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

2023 Poultry Science 102:102476
https://doi.org/10.1016/j.psj.2023.102476

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

Received October 12, 2022.
Accepted January 1, 2023.

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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.

	Treatments[1]			
Analyzed composition (g/kg[2])	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 $InsP_{3-6}$ 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 (**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

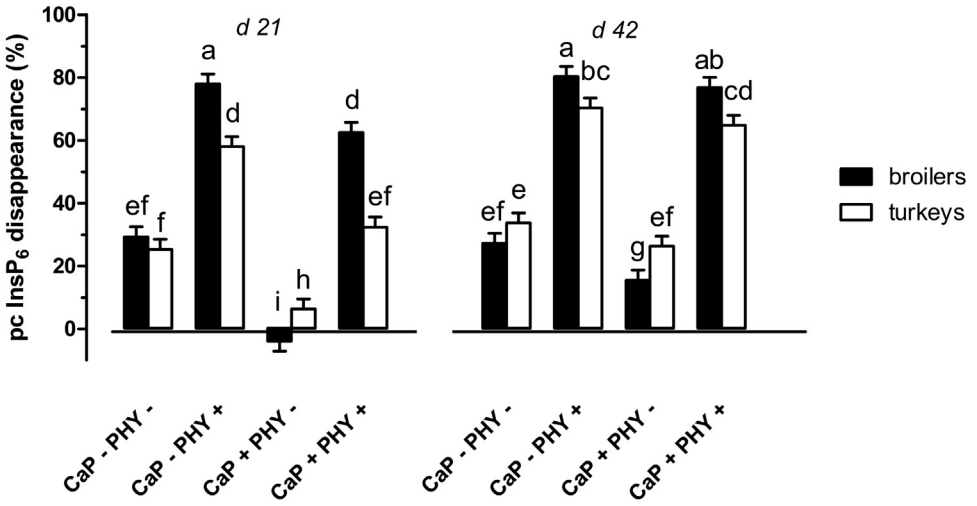

Figure 1. Effect of Ca and P level (CaP) and phytase supplementation (PHY) on prececal $InsP_6$ disappearance (LSmeans ± SEM) of broilers and turkeys fed experimental diets at 21 d (Novotny et al., 2023) and 42 d of age. Bars not sharing the same letters are significantly different according to a one-factorial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

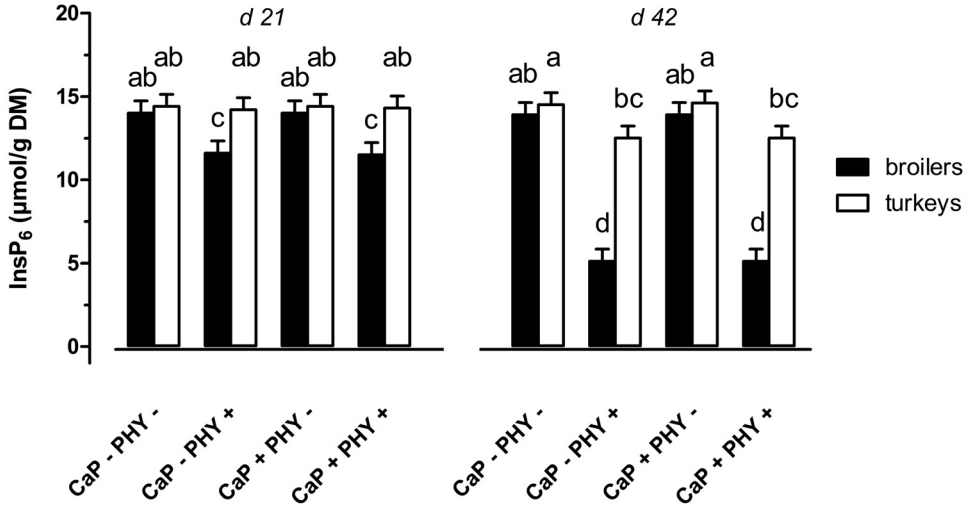

Figure 2. Effect of Ca and P level (CaP) and phytase supplementation (PHY) on $InsP_6$ concentration in the crop content (LSmeans ± SEM) of broilers and turkeys fed experimental diets at 21 d (Novotny et al., 2023) and 42 d of age. Bars not sharing the same letters are significantly different according 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.

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

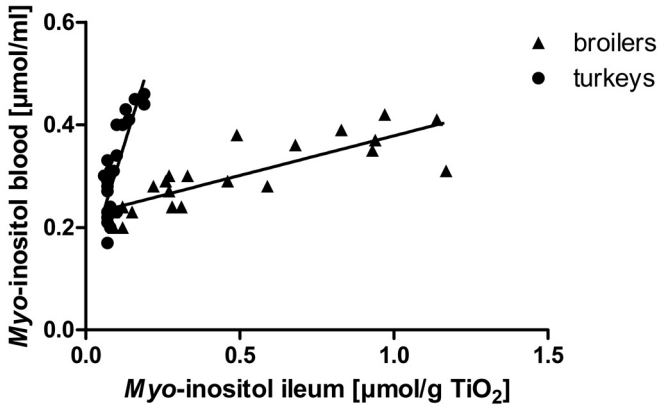

Figure 3. *Myo*-inositol concentrations in ileum digesta (per g TiO_2) and blood of broilers and turkeys fed the experimental diets at 42 d of age. Dots represent individual pen values for the ileum data and individual bird values for the blood data. Linear regression broilers: y = 0.15 x + 0.22, r^2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

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.

ACKNOWLEDGMENTS

This work was partly funded by Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) − 328017493/GRK 2366 (Sino-German International Research Training Group AMAIZE-P).

DISCLOSURES

All authors declare that they have no conflict of interest.

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