3,4,6)P_5 in the feed below the limit of quantification. At this age, broiler chickens and turkeys also showed the same extent of mucosal phosphatase activity in the jejunum (chapter 5.2, Table 3). Interestingly, 6-week old turkeys, fed diets without phytase supplementation and with Ins(1,2,3,4,6)P_5 in the feed below the limit of quantification, showed higher extent of mucosal phosphatase activity in the jejunum than broiler chickens of the same age fed the same diets, but had lower Ins(1,2,3,4,6)P_5 concentrations in the terminal ileum (chapter 5.3, Table 3, Table 6). This indicates that turkeys possibly also have endogenous mucosal 5-phytases, but it also indicates that turkeys and broiler chickens might have a different composition of various types of endogenous mucosal phosphatases.

4.3 *Myo*-inositol

The role in the metabolism of *myo*-inositol, the final product of phytate degradation, is complex (Huber, 2016; Gonzalez-Uarquin et al., 2020). Dietary *myo*-inositol has been studied in many different species and was reported to influence lipid metabolism, bone formation, skeletal muscle metabolism, reproductive system, peripheral nerve function, and central nerve system (Gonzalez-Uarquin et al., 2020). Generally, the mode of action is not yet fully understood and frequently data on the effect of dietary *myo*-inositol is

inconsistent (Gonzalez-Uarquin et al., 2020). In a similar fashion, dietary *myo*-inositol in chicken has been attributed to increased weight gain under certain conditions (Żyła et al., 2004; Pirgozliev et al., 2007; Żyła et al., 2013) or decreased feed intake per weight gain (Cowieson et al., 2013; Sommerfeld et al., 2018b). However, other studies could not confirm such effects of dietary *myo*-inositol. At any rate, explanations given for effects of dietary *myo*-inositol on chicken performance were speculative and could not be substantiated yet.

An observation that has been made by all studies reviewed by the author, is that an increase in *myo*-inositol concentration in the digestive tract coincided with an increase in *myo*-inositol concentration in the blood of chickens (Sommerfeld et al., 2018a; Sommerfeld et al., 2018b; Pirgozliev et al., 2019; Whitfield et al., 2022). In the studies included in this work (chapter 5.2 and 5.3), the same coincidence of increased *myo*-inositol concentrations in the blood plasma and *myo*-inositol concentration in the ileum of both turkeys and broiler chickens was observed. The slope of the regression line was much higher in turkeys than in broiler chickens, both at d 21 and d 42 (Figure 2). Hence, increased plasma *myo*-inositol concentrations in turkeys and broiler chickens were most likely due to an increased absorption of phytate-released *myo*-inositol from the intestine. When plotting *myo*-inositol concentration in blood plasma against precaecal $InsP_6$ disappearance, inclination of regression line is, once again, greater for data of turkeys than that of broiler chickens (Figure 3). This could mean that a larger proportion of phytate, where the first degradation step has taken place, was completely dephosphorylated in turkeys than in broiler chickens. Or it could mean that absorption of phytate-released *myo*-inositol was more efficient in turkeys than broiler chickens. A combination of both explanations could also be possible.

Dietary *myo*-inositol supplementation and high inclusion rates of phytase to the diets appear to lead to a higher *myo*-inositol absorption in chickens and turkeys than without these dietary interventions. Also, the magnitude of this increased absorption might be

greater in turkeys than broiler chickens. However, the effects of higher *myo*-inositol absorption are unknown and require further detailed research.

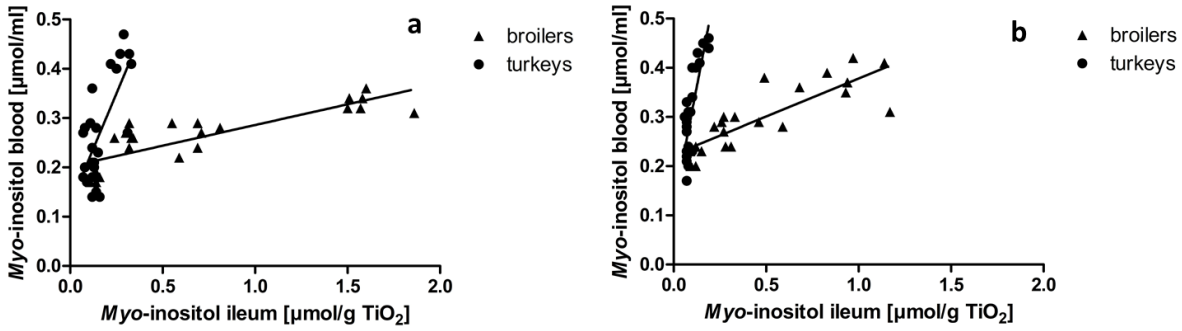

*Figure 2. Myo-inositol concentration in blood and ileum digesta (per g TiO_2) of turkeys and broiler chickens at 21 d of age (***a***) and 42 d of age (***b***). Linear regression turkeys d 21: y = 0.89 x + 0.13, r^2 = 0.53. Linear regression broiler chickens d 21: y = 0.08 x + 0.20, r^2 = 0.69. Linear regression turkeys d 42: y = 1.95 x + 0.12, r^2 = 0.69. Linear regression broiler chickens d 42: y = 0.15 x + 0.22, r^2 = 0.69. From chapters 5.2 and 5.3.*

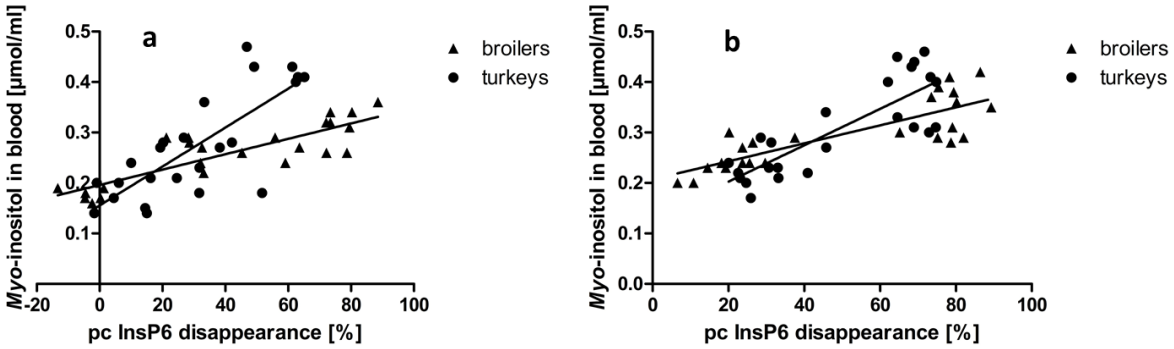

*Figure 3. Myo-inositol concentration in blood and precaecal $InsP_6$ disappearance in turkeys and broiler chickens at 21 d of age (***a***) and 42 d of age (***b***). Linear regression turkeys d 21: y = 0.0039 x + 0.16, r^2 = 0.61. Linear regression broiler chickens d 21: y = 0.0015 x + 0.20, r^2 = 0.72. Linear regression turkeys d 42: y = 0.0036 x + 0.13, r^2 = 0.67. Linear regression broiler chickens d 42: y = 0.0018 x + 0.21, r^2 = 0.68. Data from chapters 5.2 and 5.3*

4.4 Amino acid digestibility

As described in chapter 5.2, the increase in AA digestibility caused by phytase supplementation in broilers was only significant when the P and Ca level of the diet was low (in 9 out of 17 analysed AA). When considering differences which tended to be significant ($P < 0.100$, data not shown), precaecal digestibility of 14 out of 17 AA was increased by phytase supplementation in broilers fed low-P and low-Ca diets. In broilers fed with high dietary P and Ca levels, precaecal digestibility was increased in 3 out of 17 AA by phytase supplementation. Considering numerical differences, precaecal AA digestibility in broilers was always increased by supplemented phytase. This indicates that there might have been an increase in precaecal AA digestibility by phytase supplementation in broilers fed high-P and high-Ca diets, albeit this increase must have been much smaller than in low-P and low-Ca diets.

In turkeys, digestibility was reduced in 15 out of 17 AA when considering differences which tended to be significant ($P < 0.100$, data not shown), when fed low-P and low-Ca diets, and increased in all AA when turkeys were fed high-P and high-Ca diets. This corroborates a general effect of phytase supplementation on digestibility of all AA, depending on species and dietary P and Ca level.

In chapter 5.2, a shorter retention time due to a relatively shorter small intestine was hypothesised as an explanation for the lower precaecal AA digestibility in turkeys fed low-P and low-Ca diets with phytase supplementation compared to turkeys fed low-P and low-Ca diets without phytase supplementation. These considerations were based on observed differences in length of the mall intestine and are therefore only vague estimates. This emphasises how important reliable data on retention time is for a better understanding of effects of phytate degradation on digestibility of other nutrients. The method by Sharma et al. (2023) mentioned in chapter 4.2.3.2 using ingestible microdevices to monitor gastrointestinal dynamics could potentially developed further to elucidate flow dynamics in the digestive tract of poultry.

4.5 Strategies to increase phytate degradation

Generally, more studies on phytase efficiency with low-Ca diets and without mineral P supplementation are required in order to reliably predict minimum required phytase activity for any given diet composition. More data on phytate degradation and P digestibility of older animals, especially turkeys, are required. Here, the potential to improve P efficiency is greatest. As requirement for dietary P concentration is generally lower, omitting supplementation of mineral P is easier to achieve. Due to the much larger feed intake of older birds, this would have a considerable impact on the total P employed for a given fattening period and consequently on the P emissions of said period (Rodehutscord et al., 2003).

The strategy of dietary supplementation of phosphate releasing enzymes could be improved further. As elaborated in chapter 4.2.3.2, the main site of exogenous microbial phytase activity in the digestive tract appears to be different for broiler chickens and turkeys. This could have implications for desirable traits of the employed phytases. For example, a different pH range of high activity might be required in turkeys compared to broiler chickens. This aspect would require dedicated attention.

The characteristic of exogenous microbial 6-phytases to lead to an accumulation of Ins(1,2,5,6)P_4 in the small intestine is well documented in chickens (chapter 5.2) and also documented in turkeys (Olukosi et al., 2020; chapter 5.2, Table 6; chapter 5.3, Table 6). Combined supplementation of multiple phytases to facilitate a higher degree of phytate degradation has been tried (Ennis et al., 2020) but without satisfactory success. A more promising strategy would be to develop and produce phosphatases specialised on dephosphorylating lower inositol phosphates and employ those in combination with exogenous microbial phytases. This would be very laborious and arguably it would be more cost effective to increase phytase supplementation as Kriseldi et al. (2021b) showed that phytase activities of 4500 FTU/kg and more can also solve this issue.

For production systems were exogenous microbial phytases cannot be employed (for example in organic poultry production in the European Union) an approach to improve digestibility of plant-based P would be a breeding program for poultry strains with

extraordinary high endogenous mucosal phytase and phosphatase activity. Research on breeding for P use efficiency has already been conducted (Zhang et al., 2003; de Verdal et al., 2011; Beck et al., 2014; Beck et al., 2016; Ponsuksili et al., 2021) but focusing on P use efficiency, a very general trait influenced by many factors, might be the reason why heritability was not high. Focusing at first on a more specific trait like intestinal phytase and phosphatase activity might be beneficial.

4.6 Conclusions

Generally, phytate degradation in turkeys and broiler chicken appears to be subjected to the same effectors. Thus, fundamental findings, such as the inhibitory effect of high P and Ca supplementation on phytate degradation can be assumed to be valid across species. However, phytate degradation in the digestive tract is a complex system and many factors affecting phytate degradation differ slightly between turkeys and broiler chickens. Similar to *in vitro* trials, findings from other poultry species can contribute to a better trial design and better understanding of the obtained results, but cannot completely replace trials with the target species.

In conventional poultry meat production, supplementation of mineral P should be completely replaced by supplementation of phytase whenever possible. For this, more data on high phytase dose effects in different feed matrices are required to obtain the capability to safely predict phytate-P digestibility in order to formulate highly P-efficient conventional poultry diets.

Future research on endogenous mucosal non-phytase-phosphatase activity should be conducted using lower *myo*-inositol phosphates as substrate, as pH optimum and overall activity of phosphatases are substrate specific. More data on retention time in the different sections of the digestive tract is required. Especially in respect to possible effects on retention time of dietary treatments and inter-species differences.

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5.1 Manuscript 1

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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.

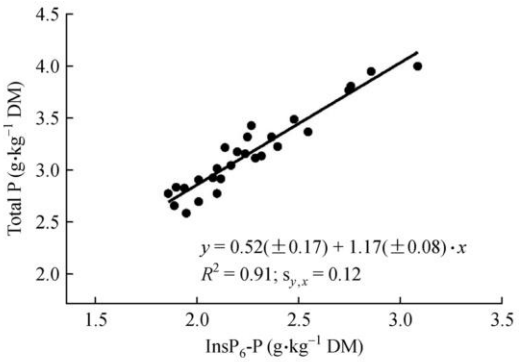

Fig. 1 Relationship between concentrations of total P and $InsP_6$-P in 27 maize samples that comprised common hybrids as well as specialty maize with high oil content. Data from Rodehutscord et al.[2].

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].

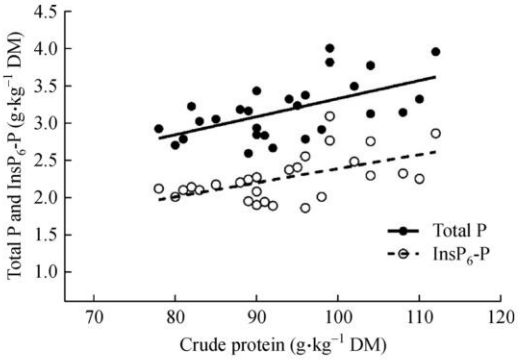

Fig. 2 Relationship between concentrations of crude protein, total P and $InsP_6$-P in the 27 maize hybrids of the GrainUp project. Data from Rodehutscord et al.[2].

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

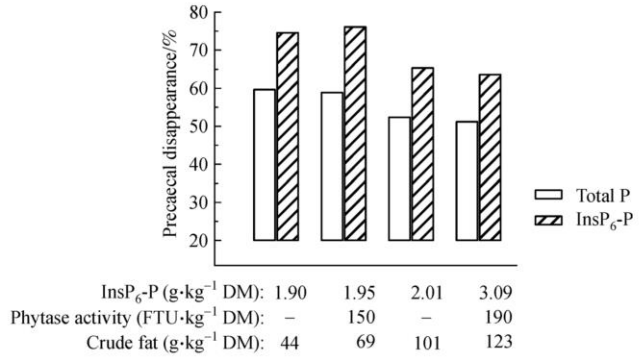

Fig. 3 Disappearance of total P and $InsP_6$-P by the end of the ileum in broiler chickens fed maize-based diets containing maize genotypes with different concentrations of $InsP_6$-P, crude fat and intrinsic phytase activity. Data from Ingelmann et al.[32].

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

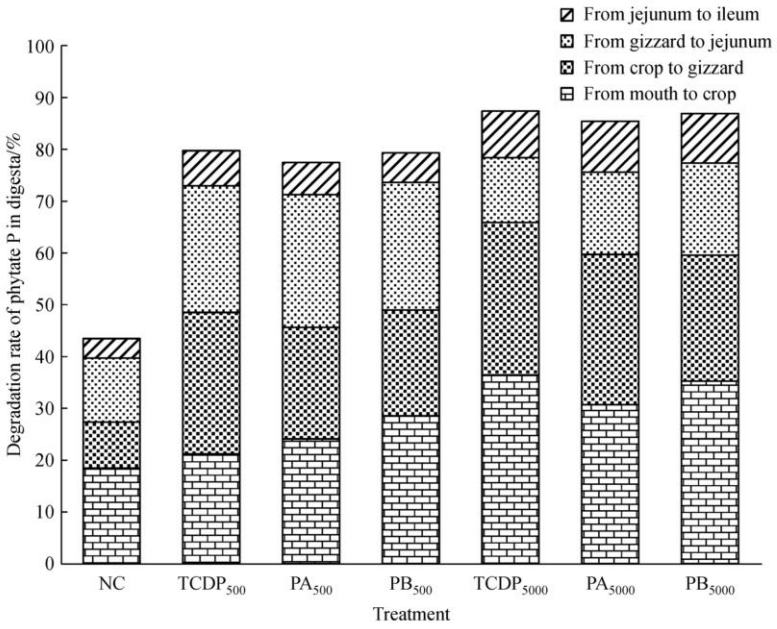

Fig. 4 Degradation rate (%) of phytate P in digesta of laying hens fed with or without added phytase from various sources. NC = negative control diet; $TCDP_{500}$ = transgenic maize-derived phytase at 500 FTU·kg^{-1} of diet; PA_{500} = commercial phytase source A (Natuphos, BASF AG, Ludwigshafen, Germany) 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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5.2 Manuscript 2

Comparison of mucosal phosphatase activity, phytate degradation, and nutrient digestibility in 3-week-old turkeys and broilers at different dietary levels of phosphorus and phytase

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ABSTRACT A comparison between 3-wk-old female turkeys (B.U.T. 6) and broilers (Ross 308) was performed to study the effects of species, dietary P, Ca, and phytase levels on gut mucosal phosphatase activity, *myo*-inositol hexakisphosphate ($InsP_6$) degradation along the digestive tract, digestibility of P, Ca, and amino acids, and concentrations of *myo*-inositol in the digesta and blood. The experimental diets were corn-soybean meal-based and identical for both species. Two dietary P and Ca concentrations (CaP−: 4.1 g P/kg, 5.5 g Ca/kg and CaP+: 9.0 g P/kg, 12.0 g Ca/kg) and 2 levels of phytase supplementation (0 and 1,500 FTU/kg) were used in a 2 × 2 factorial design and fed to the animals for 7 d in their third week of age. Each diet was randomly assigned to 6 broiler and 6 turkey pens, with 10 birds each. After slaughter, blood, digesta from the crop, gizzard, duodenum, lower ileum, and mucosa from the jejunum were collected. When fed CaP− without phytase supplementation, there were no differences between species in gut mucosal phosphatase activity, prececal $InsP_6$ disappearance, and P and Ca digestibility, indicating a similar intrinsic capacity for phytate degradation in both species. When fed CaP+ without phytase supplementation, turkeys showed higher prececal $InsP_6$ disappearance than broilers. Phytase supplementation increased prececal $InsP_6$ disappearance and digestibility of P and Ca in both species. However, the phytase-induced increase in prececal $InsP_6$ disappearance was more pronounced in broilers than in turkeys, possibly due to more adequate conditions for phytase activity in the broiler crop. In broilers, phytase supplementation increased amino acid digestibility overall, whereas, in turkeys, it increased with CaP+ and decreased with CaP−. In addition, the relationship between *myo*-inositol concentration in the ileum and blood differed between species, indicating differences in *myo*-inositol metabolism. It was concluded that 3-week-old turkeys and broilers differ in nutrient digestibility and InsP degradation in some segments of the digestive tract but have similar endogenous $InsP_6$ degradation when fed low P and Ca diets.

Key words: phytate degradation, *myo*-inositol, broiler, turkey, digestibility

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INTRODUCTION

Most P in plant seeds is bound in the form of phytate (any salt of *myo*-inositol hexakisphosphate (**$InsP_6$**)). Phytases and other phosphatases must hydrolyze $InsP_6$ before $InsP_6$-P can be absorbed in the digestive tract of animals. In non-ruminants, endogenous phytase activity is considered to be inadequate, leading to limited $InsP_6$ degradation in the digestive tract. However, the potential of broilers to degrade $InsP_6$ by the end of the ileum is high (64−76% prececal $InsP_6$ disappearance) (Tamin et al., 2004; Zeller et al., 2015a; Ingelmann et al., 2019), with substantial contributions from endogenous gut mucosal phytase and phosphatases (Sommerfeld et al., 2019). However, supplementation with mineral P sources reduced the potential to degrade $InsP_6$ (Shastak et al., 2014; Zeller et al., 2015b; Sommerfeld et al., 2018; Sommerfeld et al., 2019), and the inclusion of mineral P in poultry feed remains an industry standard. This leads to the excretion of unused phytate P, which can cause eutrophication of water bodies (Sharpley, 1999). Mineral P supplements are produced from mined phosphate rock, which is a finite resource. To reduce or avoid the inclusion of unsustainable and expensive mineral P and to reduce P excretion, a common strategy is to supplement poultry feed with

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exogenous phytases. These enzymes release P from $InsP_6$ and may also increase the digestibility of amino acids (**AA**), and bioavailability of cations such as Zn, Fe, Mn, Ca, and Mg chelated with phytate (Rodehutscord et al., 2022).

Several studies have been conducted to better understand the process of $InsP_6$ degradation in broiler chickens. However, such studies are scarce for turkeys. In comparative studies between turkeys and broilers, prececal $InsP_6$ disappearance was much lower in turkeys than broilers (5.5−15.3% in turkeys vs. 63.8−76.3% in broilers) when exogenous phytase was not supplemented (Ingelmann et al., 2019). Correspondingly, upon exogenous phytase supplementation, prececal $InsP_6$ disappearance increased more in turkeys than in broilers, although it remained lower in turkeys (Ingelmann et al., 2019; Olukosi et al., 2020). Other studies have also reported that turkeys were more responsive to phytase supplementation than broilers (Pirgozliev et al., 2007). This finding suggests the existence of fundamental differences between broiler chickens and turkeys. However, species-specific feeds were used in all studies published on this subject, which differed markedly in ingredient composition and nutrient concentrations, such as total P, $InsP_6$-P, Ca, and crude protein. Because of the interactions between $InsP_6$ degradation and dietary Ca and P concentrations, such differences in diet composition may confound possible species effects. In a study that used the same diet without mineral P supplementation in broiler chickens and turkeys, total tract P retention was 58% in broilers and 39% in turkeys at 4 wk of age (Rodehutscord and Dieckmann, 2005). However, $InsP_6$ degradation was not investigated in this study.

The main objective of this study was to compare prececal $InsP_6$ disappearance in broilers and turkeys, including the effects of dietary Ca, P, and phytase concentrations, using the same feed for both species. It was hypothesized that broilers would have a higher capability to degrade $InsP_6$ than turkeys. Another objective was to determine the traits (gut length, pH of digestive tract contents, and jejunal mucosal phosphatase activity) that might explain potential species differences in their abilities to degrade $InsP_6$ and utilize $InsP_6$-P.

MATERIALS AND METHODS

Birds and Housing

The trial was conducted at the Experimental Station "Unterer Lindenhof" of the University of Hohenheim, Germany. This study was approved in accordance with the German Animal Welfare Legislation by Regierungspräsidium Tübingen, Germany (Project No. HOH 59/19 TE).

A total of 530 female Ross 308 broiler hatchlings and 530 female B.U.T. 6 turkey hatchlings were obtained from commercial hatcheries (Brüterei Süd ZN der BWE-Brüterei Weser-Ems GmbH & Co. KG, Regenstauf, Germany and Gebrüder Böcker Putenbrüterei GmbH, Wallhausen, Germany) and placed in floor pens on wood shavings (broilers in 2 pens, turkeys in 4 pens). During the 14 d post-hatch period, broilers were fed a pelleted diet that met or exceeded the supply recommendations of the Gesellschaft für Ernährungsphysiologie (1999) and contained 240 g CP/kg, 8 g P/kg, 9.6 g Ca/kg, and 12.6 MJ ME/kg, with no phytase added. Turkeys were fed a commercial granulated starter diet (Bio-PSG N10 + Ei + C, Kaisermühle Gänheim Otmar Kaiser GmbH, Arnstein-Gänheim, Germany) containing 305 g CP/kg, 10 g P/kg, 14 g Ca/kg, and 11.4 MJ ME/kg until d 8. From d 8 to 14, turkeys were fed a pelleted starter diet formulated to meet or exceed the feeding recommendations of the Gesellschaft für Ernährungsphysiologie (2004), containing 266 g CP/kg, 9 g P/kg, 14.5 g Ca/kg, and 12.3 MJ ME/kg without added phytase. On d 15, 240 birds of both species were allocated in groups of 10 to 48 perforated floor pens (1.15 m × 2.3 m for broilers and 3 m × 4 m for turkeys). The other animals remained in the pens on wood shavings for an additional 21 d, after which they underwent a similar trial reported in a companion paper (Novotny et al., 2023). Each pen was equipped with 2 feeding troughs and 1 drinker. All animals were weighed on a pen basis, and similar body weights per pen for all treatments were maintained within each species. Each pen was allocated in a randomized complete block design to 1 of 4 experimental diets, with 6 pens per diet and species, and feed and water were provided for ad libitum consumption until slaughter on d 21. The light program was 24L:0D for the first 3 d and 18L:6D from d 4 until the end of the experiment. The temperature was 34°C for the first 3 d and then gradually lowered to 26°C on d 21. The animals were inspected twice daily for abnormal behavior and overall health. No mortality was observed during the experimental period.

Experimental Diets and Treatments

The experiment was designed as a 2 × 2 × 2 factorial arrangement of treatments (2 species, 2 Ca and P levels (**CaP**), and 2 levels of phytase (**PHY**) addition). In the experimental phase, both species were fed the same experimental diets (Table 1) and were concurrently housed in the same barn in order to not confound the species comparison by differences in diet composition or environmental conditions. The experimental diets were based on corn and soybean meal and formulated to meet the supply recommendations for turkeys (Gesellschaft für Ernährungsphysiologie, 2004), except for P and Ca. The CaP+ diets were supplemented with monocalcium phosphate (**MCP**) and limestone to achieve P and Ca concentrations of 9.0 g/kg and 12.0 g/kg, respectively. No mineral phosphate was added to the CaP− diets, resulting in calculated P and Ca concentrations of 4.1 g/kg and 5.5 g/kg, respectively. Sand was used at the expense of MCP and limestone. In the PHY+ diets, a modified E. coli-derived 6-phytase (Quantum Blue, AB Vista, Marlborough, United Kingdom) was added at 1,500 FTU/kg. PHY− diets did not contain added

Table 1. Ingredient composition and calculated nutrient concentrations of the experimental diets.

Ingredient, g/kg	CaP−PHY−	CaP−PHY+	CaP+PHY−	CaP+PHY+
Corn	436.8	436.8	436.8	436.8
Soybean meal	416.5	416.5	416.5	416.5
Rapeseed meal	40.1	40.1	40.1	40.1
Soybean oil	43.2	43.2	43.2	43.2
L-lysine-sulfate	7.5	7.5	7.5	7.5
DL-methionine	3.8	3.8	3.8	3.8
L-threonine	0.7	0.7	0.7	0.7
L-valine	0.3	0.3	0.3	0.3
Choline chloride	2.0	2.0	2.0	2.0
NaCl	1.0	1.0	1.0	1.0
$NaHCO_3$	4.6	4.6	4.6	4.6
Vitamin mix[1]	2.0	2.0	2.0	2.0
Mineral mix[2]	0.5	0.5	0.5	0.5
Titanium dioxide	5.0	5.0	5.0	5.0
Limestone	7.3	7.3	12.9	12.9
Monocalcium phosphate	0.0	0.0	23.1	23.1
Sand	28.7	28.7	0.0	0.0
Calculated (g/kg):				
P	4.1	4.1	9.0	9.0
Ca	5.5	5.5	12.0	12.0
Crude protein	253	253	253	253
Phytase (FTU/kg)	0	1,500	0	1,500

[1]Vitamin mix (MIAVIT GmbH, Essen (Oldb.), Germany), provided per kg of complete diet: 10,000 IU vitamin A, 3,000 IU vitamin D3, 30 mg DL-α-Tocopherylacetate, 2.4 mg vitamin K3, 3 mg vitamin B1, 6 mg vitamin B2, 6 mg vitamin B6, 30 μg vitamin B12, 50 mg nicotinic acid, 14 mg pantothenic acid, 1 mg folic acid, 0.1 mg biotin.

[2]Mineral mix (Gelamin, Gesellschaft für Tierernährung mbH, Memmingen, Germany), provided per kg of complete diet: 50 mg calcium from calcium carbonate, 80 mg manganese from manganese-(II)-oxide, 60 mg zinc from zinc-oxide, 25 mg iron from ferrous-(II)-carbonate, 7.5 mg copper from cupric-(II)-sulphate pentahydrate, 0.6 mg iodine from calcium iodate, 0.2 mg selenium from sodium selenite.

phytase. All the diets contained 5 g/kg TiO_2 as an indigestible marker. To achieve uniformity of all diets, ingredients except MCP, limestone, sand, and PHY were mixed in one lot. This basal lot was divided into 2 parts. One part was supplemented with MCP and limestone, and the other was supplemented with sand. Each of these mixes was split into 2 parts, and one was supplemented on top with the enzyme, while the other remained without it. The diets were pelleted without steam through a 3-mm die. Mixing and pelleting were performed at the certified feed mill of the Agricultural Experimental Station of the University of Hohenheim. The formulated concentrations of P, $InsP_6$-P, Ca, and phytase activity were confirmed by the analyses (Table 2). The phytase activity in the PHY+ diets was lower than the calculated value but similar in both the phytase-containing diets (approximately 1,100 FTU/kg).

Procedures and Sampling

Animals and feed were weighed on d 14 and 21 on a pen basis, and ADG, ADFI, and gain per feed (**G:F**) were calculated. On d 21, the feed troughs of the pens were removed 2 h before slaughter according to a fixed time schedule and returned 1 h before slaughtering to standardize the gut fill. The birds were stunned using a gas mixture consisting of 35% N_2, 35% CO_2, and 30% O_2. Two stunned birds per pen were randomly selected and weighed. They were killed by decapitation and trunk blood was collected in tubes containing sodium fluoride. Blood samples were centrifuged for 10 min at 2,500 $\times g$ to obtain plasma. One of the birds was eviscerated, the small intestine and ceca were spread out on a 1 cm grid, and a picture was taken to determine the length of the intestine sections later. The jejunum was dissected from the second bird, opened longitudinally, and flushed with phosphate-buffered saline. Mucosa samples were stripped off with microscopic slides, shock-frozen in liquid nitrogen, transported on dry ice to the laboratory, and stored at −80°C until further analysis. The remaining 8 stunned birds from each pen were asphyxiated using CO_2. From all 10 birds in a pen, crop, gizzard, duodenum, and lower ileum, defined as the last two-thirds of the section between Meckel's diverticulum and 2 cm prior to the ileo-ceco-colonic junction, and ceca were excised. Digesta of the crop and gizzard were carefully obtained with a spatula, digesta of the duodenum was gently squeezed out, and the digesta of the ileum and ceca were flushed out using ice-cold double-distilled water. The samples of the respective sections were pooled on a pen basis and frozen at −20°C following the determination of pH values in the contents of the crop, gizzard, and duodenum (InLab Solids, Mettler-Toledo GmbH, Vienna, Austria).

Sample Preparation and Chemical Analyses

Digesta samples were freeze-dried, pulverized (PULVERISETTE 9; Fritsch GmbH, Idar-Oberstein, Germany), and stored in sealed containers at room temperature. Pulverized feed and digesta samples were analyzed for P, Ca, and Ti using inductively coupled plasma-optical emission spectrometry after wet

Table 2. Analyzed composition of the experimental diets.

	Treatments[1]			
Analyzed composition (g/kg[2])	CaP− PHY−	CaP− PHY+	CaP+ PHY−	CaP+ PHY+
$InsP_6$ (mmol/kg as fed)	12.7	12.9	12.4	12.4
$InsP_5$ (mmol/kg as fed)	1.6	1.7	1.6	1.6
Myo-inositol (μmol/g)	1.8	1.8	1.8	1.8
$InsP_6$-P	2.5	2.4	2.4	2.4
P	4.5	4.5	9.7	9.9
Ca	5.7	5.7	12.2	12.3
Crude protein	264	264	257	264
Arg	17.5	17.9	17.7	17.8
His	7.3	7.4	7.4	7.5
Ile	11.6	12.0	11.8	11.8
Leu	22.1	22.5	22.4	22.5
Lys	18.9	19.5	19.2	19.4
Met[3]	7.7	7.9	7.9	7.9
Phe	13.1	13.3	13.3	13.4
Thr	10.8	11.0	11.0	11.1
Val	12.9	13.2	13.0	13.1
Phytase (FTU/kg)	< 50	1130	< 50	1080

[1]Calculated composition: CaP−, 4.1 g P/kg and 5.5 g Ca/kg; CaP+, 9.0 g P/kg and 12.0 g Ca/kg; PHY−, no supplemented phytase; PHY+: 1,500 FTU/kg supplemented phytase.
[2]Unless stated otherwise.
[3]Methionine determined as methionine sulfone.

digestion (Zeller et al., 2015a). Extraction and measurement of $InsP_{3\text{-}6}$ isomers were carried out using the method of Zeller et al. (2015a) with modifications described by Sommerfeld et al. (2018) and using high-performance ion chromatography (ICS-3000 system, Dionex, Idstein, Germany). Using this methodology makes separating enantiomers impossible; therefore, we could not distinguish between the D- and L-forms. Furthermore, discrimination of isomers Ins(1,2,6)P_3, Ins(1,4,5)P_3, and Ins(2,4,5)P_3 was not possible because of coelution; therefore, we used the term $InsP_{3x}$ for these $InsP_3$ isomers of unknown proportions. *Myo*-inositol (**MI**) in the feed, digesta, and plasma samples was analyzed according to Sommerfeld et al. (2018) using a gas chromatograph/mass spectrometer (Agilent 5977A, Waldbronn, Germany) following a two-step derivatization of the samples. Phytase activity in the feed was analyzed by AB Vista Lab Service (Ystrad Mynach, Wales, UK) using a validated product-specific ELISA method. The results were calculated from a calibration curve with a known activity as determined by the Quantum Blue product analysis, and the activity was expressed as FTU/kg feed. Amino acids were analyzed according to Rodehutscord et al. (2004) using an L-8900 amino acid analyzer (VWR, Hitachi Ltd, Tokyo, Japan) following sample oxidation and acid hydrolysis.

Mucosal Phosphatase Activity Measurement

For mucosal phosphatase activity measurement, the brush-border membrane (**BBM**) was enriched in the mucosa samples, according to Huber et al. (2015). In brief, mucosal samples of the jejunum were ground using a mortar and pestle under liquid nitrogen and mixed with 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid/mannitol buffer (HEPES 2 mmol, mannitol 50 mmol, PMSF 25 mmol). Using a glass potter and homogenizer (homogen^{plus}, shuett-biotec GmbH, Göttingen, Germany), samples were homogenized, and enterocytes in the mucosa sample were sheared off at the tight junctions, separating BBM from the basolateral membrane. After this step, the mucosal homogenates were mixed with 1 M $MgCl_2$ to precipitate the basolateral membranes, which were subsequently removed by centrifugation. Precipitates containing enriched BBM after the final high-speed centrifugation were resuspended in HEPES/mannitol buffer with protease inhibitors. Aliquots (50 μL) of BBM homogenates were frozen and stored at −80°C until analysis. Protein concentrations of the BBM preparations were determined in triplicate using the Bradford assay (Bradford Reagent, 5 ×, SERVA, Heidelberg, Germany). The activity of phosphatases, including phytases, associated with BBM was measured as described by Gonzales-Uarquin et al. (2020) with modifications. Briefly, BBM preparation (equivalent to 160 μg protein) was added to a mixture of double-distilled water containing 25 μg of sodium phytate (Sirius Fine Chemicals SiChem GmbH, Bremen, Germany) and buffer (pH 5.5 buffer from a test kit (K-PHYT 05/17 assay; Megazyme International, Ireland). After 15 min of incubation at 40°C, the reaction was stopped by adding trichloric acid. A second aliquot of the BBM preparation from the same animal was incubated in the same way, but the stop reagent trichloric acid was added beforehand, thus creating a blind value. Following incubation, free phosphate ($\mathbf{P_i}$) was determined photometrically (655 nm, 40°C, Infinite 200 PRO M NANO+, Tecan Trading AG, Switzerland) using the method described in the K-PHYT test kit. The released P_i was calculated by subtracting the respective blind values from the original measurement. The activity of BBM-associated phosphatase is the amount of P_i released per gram of BBM protein per minute

incubation time at pH 5.5. If insufficient BBM preparation was available for the determination of a blind value, the mean of all blind values of the respective treatment was used to calculate the released P_i.

Calculations and Statistical Analysis

Prececal digestibility of P, Ca, AA, and $InsP_6$ disappearance was calculated using the marker method and the following equation:

$$y(X) = 100 - 100 \times (X_{digesta} \times TiO_{2\ feed})/(TiO_{2\ digesta} \times X_{feed})$$

where y is the disappearance or digestibility of X in %; X is the concentration of $InsP_6$, P, Ca, or AA in the feed and digesta; and TiO_2 is the concentration of TiO_2 in the feed and digesta.

The data were analyzed with a 3-way ANOVA using the MIXED procedure of the software package SAS (version 9.4; SAS Institute Inc., Cary, NC). Non-normally distributed data were log-transformed. The results are presented as LSmeans and pooled SEM of the untransformed data. The pen was considered the experimental unit. The following model was used.

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \delta_l + \varepsilon_{ijkl}$$

where y_{ijkl} = response variable, μ = overall mean, α_i = effect of species (fixed), β_j = effect of CaP (fixed), γ_k = effect of PHY (fixed), $(\alpha\beta)_{ij}$ = interaction between species and CaP (fixed), $(\alpha\gamma)_{ik}$ = interaction between species and PHY (fixed), $(\beta\gamma)_{jk}$ = interaction between CaP and PHY (fixed), $(\alpha\beta\gamma)_{ijk}$ is the 3-way interaction between species, CaP, and PHY (fixed), δ_l = effect of block (random), and ε_{ijkl} = residual error. Statistical significance was set at $P < 0.05$.

RESULTS

Analyses of gastrointestinal pH, mucosal enzyme activity, prececal $InsP_6$ disappearance, and P and Ca digestibility are shown in Table 3. The pH of the crop content was higher in turkeys than in broilers. It was lower overall in CaP+ treatments than in CaP− treatments, but this effect was greater in turkeys, resulting in a species × CaP interaction (CaP+ turkey: 6.6, CaP+ broiler: 6.0, turkey: 5.7, CaP− broiler: 5.6; $P < 0.001$). In the gizzard content, species and CaP also significantly affected the pH value, but an interaction did not exist. Gizzard pH was lower in turkeys than in broilers (3.8 vs. 4.0, $P < 0.001$) and higher in CaP+ (3.9) than in CaP− (3.8, $P = 0.025$). The pH value of the duodenum content was higher in broilers (6.3) than in turkeys (6.1, $P < 0.001$) but was not affected by the other factors. The phosphatase activity in the jejunal mucosa was only affected by added phytase and was higher in PHY+ (4.7 μmol P_i/g/min) than in PHY− (3.6 μmol/g/min) ($P = 0.043$). Prececal $InsP_6$ disappearance was affected by a 3-way interaction ($P = 0.013$). $InsP_6$ disappearance in broilers did not differ from turkeys at CaP−PHY−, but it was higher in turkeys than in broilers (6.3% vs. −3.9%) at CaP+PHY−. In the treatments with added phytase, broilers had a higher $InsP_6$ disappearance than turkeys, and the level was lower overall in CaP+ than in CaP−.

Prececal P digestibility was also significantly affected by a 3-way interaction ($P = 0.020$). In all PHY−

Table 3. Effect of Ca and P level (CaP) and phytase supplementation (PHY) on prececal calcium (Ca) and phosphorus (P) digestibility, prececal (pc) $InsP_6$ disappearance, jejunal endogenous mucosal phosphatase activity, and pH in the crop, gizzard, and duodenum of broilers and turkeys at 21 d of age.

Species	Treatment[1]		pH crop	pH gizzard	pH duodenum	Mucosal phosphatase activity (μmol P_i/g BBM protein/min.)	pc $InsP_6$ disappearance (%)	pc P digestibility (%)	pc Ca digestibility (%)
Broiler	CaP−	PHY−	5.8	4.0	6.3	3.5	29.3[e]	41.1[e]	45.5
		PHY+	6.1	3.9	6.3	4.8	77.9[a]	70.8[a]	57.1
	CaP+	PHY−	5.6	4.0	6.3	3.6	−3.9[e]	44.8[e]	28.1
		PHY+	5.6	4.1	6.3	4.6	62.5[b]	49.8[d]	27.8
Turkey	CaP−	PHY−	6.6	3.8	6.2	4.5	25.3[e]	44.7[e]	43.2
		PHY+	6.5	3.6	6.1	5.7	58.0[b]	61.4[b]	52.7
	CaP+	PHY−	5.7	3.8	6.1	2.9	6.3[d]	56.2[c]	39.5
		PHY+	5.8	3.8	6.1	3.9	32.4[c]	57.5[bc]	39.2
SEM			0.07	0.08	0.04	0.80	3.35	1.87	2.51
P-values									
Species			< 0.001	< 0.001	< 0.001	0.836	< 0.001	0.001	0.003
CaP			< 0.001	0.025	0.232	0.104	< 0.001	0.016	< 0.001
PHY			0.318	0.364	0.803	0.043	< 0.001	< 0.001	< 0.001
Species × CaP			< 0.001	0.936	0.341	0.131	0.673	< 0.001	< 0.001
Species × PHY			0.300	0.761	0.561	0.965	< 0.001	< 0.001	0.673
CaP × PHY			0.376	0.106	0.739	0.837	0.237	< 0.001	< 0.001
Species × CaP × PHY			0.059	0.598	0.868	0.989	0.013	0.020	0.682

[1]Calculated composition: CaP−, 4.1 g P/kg and 5.5 g Ca/kg; CaP+, 9.0 g P/kg and 12.0 g Ca/kg; PHY−, no supplemented phytase; PHY+: 1,500 FTU/kg supplemented phytase.

[a-e]Means within a column not sharing a common superscript differ ($P < 0.05$).

Table 4. Effect of Ca and P level (CaP) and phytase supplementation (PHY) on the concentrations of *myo*-inositol and inositol phosphates (μmol/g DM) in the crop of broilers and turkeys at 21 d of age.

Species	Treatment[1]		$InsP_6$	$Ins(1,2,4,5,6)P_5$	$Ins(1,2,3,4,5)P_5$	$Ins(1,2,3,4,6)P_5$	$Ins(1,2,5,6)P_4$	*Myo*-inositol
Broiler	CaP−	PHY−	14.0	1.2	0.7[b]	0.3	< loq[2]	2.0
		PHY+	11.6	0.8	0.6[bcd]	< loq	2.4	2.2
	CaP+	PHY−	14.0	1.2	0.6[cd]	0.2	< loq	2.0
		PHY+	11.5	0.7	0.7[a]	< loq	2.6	2.0
Turkey	CaP−	PHY−	14.4	1.3	0.6[cd]	0.2	< loq	2.2
		PHY+	14.2	1.2	0.7[bc]	< loq	0.3	2.2
	CaP+	PHY−	14.4	1.2	0.6[d]	0.2	< loq	2.2
		PHY+	14.3	1.2	0.7[bc]	0.2	0.3	2.2
SEM			0.34	0.05	0.02	0.02	0.27	0.07
P-values								
Species			< 0.001	< 0.001	0.010	0.352	< 0.001	0.001
CaP			1.000	0.313	0.499	0.071	0.688	0.136
PHY			< 0.001	< 0.001	0.002	1.000		0.136
Species × CaP			0.807	0.313	0.181	0.352	0.829	0.136
Species × PHY			< 0.001	< 0.001	1.000			0.136
CaP × PHY			0.917	0.735	0.002			0.614
Species × CaP × PHY			0.834	0.313	0.010			0.614

[1]Calculated composition: CaP−, 4.1 g P/kg and 5.5 g Ca/kg; CaP+, 9.0 g P/kg and 12.0 g Ca/kg; PHY−, no supplemented phytase; PHY+: 1,500 FTU/kg supplemented phytase.
[2]loq = limit of quantification (0.3 μmol/g DM).
[a-c]Means within a column not sharing a common superscript differ ($P < 0.05$).

treatments, prececal P digestibility was lower than in PHY+ treatments, except in turkeys fed CaP+, where phytase supplementation showed no effect. Prececal Ca digestibility was not significantly different between turkeys and broilers in CaP− treatments but was reduced by CaP+ to a greater extent in broilers than in turkeys (species × CaP: $P < 0.001$). Furthermore, phytase supplementation increased prececal Ca digestibility only when the CaP level was low (CaP × PHY: $P < 0.001$).

The $InsP_6$ and $Ins(1,2,4,5,6)P_5$ concentrations in crop content were significantly affected by the species × PHY interaction ($P < 0.001$, Table 4), indicating that phytase addition caused a reduction in the concentration of these molecules in broilers but barely in turkeys. $Ins(1,2,5,6)P_4$ was only detected in the PHY+ treatments, and its concentration was higher in broilers than in turkeys ($P < 0.001$). The MI concentration in the crop content was slightly but significantly higher in turkeys than in broilers ($P = 0.001$).

The $InsP_6$ concentration in the gizzard content was lower in turkeys than in broilers in PHY− diets (5.7 μmol/g vs. 6.8 μmol/g) and reduced to a similar level (0.8 μmol/g and 0.6 μmol/g) in PHY+ diets (species × PHY: $P < 0.001$) (Table 5). The $InsP_6$ concentration was also affected by CaP x PHY interaction ($P = 0.003$), and it was lower in CaP+PHY− (6.0 μmol/g) than in CaP−PHY− (6.6 μmol/g). In the treatments with supplemented phytase, the $InsP_6$ concentration was higher in CaP+ (0.8 μmol/g) than CaP

Table 5. Effect of Ca and P level (CaP) and phytase supplementation (PHY) on the concentrations of *myo*-inositol and inositol phosphates (μmol/g DM) in the gizzard of broilers and turkeys at 21 d of age.

Species	Treatment[1]		$InsP_6$	$Ins(1,2,4,5,6)P_5$	$Ins(1,2,3,4,5)P_5$	$Ins(1,2,5,6)P_4$	$InsP_{3x}$[2]	*Myo*-inositol
Broiler	CaP−	PHY−	7.0	0.4	0.3	n.d.[3]	n.d.	1.7
		PHY+	0.5	n.d.	n.d.	2.3	< loq[4]	2.7
	CaP+	PHY−	6.6	0.5	0.2	n.d.	n.d.	1.1
		PHY+	0.7	n.d.	0.1	3.9	< loq	1.2
Turkey	CaP−	PHY−	6.1	0.4	0.2	n.d.	n.d.	0.6
		PHY+	0.6	n.d.	n.d.	3.3	< loq	1.1
	CaP+	PHY−	5.3	0.4	0.2	n.d.	n.d.	0.6
		PHY+	1.0	n.d.	0.3	3.6	n.d.	0.6
SEM			0.21	0.03	0.03	0.24		0.07
P-values								
Species			0.004	0.018	1.000	0.172		< 0.001
CaP			0.374	0.771	0.367	0.001		< 0.001
PHY			< 0.001		0.546			< 0.001
Species × CaP			0.751	0.771	0.546	0.012		< 0.001
Species × PHY			< 0.001		0.001			0.006
CaP × PHY			0.003					< 0.001
Species × CaP × PHY			0.344					0.060

[1]Calculated composition: CaP−, 4.1 g P/kg and 5.5 g Ca/kg; CaP+, 9.0 g P/kg and 12.0 g Ca/kg; PHY−, no supplemented phytase; PHY+: 1,500 FTU/kg supplemented phytase.
[2]$Ins(1,2,6)P_3$, $Ins(1,4,5)P_3$, and $Ins(2,4,5)P_3$ could not be differentiated due to co-elution and are thus referred to as $InsP_{3x}$.
[3]n.d. = not detectable (< 0.1 μmol/g DM).
[4]loq = limit of quantification (0.3 μmol/g DM).

− (0.5 μmol/g). Ins(1,2,5,6)P_4 was detected in the gizzard content only in the PHY+ treatment. The MI concentration in the gizzard content was affected by all two-way interactions. It was increased by the addition of phytase to a greater extent in broilers than in turkeys ($P = 0.006$ for the interaction) in CaP− but not CaP+ ($P < 0.001$ for the interaction).

In the ileum, Ins(1,2,4,5,6)P_5 concentrations were lowest in CaP− and unaffected by phytase or species, whereas in CaP+, concentrations where reduced by phytase in broilers but not in turkeys ($P < 0.001$, Table 6). Ins(1,2,3,4,5)P_5 concentrations in the ileum were significantly affected by a CaP × PHY interaction ($P < 0.001$, Table 6) and were highest in CaP+PHY+ treatments, followed by CaP+PHY− and CaP−PHY+. CaP−PHY− was not different from CaP−PHY+ but lower than that of CaP+PHY−. The concentration of Ins(1,2,5,6)P_4 in the ileum was the highest in broilers fed CaP+PHY+, and the PHY effect was lower in turkeys than in broilers ($P < 0.001$). InsP_{3x} in the ileum content was only detected in CaP+PHY+ treatments, and the concentration was higher in broilers than in turkeys ($P < 0.001$). The MI concentration in the ileum content was affected by a three-way interaction ($P < 0.001$). Broilers in the PHY+ treatments had higher MI concentrations than those in the PHY− treatments, with the highest concentration in CaP−. The MI concentration in the ileum of turkeys was lower than that in broilers. The MI concentration in the blood was significantly affected by the 3-way interaction ($P < 0.001$). The concentration was the lowest in CaP+PHY−. It was higher when CaP was low, PHY was added, or both. At CaP−PHY+, the highest plasma MI concentration was measured in both species, with the highest value in turkeys.

Performance traits were significantly affected by 3-way interactions (Table 7). Throughout all treatments, ADG ($P < 0.001$) and ADFI ($P < 0.036$) of broilers were higher than those of the turkeys. ADG and ADFI were lower in the CaP−PHY− treatments in each species than in the other treatments. The G:F ratio was generally higher in broilers than in turkeys. In broilers, G:F was similar in all treatments except for CaP−PHY−, which was lower. The G:F ratio in turkeys was lower in both CaP− treatments than in both CaP+ treatments ($P < 0.004$).

The small intestine in total was longer in broilers than in turkeys ($P < 0.001$, Table 7). It was longer in CaP+ than CaP− treatments ($P = 0.023$), irrespective of species. When expressed per kilogram of BW, the small intestine was longer in turkeys than broilers ($P < 0.001$), not affected by CaP level, but shorter in PHY+ than PHY− treatments ($P = 0.017$).

The prececal digestibility of all analyzed AA was significantly affected by 3-way interactions ($P \leq 0.035$, Tables 8 and 9). In broilers, prececal AA digestibility of most AA was higher in CaP−PHY+ (Ile, Leu, Lys, Met, Phe, Val, Tyr, Ala, and Ser) or CaP+PHY+ (His, Met, Thr, Cys, Ala, and Pro) than in CaP−PHY−. Prececal digestibility of Glx and Gly was not significantly

Table 6. Effect of Ca and P level (CaP) and phytase supplementation (PHY) on the concentrations of TiO_2 (mg/g), *myo*-inositol, and inositol phosphates in the ileum (μmol/mg TiO_2) and *myo*-inositol in blood plasma (μmol/ml) of broilers and turkeys at 21 d of age.

										Myo-inositol	
Species	Treatment[1]	TiO_2	$InsP_6$	Ins(1,2,4,5,6)P_5	Ins(1,2,3,4,5)P_5	Ins(1,2,3,4,6)P_5	Ins(1,2,5,6)P_4	Ins(1,2,3,4)P_4	$InsP_{3x}$[2]	Ileum	Blood
Broiler	CaP− PHY−	15.3^{b}	1.67^{e}	0.03^{de}	0.08	0.03	n.d.[3]	0.02	n.d.	0.67^{b}	0.27^{c}
	PHY+	15.8^{b}	0.52^{g}	0.03^{e}	0.08	n.d.	0.05	0.01	n.d.	1.60^{a}	0.33^{b}
	CaP+ PHY−	16.1^{b}	2.45^{a}	0.18^{a}	0.12	0.05	0.04	0.03	n.d.	0.14^{d}	0.18^{g}
	PHY+	17.1^{a}	0.89^{f}	0.11^{c}	0.29	< loq[4]	0.66	0.07	0.37	0.31^{c}	0.26^{e}
Turkey	CaP− PHY−	14.1^{c}	1.76^{e}	0.05^{d}	0.06	0.03	n.d.	< loq	n.d.	0.11^{d}	0.29^{c}
	PHY+	13.2^{cd}	0.99^{f}	0.04^{de}	0.11	< loq	0.04	0.01	n.d.	0.28^{c}	0.43^{a}
	CaP+ PHY−	12.4^{d}	2.21^{b}	0.15^{b}	0.10	0.05	0.02	0.01	n.d.	0.12^{d}	0.17^{e}
	PHY+	14.0^{c}	1.60^{e}	0.14^{b}	0.29	0.02	0.24	0.04	0.09	0.15^{d}	0.21^{d}
SEM		0.36	0.084	0.007	0.014	0.002	0.024	0.004	0.024	0.028	0.011
P-values											
Species		<0.001	<0.001	0.152	1.000	0.092	<0.001	0.004	<0.001	<0.001	0.055
CaP		0.181	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		<0.001	<0.001
PHY		0.015	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		<0.001	<0.001
Species × CaP		0.003	0.688	0.152	0.475	0.302	<0.001	0.004		<0.001	<0.001
Species × PHY		0.424	<0.001	0.001	0.095		<0.001	0.702		<0.001	0.260
CaP × PHY		0.003	0.232	0.002	<0.001			<0.001		<0.001	0.014
Species × CaP × PHY		0.030	0.012	<0.001	0.422					<0.001	<0.001

[1]Calculated composition: CaP−, 4.1 g P/kg and 5.5 g Ca/kg; CaP+, 9.0 g P/kg and 12.0 g Ca/kg; PHY−, no supplemented phytase; PHY+: 1,500 FTU/kg supplemented phytase.
[2]Ins(1,2,6)P_3, Ins(1,4,5)P_3, and Ins(2,4,5)P_3 could not be differentiated due to co-elution and are thus referred to as InsP_{3x}.
[3]n.d. = not detectable (<0.1 μmol/g DM corresponds to <0.006 μmol/mg TiO_2).
[4]loq = limit of quantification (<0.3 μmol/g DM corresponds to 0.018 μmol/mg TiO_2).
[a–e]Means within a column not sharing a common superscript differ ($P < 0.05$).

Table 7. Effect of Ca and P level (CaP) and phytase supplementation (PHY) on average daily gain (ADG), average daily feed intake (ADFI), gain to feed ratio (G:F) from 14-21 d of age, and length of small intestine and BW of broilers and turkeys (one bird per pen) at 21 d of age.

Species	Treatment[1]		ADG[2] (g/d)	ADFI (g/d)	G:F (g/g)	Small intestine length (cm)	BW of the bird (kg)	Small intestine length (cm/kg BW)
Broiler	CaP−	PHY−	66.0[b]	77.0[b]	0.86[b]	141	852[b]	166
		PHY+	76.8[a]	84.7[a]	0.91[a]	142	1014[a]	140
	CaP+	PHY−	77.1[a]	85.5[a]	0.90[a]	150	971[a]	155
		PHY+	76.6[a]	84.6[a]	0.91[a]	150	1004[a]	150
Turkey	CaP−	PHY−	34.9[d]	48.0[d]	0.73[d]	108	533[d]	204
		PHY+	38.8[c]	52.5[c]	0.74[d]	111	556[d]	200
	CaP+	PHY−	39.9[c]	52.6[c]	0.76[c]	111	578[cd]	193
		PHY+	40.3[c]	52.5[c]	0.77[c]	113	637[c]	178
SEM			0.76	0.75	0.006	3.4	26.7	7.2
P-values								
Species			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CaP			<0.001	<0.001	<0.001	0.023	0.004	0.110
PHY			<0.001	<0.001	<0.001	0.598	<0.001	0.017
Species × CaP			0.014	0.040	0.199	0.247	0.818	0.118
Species × PHY			<0.001	0.200	0.026	0.749	0.148	0.519
CaP × PHY			<0.001	<0.001	0.002	0.872	0.227	0.583
Species × CaP × PHY			<0.001	0.036	0.004	0.910	0.036	0.117

[1]Calculated composition: CaP−, 4.1 g P/kg and 5.5 g Ca/kg; CaP+, 9.0 g P/kg and 12.0 g Ca/kg; PHY−, no supplemented phytase; PHY+: 1,500 FTU/kg supplemented phytase.
[2]Average initial body weights per bird on d 14: 465 g (broilers) 295 g (turkeys).
[a-d]Means within a column not sharing a common superscript differ ($P < 0.05$).

affected in broilers. In turkeys, treatments with added phytase or CaP had lower prececal digestibility values for Arg, Ile, Leu, Met, Asp, Glu, Gly, and Pro compared to the CaP−PHY− treatment. However, the turkey CaP+Phy+ treatment had higher prececal digestibility values for Arg, His, Leu, Lys, Met, Thr, Val, Cys, Tyr, Ala, Asx, Gly, Pro, and Ser compared to the turkey CaP+PHY− treatment, reaching the same level as the turkey CaP−PHY− treatment. The treatment turkey CaP+PHY− showed the lowest prececal digestibility values of all analyzed AA.

DISCUSSION

The hypothesis that broilers exert an overall higher capability of $InsP_6$ degradation than turkeys when fed the same feed must be rejected for 3-wk-old birds as there were no differences in jejunal mucosal phosphatase activity, prececal $InsP_6$ disappearance, $InsP_{3-6}$ concentrations in the digesta along the digestive tract, and prececal P and Ca digestibility between broilers and turkeys fed the CaP−PHY− diet. Broilers showed higher prececal $InsP_6$ disappearance than turkeys only

Table 8. Effect of Ca and P level (CaP) and phytase supplementation (PHY) on prececal essential amino acid digestibility (%) of broilers and turkeys at 21 d of age.

Treatment[1]		Arg	His	Ile	Leu	Lys	Met[2]	Phe	Thr	Val
Broiler										
CaP−	PHY−	85.5[ab]	76.6[bc]	79.6[b]	79.3[bc]	84.8[bc]	90.8[bc]	80.1[bc]	71.6[bc]	77.9[bc]
	PHY+	86.9[a]	79.0[ab]	82.6[a]	82.4[a]	86.7[a]	92.2[a]	83.1[a]	74.2[ab]	80.9[a]
CaP+	PHY−	84.7[bc]	78.0[abc]	79.5[b]	79.9[abc]	84.6[bc]	91.5[ab]	80.3[bc]	72.3[abc]	78.3[bc]
	PHY+	85.9[ab]	79.7[a]	81.3[ab]	81.7[ab]	85.8[ab]	92.5[a]	82.0[ab]	74.6[a]	80.1[ab]
Turkey										
CaP−	PHY−	84.3[bc]	76.3[cd]	79.4[b]	79.0[c]	85.1[abc]	91.3[ab]	79.5[bcd]	71.9[abc]	77.5[cd]
	PHY+	82.1[de]	74.0[de]	76.9[c]	76.2[de]	83.8[cd]	89.9[cd]	77.0[de]	69.8[cd]	75.1[de]
CaP+	PHY−	80.9[e]	72.5[e]	75.9[c]	75.0[e]	82.9[d]	89.1[d]	75.6[e]	67.6[d]	73.8[e]
	PHY+	83.2[cd]	76.2[cd]	78.6[bc]	78.1[cd]	84.8[bc]	91.1[abc]	78.3[cde]	71.7[abc]	77.1[cd]
SEM		0.79	0.97	1.02	0.98	0.65	0.69	0.97	1.05	1.03
P-values										
Species		<0.001	<0.001	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	<0.001
CaP		0.039	0.806	0.230	0.415	0.163	0.944	0.178	0.637	0.454
PHY		0.140	0.028	0.063	0.056	0.031	0.029	0.064	0.017	0.036
Species × CaP		0.765	0.145	0.851	0.467	0.955	0.145	0.498	0.208	0.621
Species × PHY		0.216	0.295	0.086	0.102	0.174	0.213	0.087	0.305	0.137
CaP × PHY		0.028	0.033	0.111	0.088	0.150	0.038	0.146	0.043	0.091
Species × CaP × PHY		0.017	0.009	0.016	0.012	0.029	0.007	0.015	0.026	0.012

[1]Calculated composition: CaP−, 4.1 g P/kg and 5.5 g Ca/kg; CaP+, 9.0 g P/kg and 12.0 g Ca/kg; PHY−, no supplemented phytase; PHY+: 1,500 FTU/kg supplemented phytase.
[2]Methionine determined as methionine sulfone.
[a-e]Means within a column not sharing a common superscript differ ($P < 0.05$).

Table 9. Effect of Ca and P level (CaP) and phytase supplementation (PHY) on prececal non-essential amino acid digestibility (%) of broilers and turkeys at 21 d of age.

Treatment[1]		Cys[2]	Tyr	Ala	Asx[3]	Glx[3]	Gly	Pro	Ser
Broiler									
CaP−	PHY−	64.2^{b}	78.8^{bc}	76.3^{b}	73.8^{abc}	82.5^{abc}	71.4^{a}	77.2^{bc}	74.1^{bc}
	PHY+	68.1^{ab}	81.6^{a}	79.5^{a}	76.0^{a}	84.4^{a}	73.9^{a}	79.1^{ab}	77.0^{a}
CaP+	PHY−	68.4^{ab}	79.1^{bc}	77.0^{ab}	73.1^{bc}	82.0^{abc}	72.1^{a}	77.6^{abc}	74.4^{abc}
	Phy+	70.5^{a}	81.0^{ab}	79.1^{a}	75.2^{ab}	83.6^{ab}	74.0^{a}	79.8^{a}	76.6^{ab}
Turkey									
CaP−	PHY−	54.1^{cd}	79.1^{bc}	77.2^{ab}	74.0^{ab}	81.3^{bc}	71.4^{a}	75.5^{cd}	73.6^{cd}
	Phy+	50.0^{de}	76.8^{cd}	74.4^{bc}	71.4^{cd}	78.1^{d}	68.7^{bc}	72.6^{ef}	71.1^{de}
CaP+	PHY−	46.8^{e}	74.9^{d}	73.1^{c}	70.1^{d}	77.2^{d}	66.7^{c}	70.5^{f}	68.5^{e}
	Phy+	55.2^{c}	77.7^{c}	76.9^{ab}	73.0^{bc}	79.8^{cd}	71.1^{ab}	74.1^{de}	72.7^{cd}
SEM		1.72	0.94	1.07	1.01	0.99	1.12	0.99	1.05
P-values									
Species		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CaP		0.326	0.164	0.631	0.155	0.144	0.550	0.360	0.194
PHY		0.027	0.042	0.030	0.074	0.248	0.037	0.073	0.017
Species × CaP		0.062	0.211	0.506	0.718	0.682	0.262	0.082	0.214
Species × PHY		0.717	0.096	0.144	0.128	0.110	0.328	0.197	0.210
CaP × PHY		0.023	0.089	0.063	0.043	0.038	0.026	0.014	0.035
Species × CaP × PHY		0.003	0.019	0.011	0.035	0.023	0.008	0.021	0.010

[1]Calculated composition: CaP−, 4.1 g P/kg and 5.5 g Ca/kg; CaP+, 9.0 g P/kg and 12.0 g Ca/kg; PHY−, no supplemented phytase; PHY+: 1,500 FTU/kg supplemented phytase.

[2]Cysteine determined as cysteine sulfone.

[3]The amide residue in the side group of asparagine and glutamine is lost during acid hydrolysis and aspartic acid and glutamic acid are formed. Hence, aspartic acid together with asparagine (Asx) and glutamic acid together with glutamine (Glx) was analyzed.

[a–f]Means within a column not sharing a common superscript differ ($P < 0.05$).

when phytase was supplemented. The similarities between broilers and turkeys found in the CaP−PHY− treatments indicate that 3-wk-old broilers and turkeys had the same endogenous $InsP_6$ degradation when fed diets with low P and Ca concentrations and no phytase supplementation. These findings are inconsistent with previous comparative studies that reported higher prececal $InsP_6$ disappearance in broilers than in turkeys (Ingelmann et al., 2019; Olukosi et al., 2020). However, in these studies, species-specific diets were compared, which differed markedly in the concentrations of nutrients such as P and Ca. In chickens, adverse effects on phytate degradation have been reported for high dietary concentrations of mineral P (Olukosi et al., 2013; Shastak et al., 2014; Walk et al., 2014; Zeller et al., 2015b; Sommerfeld et al., 2018; Siegert et al., 2019) and Ca (Tamim et al., 2004; Sommerfeld et al., 2018). Possible reasons for these adverse effects are the end-product inhibition of phytase by mineral P (Greiner et al., 1993; Zeller et al., 2015b) and precipitation and subsequent inaccessibility for the enzyme caused by chelating Ca^{2+} ions (Walk et al., 2012; Sommerfeld et al., 2018). There is no indication that these mechanisms do not affect turkeys. In the present study, high CaP levels markedly reduced prececal $InsP_6$ disappearance in broilers and turkeys compared to low CaP levels, although slightly more in broilers. Hence, it is likely that in the previously mentioned comparative studies, differences in P and Ca concentrations of the feed were largely responsible for the observed differences in prececal $InsP_6$ disappearance and not the inherent capabilities of the animals.

In broilers, Rodehutscord et al. (2012) found a slight reduction of prececal P digestibility when dietary P concentrations increased incrementally to 8.1 g/kg using MCP. Further, when dietary P concentrations increased incrementally to 7.7 g/kg DM, Rodehutscord and Dieckmann (2005) reported a reduction in the percentage of retained P in broilers and an increase in the percentage of retained P in turkeys, using the same feed for both species. Thus, the high levels of supplemented P and Ca in the present trial were likely absorbed more by turkeys than broilers, as in PHY− treatments, increased dietary Ca and P concentrations led to higher prececal P digestibility in turkeys but not in broilers. In addition, Ca digestibility in the CaP+ treatments was higher in turkeys than in broilers. This led to higher Ca and P concentrations in the ileum of broilers than in turkeys in the CaP+ treatments, whereas in CaP− treatments Ca and P concentrations in the ileum were the same for both species (data not shown). Thus, the greater effect of CaP+ on prececal $InsP_6$ disappearance in broilers than in turkeys is assumed to be caused by the aforementioned adverse effects of higher P and Ca concentrations in the gut of broilers than in turkeys. This is assumed to have led to the significantly lower prececal $InsP_6$ disappearance in broilers than in turkeys in the CaP+PHY− treatment. A negative value for prececal $InsP_6$ disappearance in broilers was calculated for the CaP+PHY− treatment. Because $InsP_6$ formation in the digestive tract is unlikely to occur, this effect was likely an artefact of the marker method. Therefore, it was assumed that, in this treatment, there was very little or no prececal $InsP_6$ disappearance.

Sommerfeld et al. (2019) showed that up to 42% of prececal $InsP_6$ disappearance can be attributed to endogenous mucosal phosphatase activity in broilers. In the present study, similar jejunal mucosal phosphatase activities between turkeys and broilers in the CaP−PHY− treatment coincided with similar prececal $InsP_6$ disappearance and prececal P digestibility;

however, it remains unclear to what extent the mucosal phytase activity contributed to phytate degradation, because enzymes from resident microbiota or the feed ingredients, not analyzed by the product-specific ELISA method, might have been involved. The higher mucosal phosphatase activity caused by the supplemented phytase could have been triggered by the relatively high concentrations of Ins(1,2,5,6)P_4 found in the gizzard, which Walk et al. (2018) hypothesized is due to a triggering mechanism of lower InsP. Similar to the treatments without phytase supplementation, the contribution of mucosal phosphatase to phytate degradation is unclear.

As expected, supplementation with phytase increased prececal $InsP_6$ degradation in both species. However, the effect of phytase supplementation was much greater in broilers than in turkeys. The $InsP_6$ concentration in the crop was reduced by phytase supplementation only in broilers. When the crop was opened, we observed that feed pellets were still visible in turkeys other than broilers, whose crop content was a moist mash. Also, there was a difference in weight loss of digesta samples from the crop during freeze-drying between broilers and turkeys (broilers: 65.7%, turkeys: 36.8% weight loss, $P < 0.001$, data not shown). Thus, the difference in $InsP_6$ disappearance might have been caused by the higher water content in the crop of broilers compared to turkeys, as supplemented phytase might have lacked a medium to access phytate in turkeys. The lack of an effect of phytase supplementation on $InsP_6$ concentration in the crop of turkeys may explain the lower prececal $InsP_6$ disappearance in turkeys compared to broilers. However, differences in prececal $InsP_6$ disappearance were not as severe as the differences in crop $InsP_6$ concentration might suggest. Possible explanations for this could be the following: Lower pH in the gizzard and duodenum of turkeys compared to broilers could have led to higher $InsP_6$ solubility (Grynspan and Cheryan, 1983) and, consequently, more $InsP_6$ degradation in these sections. In addition, according to Menezes-Blackburn et al. (2015), pH was slightly closer to the optimum of the supplemented phytase in the gizzard and duodenum of turkeys than in broilers. Furthermore, differences in retention time could not be ruled out as a reason for the species effect.

In the crop of broilers, there was a reduction in $InsP_6$ and Ins(1,2,4,5,6)P_5 concentrations when supplemented with phytase. The small variation in Ins(1,2,3,4,5)P_5 concentration in the crop of all treatments indicates that the release of the first two phosphate groups from $InsP_6$ occurred at a similar pace. Elevated Ins(1,2,5,6)P_4 concentrations in the digestive tract in PHY+ treatments indicate that the supplemented phytase was limited in activity in the third degradation step. This is consistent with results from previous studies using 6-phytases in broilers (Walk et al., 2014; Zeller et al., 2015a,b; Krieg et al., 2020; Olukosi et al., 2020; Kristoffersen et al., 2021) and turkeys (Olukosi et al., 2020). However, in the terminal ileum Ins(1,2,5,6)P_4 concentrations were substantially elevated only in CaP+PHY+ treatments, indicating inhibitory effects of high CaP level on mucosal phosphatase activity.

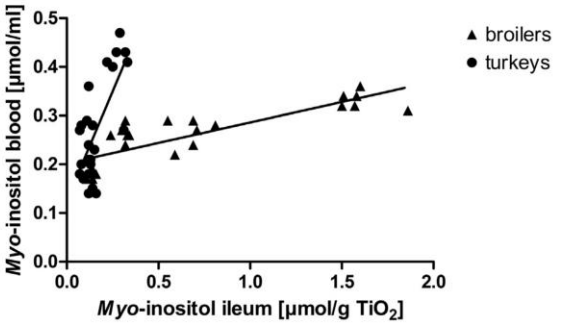

Figure 1. *Myo*-inositol concentrations in ileum digesta (per g TiO_2) and blood of broilers and turkeys fed the experimental diets at 21 d of age. Linear regression broilers: $y = 0.08\ x + 0.20$, $r^2 = 0.69$. Linear regression turkeys: $y = 0.89\ x + 0.13$, $r^2 = 0.53$.

The higher MI concentrations in the ileum of broilers compared to turkeys appear to be inconsistent with similarly high prececal $InsP_6$ disappearance and mucosal phosphatase activity, especially in the CaP−PHY− treatment. However, in the blood, MI concentrations were similar in both species, except for the treatment CaP−Phy+, where turkeys exerted a higher MI concentration than broilers, even though their prececal $InsP_6$ disappearance was lower. In addition, a linear relationship between MI concentrations in the blood and ileum was observed in both species (Figure 1); however, the regression slope was greater in turkeys than in broilers. Thus, it is speculated that the lower concentration of MI in the ileum of turkeys was caused by a higher uptake rate, a different uptake location in the small intestine, a different turnover rate in the enterocytes, or a different transport rate from the blood to organs of turkeys. A concentration-dependent increase in active MI transport in the small intestine of broilers has been previously discussed (Walk et al., 2018), but the authors are unaware of comparable information on turkeys.

P digestibility increased with phytase supplementation following an increase in $InsP_6$ degradation. In addition, Ca digestibility increased with phytase supplementation, possibly due to fewer Ca^{2+} ions forming complexes with phytate and a higher metabolic Ca demand when more P is absorbed and retained in bones.

As reviewed by Rodehutscord et al. (2022), phytase supplementation increased AA digestibility in some, but not all, studies. In the present study, AA digestibility in broilers was significantly increased in 9 out of 17 analyzed AA, but only when the CaP level was low. This indicates that AA digestibility in broilers was affected by prececal $InsP_6$ disappearance and unknown factors linked to dietary P and Ca concentrations. In turkeys, the CaP level and supplemented phytase effects were considerably different, as an increase in prececal AA digestibility due to phytase supplementation was only seen when the CaP level was high (14 out of 17 analyzed AA). In contrast, when the CaP level was low, phytase

supplementation decreased prececal AA digestibility in 8 out of 17 analyzed AA. This might have been related to different digesta passage rate owing to differences in small intestine lengths between PHY+ and PHY− (Table 7). In CaP+ treatments such effect might have been compensated by higher absolute length of the small intestine. In any case, differences indicate that prececal AA digestibility is affected differently by P, Ca, and $InsP_6$ concentrations in turkeys and broilers.

As 2 species were compared that differ markedly in their maturation rates (Zuidhof et al., 2014; Tůmová et al., 2020), it is necessary to evaluate whether observed differences in phytate degradation and nutrient digestibility can be attributed to species effects or if effects of different physiological development were involved. Thus, a second trial was conducted with 6 wk-old turkeys and broilers, receiving similar dietary treatments as in the present trial (Novotny et al., 2023). Many findings of the present study were confirmed in 6 wk-old birds. However, it was found that age affected turkeys and broilers differently regarding endogenous mucosal phosphatase activity and prececal $InsP_6$ disappearance, both increasing with age in turkeys. This resulted in higher prececal $InsP_6$ disappearance in 6 wk-old turkeys than 6 wk-old broilers, when no phytase was supplemented. As such, higher mucosal phosphatase activity coincided with higher prececal $InsP_6$ disappearance. Comparisons are being discussed in more detail in the companion paper (Novotny et al., 2023).

In conclusion, there was no difference in the endogenous $InsP_6$ degradation capabilities between 3-wk-old broilers and turkeys. Previously reported differences between species were possibly owing to differences in diet composition. Furthermore, the effect of phytase supplementation on prececal $InsP_6$ disappearance was greater in broilers than in turkeys, which might be linked to better crop conditions. Also, the uptake and metabolism of MI, as well as the effects of phytase supplementation on AA digestibility, deserve further research, as apparent species differences in these traits require elucidation. Further research is also necessary to clarify the relevance of age of the birds for the studied traits.

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DISCLOSURES

The authors declare no conflicts of interest.

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5.3 Manuscript 3

Mucosal phosphatase activity, phytate degradation, and mineral digestibility in 6-week-old turkeys and broilers at different dietary levels of phosphorus and phytase and comparison with 3-week-old animals

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ABSTRACT Female turkeys (B.U.T. 6) and broilers (Ross 308) were compared at 6 wk of age to evaluate the effects of species, dietary P, Ca, and phytase levels on *myo*-inositol hexakisphosphate ($InsP_6$) degradation along the digestive tract, gut mucosal phosphatase activity, P and Ca digestibility, and *myo*-inositol concentrations in the digesta and blood. The environmental conditions and experimental corn-soybean meal-based diets were the same for both species. Four diets with either combination of 2 levels of P and Ca (CaP−: 4.0 g P/kg, 5.4 g Ca/kg and CaP+: 6.0 g P/kg, 8.0 g Ca/kg) and 2 levels of phytase supplementation (0 and 1,500 FTU/kg) were fed to the animals for 7 d at their sixth wk of age. Each diet was randomly assigned to 6 pens per species, with 10 birds each. After slaughter, blood, digesta from the crop, gizzard, duodenum, lower ileum, and jejunal mucosa were collected. Endogenous mucosal phosphatase activity in the jejunum was higher in turkeys than in broilers. Prececal $InsP_6$ disappearance was also higher in turkeys than in broilers when phytase was not supplemented. Phytase supplementation led to a higher prececal $InsP_6$ disappearance in broilers than in turkeys, likely due to different crop conditions such as moisture content. However, prececal P digestibility was higher in turkeys than broilers. Different relationships between *myo*-inositol concentration in the ileum digesta and blood were found, depending on the species. A comparison of the results with those obtained in 3-wk-old birds of a companion study showed that in diets with low Ca and P levels, prececal $InsP_6$ disappearance increased with age in turkeys, but not in broilers. This coincided with changes in the conditions of the digestive tract, such as the water content in the crop, gizzard pH, and mucosal phosphatase activity. In conclusion, occurrence of differences in phytate degradation between turkeys and broilers, fed the same feed, depended on age and can be explained by different physiological development of the digestive tract.

Key words: phytate degradation, digesta, age, broiler, turkey

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INTRODUCTION

Utilization of plant-based P is a major issue in poultry nutrition, as the majority of this element is bound to phytate (any salt of *myo*-inositol hexakisphosphate [**$InsP_6$**]), which requires enzymatic hydrolysis before the P can be absorbed in the digestive tract. The capacity of poultry to hydrolyze $InsP_6$ via endogenous phytases is debatable. The high potential of endogenous $InsP_6$ degradation has repeatedly been shown in broilers (Rodehutscord et al., 2022) with endogenous mucosal phytases and phosphatases as major contributing factors (Sommerfeld et al., 2019). Turkeys have been shown to have lower $InsP_6$ degradation than broilers (Ingelmann et al., 2019; Olukosi et al., 2020). However, in these studies, species-specific experimental feeds were used, with substantial differences in dietary P and Ca concentrations. In a study using the same feed for different poultry species, total tract P retention when fed a low-P diet was 58% and 39% for broilers and turkeys, respectively (Rodehutscord and Dieckmann, 2005). However, $InsP_6$ degradation was not studied. Thus, different endogenous $InsP_6$ degradation in broilers and turkeys is likely to exist, but this has not yet been proven. Another factor that may influence endogenous $InsP_6$ degradation is the age or age-dependent physiological

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development of animals (Morgan et al., 2015; Olukosi et al., 2020; Kriseldi et al., 2021).

The objective of this study was to compare the prececal $InsP_6$ disappearance in 6-wk-old broilers and turkeys with respect to the effects of dietary Ca and P (**CaP**) concentrations and phytase supplementation (**PHY**) in order to elucidate which physiological differences between broilers and turkeys are causative for the dissimilarities previously found. Thus, the comparison was to be made using the same feed for both species. As two species with different maturation rates were compared (Zuidhof et al., 2014; Tůmová et al., 2020), it was unclear whether the observed differences could be solely attributed to species effects or if physiological development effects were confounding. Therefore, the results were compared with those obtained from 3-wk-old broilers and turkeys of a companion study (Novotny et al., 2023) to assess whether age affected the outcome of this species comparison. Another objective was to review the traits that might explain different endogenous $InsP_6$ degradation in broilers and turkeys, such as gut length, pH of the digestive tract, jejunal mucosal phosphatase activity, and changes between the third and sixth wk of age. It was hypothesized that $InsP_6$ degradation in broilers and turkeys is differently affected by age.

MATERIALS AND METHODS

The trial protocol was approved in accordance with German animal welfare legislation by the Regierungspräsidium Tübingen, Germany (Project No. HOH 59/19 TE) and was carried out at the experimental station "Unterer Lindenhof" of the University of Hohenheim, Germany. This was an extension of the trial described by Novotny et al. (2023). All procedures and analyses were the same as applied to 3-wk-old birds in that study and are therefore described only briefly in the present study.

Birds and Housing

A total of 530 female Ross 308 broiler hatchlings and 530 female B.U.T. 6 turkey hatchlings were raised in floor pens on wood shavings, and 240 birds from each species were used in the present study. Until 35 d of age, the animals were fed species-specific diets formulated according to the respective supply recommendations (Gesellschaft für Ernährungsphysiologie, 1999, 2004) without supplemented phytase. On d 35 of age, the birds were allocated to perforated floor pens (24 per species; 1.15 m × 2.3 m for broilers and 3 m × 4 m for turkeys, all in the same barn) with 10 birds per pen. Each pen was assigned to 1 of 4 experimental diets in a randomized complete block design, with 6 pens per diet and species. Feed and water were provided for ad libitum consumption until slaughter on d 42.

Experimental Diets and Treatments

A 2 × 2 × 2 factorial arrangement of treatments (2 species, 2 CaP levels, and 2 levels of PHY addition) was chosen. Both species received the same experimental diet from d 35 of age until slaughter (Table 1). Corn-soybean meal-based diets were formulated to meet the supply recommendations for turkeys (Gesellschaft für Ernährungsphysiologie, 2004), with the exception of P and Ca. The CaP− diets had no mineral phosphate added, resulting in calculated P and Ca concentrations

Table 1. Ingredient composition and calculated nutrient concentrations of the experimental diets.

Ingredient, g/kg	CaP−PHY−	CaP−PHY+	CaP+PHY−	CaP+PHY+
Corn	487.0	487.0	487.0	487.0
Soybean meal	392.3	392.3	392.3	392.3
Rapeseed meal	30.0	30.0	30.0	30.0
Soybean oil	48.2	48.2	48.2	48.2
L-lysine-sulfate	5.8	5.8	5.8	5.8
DL-methionine	2.9	2.9	2.9	2.9
L-threonine	0.3	0.3	0.3	0.3
Choline chloride	2.0	2.0	2.0	2.0
NaCl	1.0	1.0	1.0	1.0
$NaHCO_3$	4.0	4.0	4.0	4.0
Vitamin mix[1]	2.0	2.0	2.0	2.0
Mineral mix[2]	0.5	0.5	0.5	0.5
Titanium dioxide	5.0	5.0	5.0	5.0
Limestone	7.2	7.2	9.5	9.5
Monocalcium phosphate	0.0	0.0	9.5	9.5
Sand	11.8	11.8	0.0	0.0
Calculated (g/kg):				
P	4.0	4.0	6.0	6.0
Ca	5.4	5.4	8.0	8.0
Crude protein	242	242	242	242
Phytase (FTU/kg)	0	1,500	0	1,500

[1]Vitamin mix (MIAVIT GmbH, Essen (Oldb.), Germany), provided per kg of complete diet: 10,000 IU vitamin A, 3,000 IU vitamin D3, 30 mg DL-α-Tocopherylacetate, 2.4 mg vitamin K3, 3 mg vitamin B1, 6 mg vitamin B2, 6 mg vitamin B6, 30 μg vitamin B12, 50 mg nicotinic acid, 14 mg pantothenic acid, 1 mg folic acid, 0.1 mg biotin.

[2]Mineral mix (Gelamin, Gesellschaft für Tierernährung mbH, Memmingen, Germany), provided per kg of complete diet: 50 mg calcium from calcium carbonate, 80 mg manganese from manganese-(II)-oxide, 60 mg zinc from zinc oxide, 25 mg iron from ferrous-(II)-carbonate, 7.5 mg copper from cupric-(II)-sulfate pentahydrate, 0.6 mg iodine from calcium iodate, 0.2 mg selenium from sodium selenite.

Table 2. Analyzed composition of the experimental diets.

Analyzed composition (g/kg[2])	Treatments[1]			
	CaP− PHY−	CaP− PHY+	CaP+ PHY−	CaP+ PHY+
$InsP_6$ (μmol/g)	14.0	13.6	13.8	13.8
$InsP_5$ (μmol/g)	1.6	1.7	1.6	1.6
Myo-inositol (μmol/g)	1.7	1.8	1.7	1.8
$InsP_6$-P	2.6	2.6	2.6	2.5
P	4.4	4.3	6.6	6.6
Ca	5.5	5.3	8.2	8.1
Crude protein	247	243	247	243
Phytase (FTU/kg)	<50	1,110	<50	1,130

[1]Calculated composition: CaP−, 4.0 g P/kg and 5.4 g Ca/kg; CaP+, 6.0 g P/kg and 8.0 g Ca/kg; PHY−, no supplemented phytase; PHY+: 1,500 FTU/kg supplemented phytase.[2]Unless stated otherwise.

of 4.0 g/kg and 5.4 g/kg, respectively. Sand was used as the filler which was replaced by monocalcium phosphate (**MCP**) and limestone in the other diets. The CaP+ diets were supplemented with MCP and limestone to achieve P and Ca concentrations of 6.0 g/kg and 8.0 g/kg, respectively. The diets were either not supplemented with phytase on top (PHY−) or supplemented with 1,500 FTU/kg of a modified *E. coli*-derived 6-phytase (Quantum Blue, AB Vista, Marlborough, United Kingdom) (PHY+). All diets contained 5 g/kg TiO_2 as an indigestible marker and were mixed and pelleted through a 3-mm die. Formulated levels of P, $InsP_6$-P, and Ca were confirmed by analyses (Table 2). The analyzed phytase activity in the PHY+ diets was slightly lower than the calculated value (approximately 1,120 FTU/kg) but consistent in both diets.

Procedures and Sampling

Animals and feed were weighed on d 35 and 42 to calculate ADG, ADFI, and gain per feed (**G:F**), considering the removed animals. On d 42, 1 h of feed deprivation was followed by 1 h of feeding prior to slaughter to standardize the gut fill. The animals were then stunned with a gas mixture (35% N_2, 35% CO_2, and 30% O_2). Two stunned birds per pen were randomly selected, marked, and weighed individually. The birds were killed by decapitation, trunk blood was collected, and plasma was obtained. One of the birds was used for the determination of intestinal section lengths using a 1-cm grit. The jejunum was dissected from the second bird and the mucosa was obtained. The remaining birds were asphyxiated with CO_2 after stunning. Digesta samples of crop, gizzard, duodenum, and lower two-thirds of the ileum were collected from all birds in a pen. The samples of each section were pooled on a by pen basis and frozen at −20°C after determination of the pH values of the crop, gizzard, and duodenum contents.

Sample Preparation and Chemical Analyses

The digesta samples were freeze-dried, pulverized, and stored in sealed containers at room temperature. Pulverized feed and digesta samples were analyzed for P, Ca, Ti, and $InsP_{3\text{-}6}$ isomers (Zeller et al., 2015a; Sommerfeld et al., 2018) and *myo*-inositol (**MI**) was analyzed in the feed, digesta, and plasma samples (Sommerfeld et al., 2018). The activity of exogenous phytase in the feed was analyzed by AB Vista Lab Service (Ystrad Mynach, Wales, UK) using a validated product-specific ELISA method. Mucosal phosphatase activity was measured in enriched brush-border membrane samples according to the methods of Huber et al. (2015), with modifications described in the companion paper (Novotny et al., 2023). In brief, brush-border membrane sample aliquots containing 160 μg protein were incubated with 25 μg of sodium phytate (Sirius Fine Chemicals SiChem GmbH, Bremen, Germany) at pH 5.5 for 15 min. Free phosphate ($\mathbf{P_i}$) was determined photometrically (655 nm, 40°C, Infinite 200 PRO M NANO+, Tecan Trading AG, Switzerland). The activity of mucosa associated phosphatases (including phytase, as substrate was sodium phytate) is the released P_i per g brush-border membrane protein per minute incubation time at pH 5.5 and 40°C.

Calculations and Statistical Analysis

Prececal digestibility of P, Ca, AA, and $InsP_6$ disappearance were calculated using the marker method and the following equation:

$$y(X) = 100 - 100 \times (X_{digesta} \times TiO_{2\,feed})/(TiO_{2\,digesta} \times X_{feed})$$

where y is the disappearance or digestibility of X in %; X is the concentration of $InsP_6$, P, or Ca in the feed and digesta; and TiO_2 is the concentration of TiO_2 in the feed and digesta.

The data were analyzed with a 3-way ANOVA using the MIXED procedure and pairwise t-tests using the software package SAS (version 9.4; SAS Institute Inc., Cary, NC). Data without normal distribution were log-transformed. The results are presented as LSmeans and pooled SEM of the untransformed data. The pen was considered the experimental unit. The following model was used.

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \delta_l + \varepsilon_{ijkl}$$

where y_{ijkl} = response variable, μ = overall mean, α_i = effect of species (fixed), β_j = effect of CaP (fixed), γ_k = effect of PHY (fixed), $(\alpha\beta)_{ij}$ = interaction between species and CaP (fixed), $(\alpha\gamma)_{ik}$ = interaction between species and PHY (fixed), $(\beta\gamma)_{jk}$ = interaction between CaP and PHY (fixed), $(\alpha\beta\gamma)_{ijk}$ is the 3-way interaction between species, CaP, and PHY (fixed), δ_l = effect of block (random), and ε_{ijkl} = residual error. Statistical significance was set at $P < 0.05$.

RESULTS

Crop pH was lower in broilers than in turkeys ($P < 0.001$, Table 3). For both species, the pH of the crop was slightly lower in the treatments with high CaP level ($P = 0.030$). In the gizzard, pH was higher in broilers than in turkeys ($P < 0.001$, Table 3). In the duodenum, pH was slightly lower in broilers than in turkeys for all treatments, except for CaP+Phy+, in which there was no difference in pH between broilers and turkeys, resulting in a 3-way interaction ($P = 0.028$). Jejunal mucosal phosphatase activity was only affected by phytase supplementation in turkeys, where it was generally higher than that in broilers, resulting in a species × PHY interaction ($P = 0.010$). When the CaP level was high, jejunal mucosal phosphatase activity was lower than that at low CaP level, irrespective of species or phytase supplementation ($P = 0.034$). Prececal $InsP_6$ disappearance was lower in broilers than in turkeys when no phytase was supplemented. When phytase was supplemented, prececal $InsP_6$ disappearance was higher in broilers than in turkeys and overall higher than that without phytase supplementation, resulting in a species × PHY interaction ($P < 0.001$). At the high CaP level, prececal $InsP_6$ disappearance was lower than that at the low CaP level, irrespective of species or phytase supplementation ($P < 0.001$). Prececal P digestibility was higher in turkeys than in broilers, and it increased in turkeys with high CaP, but did not change in broilers, resulting in a species × CaP interaction ($P = 0.004$). Prececal P digestibility was also affected by species × PHY interaction ($P < 0.001$), with the lowest prececal P digestibility in broiler PHY− treatments, a higher digestibility in turkey PHY−, followed by broiler PHY+, and highest in turkey PHY+. Furthermore, prececal P digestibility was affected by the CaP × PHY interaction ($P < 0.001$). For prececal Ca digestibility, there was a trend for the species × CaP interaction ($P = 0.050$), where turkey treatments had the highest prececal Ca digestibility, followed by broiler CaP−, and broiler CaP+ with the lowest prececal Ca digestibility. Treatments with phytase supplementation always showed higher prececal Ca digestibility than those without phytase supplementation, irrespective of species or CaP level ($P < 0.001$).

Table 3. Effect of Ca and P level (CaP) and phytase supplementation (PHY) on pH in crop, gizzard, and duodenum, jejunal mucosal phosphatase activity (MPA), prececal (pc) Ca and P digestibility, and prececal $InsP_6$ disappearance of broilers and turkeys at 42 d of age (n = 6 pens per treatment).

Species	Treatment[1]		pH crop	pH gizzard	pH duodenum	MPA (μmol P_i/g BBM protein/ min)	pc $InsP_6$ disappearance (%)	pc P digestibility (%)	pc Ca digestibility (%)
Broiler	CaP−	PHY−	5.4	4.2	5.8[b]	1.1	27.2	29.2	26.1
		PHY+	5.3	4.4	5.8[b]	1.9	80.3	67.9	31.0
	CaP+	PHY−	5.1	4.4	5.8[b]	0.8	15.5	36.5	20.8
		PHY+	5.1	4.4	5.9[a]	1.6	76.8	60.0	27.3
Turkey	CaP−	PHY−	6.0	3.8	6.0[a]	4.6	33.7	38.4	45.7
		PHY+	5.9	3.6	6.0[a]	8.0	70.3	66.7	56.9
	CaP+	PHY−	5.8	3.8	5.9[a]	3.7	26.3	47.6	48.0
		PHY+	5.7	3.7	5.9[a]	6.2	64.8	67.3	57.5
SEM			0.15	0.10	0.03	0.77	2.77	1.57	2.65
Broiler	CaP−							48.6[c]	
	CaP+							48.3[c]	
Turkey	CaP−							52.5[b]	
	CaP+							57.4[a]	
Broiler	PHY−					0.9[c]	21.4[d]	32.8[d]	
	PHY+					1.7[c]	78.6[a]	64.0[b]	
Turkey	PHY−					4.2[b]	30.0[c]	43.0[c]	
	PHY+					7.1[a]	67.5[b]	67.0[a]	
	CaP−	PHY−						33.8[d]	
		PHY+						67.3[a]	
	CaP+	PHY−						42.0[c]	
		PHY+						63.6[b]	
SEM						0.67	2.42	1.30	
P-value									
Species			< 0.001	< 0.001	< 0.001	< 0.001	0.385	< 0.001	< 0.001
CaP			0.030	0.082	0.396	0.034	< 0.001	0.009	0.319
PHY			0.512	0.893	0.396	< 0.001	< 0.001	< 0.001	< 0.001
Species × CaP			0.986	0.845	0.031	0.185	0.674	0.004	0.050
Species × PHY			0.959	0.089	0.125	0.010	< 0.001	< 0.001	0.123
CaP × PHY			0.506	0.741	0.008	0.511	0.070	< 0.001	0.991
Species × CaP × PHY			0.831	0.296	0.028	0.527	0.268	0.058	0.599

[1]Calculated composition: CaP−, 4.0 g P/kg and 5.4 g Ca/kg; CaP+, 6.0 g P/kg and 8.0 g Ca/kg; PHY−, no supplemented phytase; PHY+: 1,500 FTU/kg supplemented phytase.

[a–d]Means within a column and within a significant interaction not sharing a common superscript differ significantly ($P < 0.05$).

Table 4. Effect of Ca and P level (CaP) and phytase supplementation (PHY) on the concentrations of *myo*-inositol and inositol phosphates[1] (μmol/g DM) in the crop of broilers and turkeys at 42 d of age (n = 6 pens per treatment).

Species	Treatment[2]		$InsP_6$	Ins(1,2,4,5,6)P_5	Ins(1,2,3,4,5)P_5	Ins(1,2,5,6)P_4	*Myo*-inositol
Broiler	CaP−	PHY−	13.9	1.1	0.6	<loq[3]	2.2
		PHY+	5.1	0.3	0.4	3.7	2.4
	CaP+	PHY−	13.9	1.2	0.6	<loq	2.0
		PHY+	5.1	0.3	0.4	4.3	2.2
Turkey	CaP−	PHY−	14.5	1.2	0.6	<loq	2.1
		PHY+	12.5	0.9	0.6	1.3	2.2
	CaP+	PHY−	14.6	1.2	0.6	<loq	1.9
		PHY+	12.5	0.9	0.6	1.4	1.8
SEM			0.97	0.07	0.05	0.47	0.08
Broiler	PHY−		13.9[ab]	1.1[a]	0.6[a]		
	PHY+		5.1[c]	0.3[c]	0.4[b]		
Turkey	PHY−		14.6[a]	1.2[a]	0.6[a]		
	PHY+		12.5[b]	0.9[b]	0.6[a]		
SEM			0.73	0.05	0.04		
P-value							
Species			< 0.001	< 0.001	0.001	< 0.001	0.002
CaP			0.957	0.674	0.623	0.530	< 0.001
PHY			< 0.001	< 0.001	< 0.001		0.060
Species × CaP			0.941	0.815	0.806	0.552	0.355
Species × PHY			< 0.001	< 0.001	< 0.001		0.060
CaP × PHY			0.987	0.815	0.806		0.617
Species × CaP × PHY			0.947	0.963	1.000		0.355

[1]Inositol phosphates at or below 0.2 μmol/g DM in concentration are not presented.
[2]Calculated composition: CaP−, 4.0 g P/kg and 5.4 g Ca/kg; CaP+, 6.0 g P/kg and 8.0 g Ca/kg; PHY−, no supplemented phytase; PHY+: 1,500 FTU/kg supplemented phytase.
[3]loq = limit of quantification (<0.2 μmol/g DM).
[a-c]Means within a column and within a significant interaction not sharing a common superscript differ significantly ($P < 0.05$).

In the crop of turkeys fed with phytase, $InsP_6$ and Ins(1,2,4,5,6)P_5 concentrations were lower than in turkeys without phytase supplementation (Table 4). In broilers with phytase supplementation, $InsP_6$ and Ins(1,2,4,5,6)P_5 concentrations were even lower than in turkeys with phytase supplementation, resulting in a species × PHY interaction ($P < 0.001$). Ins(1,2,5,6)P_4 was only quantifiable in broiler and turkey crops in treatments supplemented with phytase. Of these, broilers had higher Ins(1,2,5,6)P_4 concentrations in the crop than turkeys ($P < 0.001$). *Myo*-inositol concentrations in the crop were lower in birds fed diets with high CaP level than in those fed diets with low CaP level ($P < 0.001$).

In the gizzard content, $InsP_6$ and Ins(1,2,4,5,6)P_5 concentrations were lower in treatments with phytase supplementation than in treatments without phytase supplementation, irrespective of species ($P < 0.001$, Table 5). In treatments with high CaP level, $InsP_6$ and Ins(1,2,4,5,6)P_5 concentrations were higher than those in treatments with low CaP level, irrespective of the species ($P = 0.008$ and $P = 0.005$, respectively). Ins(1,2,5,6)P_4 was only quantifiable in the gizzards of broilers and turkeys in treatments supplemented with phytase. Concentrations tended to be higher in treatments with high CaP level than in those with low CaP level ($P = 0.051$). The *myo*-inositol concentration in the gizzard of turkeys was lower than that in broilers and unaffected by phytase supplementation, whereas in broilers, the concentration was higher with phytase supplementation than without phytase supplementation, resulting in a species × PHY interaction ($P < 0.001$). Furthermore, MI concentrations were unaffected by CaP level in turkeys. However, concentrations were higher when the CaP level was low than when it was high in broilers, resulting in a species × CaP interaction ($P = 0.049$).

In the ileum, the concentrations of all InsP were divided by the respective TiO_2 concentrations to normalize for different DM digestibility in turkeys and broilers. Ins(1,2,4,5,6)P_5 concentrations were lower in turkeys than in broilers when phytase was not supplemented (Table 6). When phytase was supplemented, Ins(1,2,4,5,6)P_5 concentrations were reduced to the same concentration in turkeys and broilers at CaP− level and to a lower concentration in broilers than in turkeys at CaP+ level, resulting in a three-way interaction ($P < 0.001$). Ins(1,2,3,4,5)P_5 concentrations were lower in turkeys than in broilers without phytase supplementation, with higher concentrations in both species in CaP+ than in CaP−. With phytase supplementation, Ins(1,2,3,4,5)P_5 concentrations increased in turkeys but decreased in broilers in CaP− and did not change in CaP+, resulting in a three-way interaction ($P = 0.032$). Ins(1,2,3,4,6)P_5 concentrations were higher in broilers than in turkeys, and higher in CaP+ than in CaP− ($P < 0.001$). Ins(1,2,5,6)P_4 concentrations were lower in PHY− than in PHY+ ($P < 0.001$), lower in CaP− than in CaP+ ($P < 0.001$), and lower in turkeys than in broilers. $InsP_{3x}$ was found in the broiler CaP+PHY+ treatment, but was not detectable in any other treatment. There was a significant three-way interaction between the MI concentrations in the ileum ($P < 0.001$). The MI concentration was the highest in broilers of the CaP−PHY+ treatment, followed by the broilers of the CaP+PHY+ treatment. The broiler CaP−PHY− treatment had lower concentrations than these treatments, but higher concentrations than broiler CaP+PHY−. The MI concentrations in all turkey treatments were at the same

Table 5. Effect of Ca and P level (CaP) and phytase supplementation (PHY) on the concentrations of *myo*-inositol and inositol phosphates[1] (μmol/g DM) in the gizzard of broilers and turkeys at 42 d of age (n = 6 pens per treatment).

Species	Treatment[2]		$InsP_6$	$Ins(1,2,4,5,6)P_5$	$Ins(1,2,3,4,5)P_5$	$Ins(1,2,5,6)P_4$	*Myo*-inositol
Broiler	CaP−	PHY−	6.2	0.4	0.3	n.d.[3]	2.1
		PHY+	0.5	n.d.	n.d.	2.3	3.6
	CaP+	PHY−	7.4	0.6	0.3	n.d.	1.6
		PHY+	0.8	n.d.	0.1	2.9	3.0
Turkey	CaP−	PHY−	6.6	0.5	0.3	n.d.	0.6
		PHY+	<loq[4]	n.d.	n.d.	1.9	1.0
	CaP+	PHY−	7.2	0.5	0.3	n.d.	0.7
		PHY+	0.3	n.d.	0.3	2.8	1.0
SEM			0.28	0.03	0.03	0.37	0.22
Broiler	CaP−						2.8[a]
	CaP+						2.3[b]
Turkey	CaP−						0.8[c]
	CaP+						0.9[c]
Broiler	PHY−						1.8[b]
	PHY+						3.3[a]
Turkey	PHY−						0.6[c]
	PHY+						1.0[c]
SEM							0.16
P-value							
Species			0.622	0.590	0.196	0.480	< 0.001
CaP			0.008	0.005	0.058	0.051	0.087
PHY			< 0.001		< 0.001		< 0.001
Species × CaP			0.215	0.288	0.511	0.705	0.049
Species × PHY			0.466				< 0.001
CaP × PHY			0.102				0.677
Species × CaP × PHY							0.890

[1]Inositol phosphates at or below 0.2 μmol/g DM in concentration are not presented.
[2]Calculated composition: CaP−, 4.0 g P/kg and 5.4 g Ca/kg; CaP+, 6.0 g P/kg and 8.0 g Ca/kg; PHY−, no supplemented phytase; PHY+: 1,500 FTU/kg supplemented phytase.
[3]n.d. = not detectable (<0.1 μmol/g).
[4]loq = limit of quantification (<0.2 μmol/g).
[a–c]Means within a column and within a significant interaction not sharing a common superscript differ significantly ($P < 0.05$).

low level as the broiler CaP+PHY− treatment. However, the MI concentrations in the ileum of CaP−PHY+ turkeys were higher than those in CaP−PHY− turkeys. The MI concentrations in the blood were at the same level in turkeys and broilers without phytase supplementation. With phytase supplementation, the blood concentration was higher than that without, whereby with added phytase, turkeys had higher MI concentrations in the blood than broilers, resulting in a species × PHY interaction ($P = 0.005$). Irrespective of the species, the different combinations of CaP level and phytase led to 4 different MI concentrations in the blood, resulting in a CaP × PHY interaction ($P = 0.037$).

Average daily gain and ADFI were higher in broilers than in turkeys, and G:F was higher in turkeys than in broilers ($P < 0.001$, Table 7). Average daily gain, G:F, and ADFI were higher with phytase supplementation than without ($P < 0.001$, $P = 0.023$, and $P < 0.001$, respectively) and higher with high CaP level than with low CaP level ($P < 0.001$, $P = 0.004$, and $P < 0.001$, respectively). The absolute length of the small intestine was greater, whereas the relative length per BW was shorter in broilers than in turkeys ($P < 0.001$, Table 7).

DISCUSSION

In this study, jejunal mucosal phosphatase activity, prececal $InsP_6$ disappearance, and prececal P and Ca digestibility were higher in 6-wk-old turkeys than in broilers without phytase supplementation. This is in contrast to the results obtained with 3-wk-old turkeys and broilers, where no differences between species were found in these traits in diets without phytase (Novotny et al., 2023). This indicated that $InsP_6$ degradation in broilers and turkeys is affected differently by bird age. In addition, the causality of mucosal phosphatase activity as a main contributor to endogenous $InsP_6$ degradation in broilers and turkeys has been indicated, albeit not proven. As already discussed by Novotny et al. (simultaneously submitted to Poultry Science), these results are in contrast to those of previous comparative studies with broilers and turkeys (Ingelmann et al., 2019; Olukosi et al., 2020), likely because these studies used species-specific diets with different concentrations of nutrients, such as P and Ca. Using species-specific feeds closely resembling industry standards may be advantageous when aiming to compare the effects of different feeding strategies (supplementation of phytase, reduction of supplemented Ca and P) in different production systems (broiler or turkey production). However, such experimental setups do not allow to study species specific traits responsible for the observed differences.

In treatments with phytase supplementation, prececal $InsP_6$ disappearance was higher in broilers than in turkeys. This was also observed in 3-wk-old broilers and turkeys (Novotny et al., 2023). However, differences in prececal $InsP_6$ disappearance between 6-wk-old turkeys and broilers were smaller than between 3-wk-old turkeys

Table 6. Effect of Ca and P level (CaP) and phytase supplementation (PHY) on the concentrations of TiO_2 (mg/g DM), *myo*-inositol and inositol phosphates in the ileum (μmol/mg TiO_2) and *myo*-inositol in blood plasma (μmol/ml) of broilers and turkeys at 42 d of age (n = 6 pens per treatment; in case of *myo*-inositol in blood n = 6 animals per treatment).

Species	Treatment[1]		TiO_2	$InsP_6$	Ins(1,2,4,5,6)P_5	Ins(1,2,3,4,5)P_5	Ins(1,2,3,4,6)P_5	Ins(1,2,5,6)P_4	Ins(1,2,3,4)P_4	$InsP_{3x}$[2]	Ileum *myo*-inositol	Blood *myo*-inositol
Broiler	CaP−	PHY−	18.0	1.90	0.06^{c}	0.08^{c}	0.04	<loq[3]	0.03	n.d.[4]	0.27^{c}	0.27
		PHY+	19.0	0.52	0.03^{e}	0.05^{d}	<loq	0.05	0.01	n.d.	1.00^{a}	0.37
	CaP+	PHY−	18.5	2.21	0.13^{a}	0.10^{b}	0.05	0.03	0.03	n.d.	0.12^{de}	0.22
		PHY+	20.6	0.61	0.05^{d}	0.09^{bc}	<loq	0.20	0.02	0.13	0.50^{b}	0.32
Turkey	CaP−	PHY−	15.6	1.73	0.04^{d}	0.06^{d}	0.03	n.d.	0.01	n.d.	0.07^{e}	0.25
		PHY+	15.0	0.78	0.03^{e}	0.08^{c}	<loq	0.03	0.01	n.d.	0.16^{d}	0.43
	CaP+	PHY−	15.3	1.93	0.08^{b}	0.08^{c}	0.04	<loq	0.02	n.d.	0.08^{de}	0.21
		PHY+	17.5	0.92	0.07^{c}	0.16^{a}	<loq	0.12	0.03	n.d.	0.08^{de}	0.33
SEM			0.45	0.072	0.005	0.008	0.038	0.015	0.003	0.011	0.027	0.013
Broiler	PHY−			2.06^{a}					0.03^{a}			0.25^{c}
	PHY+			0.56^{d}					0.02^{bc}			0.35^{b}
Turkey	PHY−			1.83^{b}					0.01^{c}			0.23^{c}
	PHY+			0.85^{c}					0.02^{b}			0.38^{a}
	CaP−	PHY−	16.8^{b}						0.02^{b}			0.26^{c}
		PHY+	17.0^{b}						0.01^{c}			0.40^{a}
	CaP+	PHY−	16.9^{b}						0.02^{ab}			0.22^{d}
		PHY+	19.0^{a}						0.03^{a}			0.32^{b}
SEM			0.32	0.063					0.002			0.010
P-value												
Species			< 0.001	0.391	< 0.001	0.032	< 0.001	0.001	0.026		<0.001	0.265
CaP			0.002	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	<0.001		<0.001	<0.001
PHY			< 0.001	< 0.001	< 0.001	0.007		< 0.001	0.299		<0.001	<0.001
Species × CaP			0.950	0.658	0.527	0.046	0.545	0.058	0.149		<0.001	0.380
Species × PHY			0.212	< 0.001	< 0.001	< 0.001			<0.001		<0.001	0.005
CaP × PHY			0.005	0.068	< 0.001	< 0.001			0.001		<0.001	0.037
Species × CaP × PHY			0.155	0.277	< 0.001	0.032			0.149		0.002	0.121

[1]Calculated composition: CaP−, 4.0 g P/kg and 5.4 g Ca/kg; CaP+, 6.0 g P/kg and 8.0 g Ca/kg; PHY−, no supplemented phytase; PHY+: 1,500 FTU/kg supplemented phytase.
[2]Ins(1,2,6)P_3, Ins(1,4,5)P_3, and Ins(2,4,5)P_3 could not be differentiated due to co-elution and are thus referred to as $InsP_{3x}$.
[3]loq = limit of quantification (Ins(1,2,3,4,6)P_5: 0.3 μmol/g; Ins(1,2,3,4)P_4: 0.2 μmol/g).
[4]n.d. = not detectable (<0.1 μmol/g).
[a-e]Means within a column and within a significant interaction not sharing a common superscript differ significantly ($P < 0.05$).

Table 7. Effect of Ca and P level (CaP) and phytase supplementation (PHY) on average daily gain (ADG), average daily feed intake (ADFI), gain to feed ratio (G:F) from d 35 and d 42 of age, and length of small intestine and BW of broilers and turkeys (one bird per pen) at 42 d of age (n = 6 pens per treatment).

Species	Treatment[1]		ADG[2] (g/d)	ADFI (g/d)	G:F (g/g)	Small intestine length (cm)	BW of the bird (g)	Small intestine length (cm/kg BW)
Broiler	CaP−	PHY−	121	176	0.69	184	3,202	58
		PHY+	126	179	0.70	183	3,148	58
	CaP+	PHY−	126	181	0.70	193	3,081	63
		PHY+	131	181	0.72	191	3,190	60
Turkey	CaP−	PHY−	112	156	0.71	161	2,292	71
		PHY+	120	165	0.73	154	2,240	70
	CaP+	PHY−	121	165	0.73	152	2,274	67
		PHY+	127	168	0.75	157	2,359	67
SEM			3.0	3.5	0.007	4.6	91	2.5
P-value								
Species			< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
CaP			< 0.001	0.004	< 0.001	0.449	0.927	0.962
PHY			< 0.001	0.023	< 0.001	0.611	0.732	0.515
Species × CaP			0.261	0.394	0.504	0.067	0.475	0.057
Species × PHY			0.393	0.223	0.865	0.834	0.935	0.867
CaP × PHY			0.588	0.160	0.356	0.390	0.239	0.737
Species × CaP × PHY			0.661	0.703	0.853	0.306	0.918	0.476

[1]Calculated composition: CaP−, 4.0 g P/kg and 5.4 g Ca/kg; CaP+, 6.0 g P/kg and 8.0 g Ca/kg; PHY−, no supplemented phytase; PHY+: 1,500 FTU/kg supplemented phytase.
[2]Average initial body weights per bird on day 35: 2300 g (broilers) 1,465 g (turkeys).

and broilers, as in broilers prececal $InsP_6$ disappearance remained at a high level of approximately 80% in CaP −PHY+; while in turkeys, prececal $InsP_6$ disappearance increased from 58.0% in wk 3 to 70.3% in wk 6 in CaP −PHY+ (Figure 1). Moisturizing crop content is important for exogenous enzyme activity (Kierończyk et al., 2016). Thus, this increase in prececal $InsP_6$ disappearance between wk 3 and wk 6 in turkeys was likely caused by a higher water content in the crop of the 6-wk-old turkeys than in 3-wk-old turkeys (indicated by higher water loss during freeze-drying of crop-content samples, data not shown), leading to better substrate accessibility for the supplemented phytase. Additionally, during sampling, crops appeared to be much less developed in 3-wk-old turkeys than in 6-wk-old turkeys. As a result, in 6-wk-old turkeys, $InsP_6$ concentrations in the crop were significantly lower in PHY+ treatments than PHY− treatments, whereas there was no difference between both treatments in 3-wk-old turkeys (Table 4, $P < 0.001$; Novotny et al., 2023). Among PHY+ treatments, the reduction in $InsP_6$ concentration in the crop from wk 3 to wk 6 was greater in broilers than in turkeys (Figure 2). However, this did not lead to higher prececal $InsP_6$ disappearance in 6-wk-old broilers compared to 3-wk-old broilers. This could partly be explained by an increase in gizzard pH in broilers between wk 3 and wk 6 from pH 4.0 (Novotny et al., 2023) to pH 4.3 (Table 3), as the lower pH in wk 6 was even further from the optimum pH of the supplemented phytase (Menezes-Blackburn et al., 2015) and associated with lower phytate solubility (Grynspan and Cheryan, 1983). Furthermore, the higher phytate degradation in the crop could

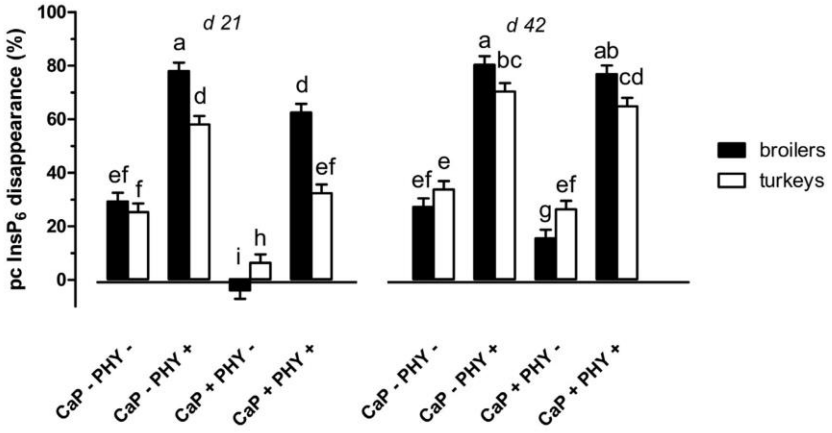

Figure 1. Effect of Ca and P level (CaP) and phytase supplementation (PHY) on prececal $InsP_6$ disappearance (LSmeans ± SEM) of broilers and turkeys fed experimental diets at 21 d (Novotny et al., 2023) and 42 d of age. Bars not sharing the same letters are significantly different according to a one-factorial ANOVA ($P < 0.05$, n = 6 pens per treatment).

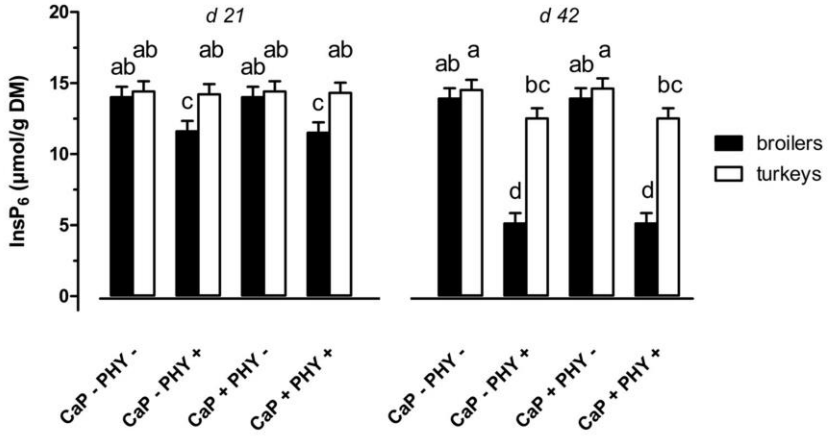

Figure 2. Effect of Ca and P level (CaP) and phytase supplementation (PHY) on $InsP_6$ concentration in the crop content (LSmeans ± SEM) of broilers and turkeys fed experimental diets at 21 d (Novotny et al., 2023) and 42 d of age. Bars not sharing the same letters are significantly different according to a one-factorial ANOVA ($P < 0.05$, n = 6 pens per treatment).

have been counteracted by lower mucosal phosphatase activity in the jejunum of older broilers. This aspect is discussed in more detail later in this text.

Different passage rates through the small intestine may have influenced phytate degradation. Although passage rates were not measured, differences in small intestine length between species decreased between wk 3, when turkeys had an almost 24% shorter small intestine than broilers (Novotny et al., 2023), and wk 6, when the small intestine was less than 17% shorter in turkeys than in broilers (Table 7). This could indicate that the retention time in the small intestine increased in turkeys relative to broilers between wk 3 and wk 6, resulting in more time for phytate degradation. Between wk 3 and wk 6, differences in dietary Ca and P concentrations were very small in CaP− treatments (5.7 g Ca/kg and 4.5 g P/kg [Novotny et al., 2023], and 5.3 g Ca/kg and 4.3 g P/kg [Table 2], respectively). However, a possible effect of dietary Ca and P concentrations on phytate degradation in CaP− treatments could not be ruled out entirely.

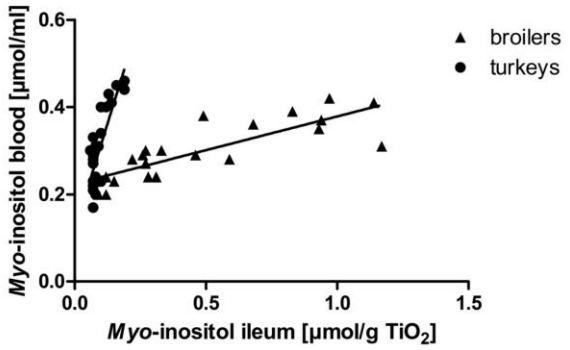

Figure 3. *Myo*-inositol concentrations in ileum digesta (per g TiO_2) and blood of broilers and turkeys fed the experimental diets at 42 d of age. Dots represent individual pen values for the ileum data and individual bird values for the blood data. Linear regression broilers: y = 0.15 x + 0.22, r^2 = 0.69. Linear regression turkeys: y = 1.95 x + 0.12, r^2 = 0.69.

The effect of CaP level, which led to reduced prececal $InsP_6$ disappearance in wk 6 when CaP level was increased, irrespective of species or phytase supplementation, can be attributed to end product inhibition of phytase by supplemented P (Greiner et al., 1993; Zeller et al., 2015b) and chelate formation caused by Ca^{2+} ions and consequent precipitation and inaccessibility for phytase (Walk et al., 2012; Sommerfeld et al., 2018). In wk 3, a high CaP level also led to lower prececal $InsP_6$ disappearance compared to low CaP level (Novotny et al., 2023) but here the effect depended on species and phytase supplementation, as the 3-way-interaction of these effects was significant. Also, in the CaP+PHY+ treatment, prececal $InsP_6$ disappearance increased in both turkeys and broilers from wk 3 to wk 6. This was most likely caused by lower dietary Ca and P concentrations in wk 6 (8.2 g Ca/kg and 6.6 g P/kg, Table 2) than in wk 3 (12.3 g Ca/kg and 9.8 g P/kg, Novotny et al., 2023), leading to lower end product inhibition of phytase by supplemented P and less chelate formation caused by Ca^{2+} ions in wk 6 compared to wk 3.

In wk 6, phytase supplementation led to an increase in prececal $InsP_6$ disappearance of approximately 57 percentage points in broilers and 38 percentage points in turkeys, irrespective of CaP level. This resulted in an absolute higher prececal $InsP_6$ disappearance in broilers than in turkeys when phytase was supplemented. However, supplementation with phytase led to an increase in prececal P digestibility of only 31 percentage points in broilers and 24 percentage points in turkeys, leaving prececal P digestibility higher in turkeys than in broilers even when phytase was supplemented. This could be due to the higher degradation of lower InsP in turkeys than in broilers, as indicated by lower concentrations of $InsP_{3\text{-}5}$-P in the ileum. The markedly higher gut mucosal phosphatase activity of turkeys than that of broilers supports this assumption. It is also possible that the P

released from phytate was absorbed at a higher proportion in turkeys than in broilers, as turkeys have shown higher P utilization than broilers when fed the same diets with high P concentrations (Rodehutscord and Dieckmann, 2005).

The MI concentration in the ileum was much lower in turkeys than in broilers, especially when phytase was supplemented, which is inconsistent with the higher degradation of lower InsP in turkeys. However, the MI concentration in the blood was higher in turkeys than in broilers when phytase was supplemented (species × PHY: $P = 0.005$, Table 6). When relating MI concentrations in the ileum digesta and blood (Figure 3), it was apparent that they were correlated in both species. However, the slope of the regression line of MI concentration in the ileum and blood of turkeys was much greater than that of broilers. Novotny et al. (2023) found very similar relations in 3-wk-old turkeys and broilers, and suggested that this may be caused by faster MI absorption or more anterior MI absorption in the digestive tract of turkeys than in broilers. A faster MI absorption in older birds is suggested by greater slope of regression lines of MI concentration in the ileum and blood of the respective species at wk 6 compared to wk 3, caused by lower MI concentration in the ileum and similar MI concentration in the blood at wk 6 compared to wk 3. This is further corroborated by the fact that prececal $InsP_6$ disappearance was higher in wk 6 than wk 3; thus, more MI should have been completely dephosphorylated. However, causalities warrant investigation in future research.

Mucosal phosphatase activity in the jejunum was not only affected by species but also by dietary CaP level, as in CaP+ treatments, mucosal phosphatase activity was lower than that in CaP−. This is consistent with the previously reported effects of dietary P on mucosal phytase/phosphatase activity (Davies et al., 1970; Onyango et al., 2006). However, in 3-wk-old birds (Novotny et al., 2023), no significant effect of dietary CaP level on mucosal phosphatase activity was observed. Further, it appeared that 6-wk-old broilers had lower jejunal mucosal phosphatase activity (average of 1.4 μmol P_i/g BBM protein/min., Table 3) compared to 3-wk-old broilers (average of 4.1 μmol P_i/g BBM protein/min., Novotny et al., 2023). In contrast, jejunal mucosal phosphatase activity appeared to be similar among 3- and 6-wk-old turkeys, except in 6-wk-old turkeys fed PHY+, in which jejunal mucosal phosphatase activity was elevated. This elevated activity could have been triggered by the high concentrations of lower InsP found in turkeys in this treatment, as discussed by Walk et al. (2018). The effect of dietary P on measured mucosal phosphatase activity, when present, could have been caused by the downregulation of the expression (or substrate affinity) of phosphatases in BBM, based on the presence of absorbable phosphate (Onyango et al., 2006). As only the average mucosal phosphatase activity in the jejunum was measured and the retention time in the small intestine was not determined, the overall contribution of endogenous mucosal phosphatase activity to phytate degradation is unknown.

In conclusion, prececal $InsP_6$ disappearance between wk 3 and wk 6 was affected by age in turkeys, but not in broilers when no mineral P was supplemented. This coincides with the observed differences in digestive tract development. Data from other age groups are required to deepen the understanding of the influence of crop, stomach, and gut development on phytate degradation. Moreover, digesta retention times in specific sections of the digestive tract require further research to better understand phytate degradation kinetics.

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DISCLOSURES

All authors declare that they have no conflict of interest.

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6 SUMMARY

A growing global human population, stagnation in available land for farming, and an increased interest in sustainable and eco-friendly food production necessitates a highly efficient and environmentally friendly food production. This includes poultry meat production, which is already one of the most efficient meat production systems in regard to required feed input. Currently, using phosphate originating from non-renewable phosphate rock as feed additive in poultry nutrition is industry standard. This leads to undesirably high phosphate concentration in the excreta, as plant-based phosphate remains unutilised. This can lead to unwanted eutrophication of waterbodies. Phytases are enzymes that can hydrolyse phosphate groups from phytate, the main contributor to plant-based phytate. Degrading phytate via enzymatic hydrolysation drastically improves digestibility of plant-based phosphate, as phytate cannot be absorbed in the digestive tract. With supplementation of microbial phytases to poultry feed, a tool is available to improve digestibility of plant-based phosphorus and reduce necessity of dietary phosphate supplementation. However, predictability of the extent of increased digestibility of plant-based phosphorus by phytase supplementation is not accurate enough to fully forego phosphate supplementation entirely.

Subject of this doctoral thesis was to study the factors that can influence phytate degradation in the digestive tract of poultry, in order to improve predictability of plant-based phosphate digestibility. The focus was put on maize-based diets, as they are very common worldwide and phytate degradation is especially challenging due to the low intrinsic phytase activity of maize. Firstly, a literature review on the current state of knowledge on phytate degradation and phosphorus digestibility of chicken fed maize-based diets was conducted. Part of this review was to compare findings on this subject specific for chicken to findings in other poultry species. There is a plethora of studies that investigated phytate degradation and phosphorus digestibility in broiler chickens but comparatively little information on turkeys. Further, there were indications of fundamental differences between broiler chickens and turkeys. Consequently, the intention was to identify reasons for these differences and to evaluate to which extent

knowledge from broiler chickens could be transferred to turkeys. Two consecutive trials comparing broiler chickens and turkeys were designed. Factors studied were: supplemented microbial phytase, dietary phosphorus and calcium concentration, age, and endogenous mucosal phosphatase activity in the jejunum. Broiler chickens and turkeys studied were kept simultaneously and under identical conditions. They also received the same diets. A total of 480 broiler chicken and 480 turkey hatchlings were obtained at the same day and raised at the experimental facility. Halve of the animals of each species took part at the first trial from day 14 to day 21 post hatch. The other halve took part in the second trial from day 35 to day 42 post hatch. This set up was chosen to additionally study the influence of physiological development, as species with different maturation rates were compared.

In 3-week-old broiler chickens and turkeys, precaecal $InsP_6$ disappearance was the same when no phytase was supplemented and dietary calcium and phosphorus level was low. This coincided with no differences in endogenous jejunal mucosal phosphatase activity. In 6-week-old turkeys, precaecal $InsP_6$ disappearance was higher than in 6-week-old broiler chickens, when no phytase was supplemented. This coincided with higher endogenous jejunal mucosal phosphatase activity in turkeys than broilers. When phytase was supplemented, precaecal $InsP_6$ disappearance was markedly increased in both species. This increase was always higher in broilers compared to turkeys of the same age. Further, increased dietary calcium and phosphorus levels led to decreased precaecal $InsP_6$ disappearance in both species at both ages. This led to the conclusion that previously reported differences in precaecal $InsP_6$ disappearance between broiler chickens and turkeys were primarily due to the higher dietary calcium and phosphorus concentrations typically used in turkey diets, and secondly due to more phytate degradation of supplemented phytase in the digestive tract of broiler chickens compared to turkeys. The latter was attributed to more favourable conditions for the supplemented phytase, such as higher moisture content, lower pH, and possibly higher retention time, in the crop of broiler chickens compared to turkeys. Although the turkeys

appeared to have compensated much of that in the more posterior parts of the digestive tract.

Endogenous jejunal mucosal phosphatase activity was higher in treatments with phytase supplementation than without. As this coincided with high concentrations of lower inositol phosphates in the digesta, these might have triggered increased expression of mucosal phosphatases on the brush border membrane. In contrast, an increase in dietary calcium and phosphorus level coincided with a decrease in endogenous jejunal mucosal phosphatase activity, numerically in 3-week-old birds, but significantly in 6-week-old birds. This might indicate a downregulation of mucosal phosphatase expression based on phosphate concentration in the small intestine.

In conclusion, fundamental mechanisms affecting phytate degradation in the digestive tract of broiler chickens and turkeys seem to be the same. However, there is one big difference in recommended dietary calcium and phosphorus levels and many small differences in important details affecting phytate degradation and phosphate digestibility between the two species. These require dedicated attention to further improve phosphorus efficiency in poultry production.

7 ZUSAMMENFASSUNG

Eine weltweit wachsende Bevölkerung bei gleichzeitiger Stagnation von verfügbarem Ackerland, sowie ein gestiegenes Interesse an nachhaltiger und umweltfreundlicher Lebensmittelproduktion erfordern effizientere Produktionsmethoden mit geringerer Umweltbeeinträchtigung. Das trifft auch für die Geflügelfleischproduktion zu, die in Bezug auf Futterverwertung bereits eine der effizientesten Fleischproduktionsmethoden ist. Momentan ist der Einsatz von Phosphat aus nicht nachhaltigem Bergbau als Futtermittelzusatzstoff Industriestandard. Das führt zu unerwünscht hohen Phosphorgehalten in den Exkrementen der so gefütterten Tiere, da das pflanzliche Phosphat weitgehend ungenutzt bleibt. Das kann zur unerwünschten Eutrophierung von Oberflächengewässern führen. Phytasen sind Enzyme, die Phosphatgruppen von Phytat, der Hauptspeicherform von Phosphat in Pflanzen, durch Hydrolyse abspalten können. Phytatabbau durch enzymatische Hydrolyse erhöht die Verdaulichkeit von pflanzlichem Phosphor erheblich, da Phytat nicht im Verdauungstrakt absorbiert werden kann. Die Supplementierung von mikrobieller Phytase mit dem Futter ist eine etablierte Methode um die Verdaulichkeit von pflanzlichem Phosphor zu erhöhen und so die Notwendigkeit mineralischen Phosphatsupplementen zu reduzieren. Allerdings kann noch nicht zuverlässig genug vorhergesagt werden, wie stark sich die Verdaulichkeit von pflanzlichem Phosphor durch Phytase-Supplementierung erhöht. Daher kann auf die Supplementierung von mineralischem Phosphat in der Praxis noch nicht völlig verzichtet werden.

Gegenstand dieser Doktorarbeit war es die Faktoren zu untersuchen, die den Phytatabbau im Verdauungstrakt von Geflügel beeinflussen, um die Vorhersagbarkeit der Verdaulichkeit von pflanzlichem Phosphat zu verbessern. Der Fokus lag auf Mais-basierten Futtermischungen, da sie weltweit sehr häufig eingesetzt werden und durch die geringe intrinsische Phytaseaktivität von Mais eine besondere Herausforderung darstellen. Zunächst wurde eine Literaturübersicht über den aktuellen Wissensstand zu Phytatabbau und Phosphorverdaulichkeit in mit Mais-basierten Rationen gefütterten Hühnern angefertigt. Teil dieser Arbeit war es außerdem, diese Hühner-spezifischen

Erkenntnisse mit Forschungsergebnissen für andere Geflügelarten zu vergleichen. Eine Erkenntnis war, dass es eine Vielzahl von Studien gab, die Phytatabbau und Phosphorverdaulichkeit in Masthühnern untersucht hat, aber vergleichsweise wenige Untersuchungen mit Mastputen. Zudem gab es Hinweise auf fundamentale Unterschiede zwischen Masthühnern und Mastputen. Daher sollten nun die Gründe für diese Unterschiede identifiziert und Möglichkeiten der Übertragbarkeit von Erkenntnissen zu Masthühnern auf Mastputen erörtert werden. Zwei aufeinander folgende Versuche mit Mastputen und Masthühnern wurden geplant. Die untersuchten Faktoren waren: supplementierte mikrobielle Phytase, Phosphor- und Calciumkonzentrationen im Futter, Alter und endogene mucosale Phosphataseaktivität im Jejunum. Masthühner und Mastputen wurden zeitgleich unter den gleichen Bedingungen gehalten und wurden mit den gleichen Versuchsfuttern gefüttert. Insgesamt wurden 480 Masthühner- und 480 Mastputen als Eintagsküken am selben Tag eingestallt und aufgezogen. Die Hälfte der Tiere der jeweiligen Spezies nahm zwischen dem 14. und dem 21. Lebenstag an einem Verdaulichkeitsversuch teil. Die übrigen Tiere nahmen zwischen dem 35. Und dem 42. Lebenstag an einem Verdaulichkeitsversuch teil. Dadurch sollte zusätzlich der Einfluss der körperlichen Entwicklung untersucht werden, da es sich um Tierarten mit unterschiedlicher Entwicklungsgeschwindigkeit handelt.

Im Alter von 3 Wochen war das praecaecale $InsP_6$-Verschwinden bei Masthühnern und Mastputen gleich hoch, wenn keine Phytase supplementiert wurde und die Calcium- und Phosphorkonzentration im Futter niedrig war. Gleichzeitig wurden ebenfalls keine Unterschiede in endogener mucosaler Phosphataseaktivität im Jejunum festgestellt. Im Alter von 6 Wochen war bei Mastputen sowohl das praecaecale $InsP_6$-Verschwinden, als auch die endogene mucosale Phosphataseaktivität im Jejunum höher als bei gleichalten Masthühnern, wenn keine Phytase supplementiert wurde. Die Supplementierung von Phytase führte in beiden Spezies zu einem deutlichen Anstieg im praecaecalen $InsP_6$-Verschwinden. Dieser Anstieg war in Mastühnern immer höher als in Mastputen des gleichen Alters. Außerdem führte eine Erhöhung der Calcium- und

Phosphorkonzentrationen im Futter zu einer Reduktion des praecaecalen $InsP_6$-Verschwindens in allen untersuchten Tieren. Daher wurde geschlussfolgert, dass die in der Literatur berichteten Unterschiede im praecaecalen $InsP_6$-Verschwinden zwischen Masthühnern und Mastputen vor allem auf die typischerweise höheren Calcium- und Phosphorkonzentrationen im Mastputenfutter, aber auch auf einen höheren Phytatabbau durch supplementierte Phytase im Verdauungstrakt von Masthühnern gegenüber Mastputen, zurückzuführen sind. Letzteres wurde für supplementierte Phytasen besseren Bedingungen, wie höherem Feuchtigkeitsgehalt, niedrigerem pH und möglicherweise längerer Retentionszeit im Kropf von Masthühnern im Vergleich zu Mastputen zugeschrieben. Jedoch konnten die Puten dies teilweise in darauffolgenden Abschnitten des Verdauungstrakts kompensieren.

Die endogene mucosale Phosphataseaktivität war höher in Behandlungen mit Phytasesupplementierung als in Behandlungen ohne. In diesen Behandlungen wurden erhöhte Konzentrationen von niederen Inositolphosphaten gemessen. Diese könnten eine erhöhte Expression von Phosphatasen an der Bürstensaummembran stimuliert haben. Im Gegensatz dazu wurden bei erhöhten Calcium- und Phosphorkonzentrationen im Futter niedrigere endogene mucosale Phosphataseaktivitäten im Jejunum gemessen. Der Unterschied war numerisch bei 3 Wochen alten Tieren und signifikant bei 6 Wochen alten Tieren. Das kann auf eine Regulierung der endogenen mucosalen Phosphatase Expression in Abhängigkeit von Phosphatkonzentration im Dünndarm hinweisen.

Abschließend kann geschlussfolgert werden, dass die grundlegenden Mechanismen, die auf Phytatabbau im Verdauungstrakt wirken, bei Masthühnern und Mastputen die gleichen zu sein scheinen. Allerdings gibt es zwischen den beiden Tierarten einen großen Unterschied bei der empfohlenen Calcium- und Phosphorkonzentration im Futter und viele kleine Unterschiede in wichtigen Details die Auswirkungen auf Phytatabbau und Phosphatverdaulichkeit haben. Diese erfordern besonderes Augenmerk um die Phosphoreffizienz in der Geflügelproduktion weiter zu verbessern.

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