

# Phytate degradation and phosphorus digestibility in broilers and turkeys fed different corn sources with or without added phytase

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**ABSTRACT** The aim of the present study was to test whether different dietary corn sources and phytase supplementation affect the prececal phosphorus digestibility (pcdP) and appearance of inositol phosphates in the lower ileum of growing broiler chickens and turkeys. Two experiments were conducted, one with broiler chickens and one with turkeys. Four corn diets were provided; these were formulated to contain low P and calcium (Ca) contents and incorporated 43% of one of the four different corn sources. Diets were either unsupplemented or supplemented with 500 FTU of an *Escherichia coli*-derived phytase/kg feed. Experimental diets were fed ad libitum from day 20 post-hatch. At 28 d of age, digesta were sampled from the lower ileum of animals to determine pcdP and pc *myo*-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate) (InsP<sub>6</sub>) degradation and to analyze the concentrations of lower inositol phosphate isomers. The pcdP of non-supplemented diets ranged from 51 to 60% and

from 22 to 28% in broilers and turkeys, respectively. A negative correlation was observed between the InsP<sub>6</sub> content of the corn source and the pcdP of diets in broilers only. Without phytase supplementation, pc InsP<sub>6</sub> degradation ranged from 64 to 76% in broilers and from 6 to 15% in turkeys. Phytase increased the pcdP by around 15% in broilers ( $P < 0.001$ ) and 9 to 17% in turkeys ( $P < 0.001$ ). In turkeys, phytase efficacy was greatest when the diets contained corn with higher contents of ether extract and InsP<sub>6</sub>. An effect of corn source on the appearance of lower InsPs in the ileal digesta was found in broilers only. These results suggest that broilers possess a greater capacity for InsP<sub>6</sub> degradation and hydrolysis of lower InsPs compared with turkeys. Furthermore, the results are influenced by the corn source used. Further research is needed to identify the factors responsible for the low level of phytate degradation in turkeys in order to improve the availability of InsP<sub>6</sub>-P and the efficacy of phytase.

**Key words:** prececal digestibility, phosphorus, phytase, corn, inositol phosphates

2019 Poultry Science 98:912–922  
<http://dx.doi.org/10.3382/ps/pey438>

## INTRODUCTION

Phosphorus (P) is an element with special relevance in poultry feeding. Corn, which represents the most important cereal in poultry feed, contains relatively low concentrations of P compared to other grains (Rodehutschord et al., 2016). However, due to its high proportion in poultry diets, it can contribute substantially to dietary P content. Most of the P in corn grain is stored in the germ, primarily as phytate (O'Dell,

1972), which means any salt of phytic acid (*myo*-inositol 1,2,3,4,5,6-hexakis [dihydrogen phosphate], InsP<sub>6</sub>). In non-ruminants, the digestibility of phytate P is limited due to the lack of sufficient endogenous phytase in the gastrointestinal tract (GIT).

The intrinsic phytase activity of corn is very low (0 to 190 FTU/kg DM) (Eeckhout and De Paepe, 1994; Rodehutschord et al., 2016). Nevertheless, there is considerable prececal (pc) InsP<sub>6</sub> degradation, often more than 50% in broilers fed low-P diets mainly based on corn (Zeller et al., 2015a,b; Leytem et al., 2008; Amerah et al., 2014; Shastak et al., 2014). This indicates a high potential of endogenous mucosal or microbial phytases to hydrolyze InsP<sub>6</sub> to less phosphorylated InsPs in the upper GIT of broilers (Zeller et al., 2015a). In turkeys, the utilization of plant P from diets based on corn is much lower than that in broilers (Rodehutschord and Dieckmann, 2005; Adebiyi and Olukosi, 2015), thus indicating differences in the ability of broilers and turkeys to hydrolyze InsP<sub>6</sub>. Nevertheless, negative effects of

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Received November 25, 2017.

Accepted August 29, 2018.

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high phytate P contents in corn have been reported on P availability in both broilers and turkeys (Kasim and Edwards, 2000; Yan et al., 2003). High phytate P contents in corn negatively affected the retention of P from corn in broilers (Kasim and Edwards, 2000), and a lower tibia ash concentration was noted in turkeys fed a conventional corn variety compared with a mutation containing less phytate and more non-phytate P (Yan et al., 2003).

Supplementation with exogenous microbial phytase can increase phytate degradation and P digestibility of diets based on corn in broilers as well as in turkeys (Lescoat et al., 2005; Kozłowski et al., 2010; Woyengo and Nyachoti, 2011; Dersjant-Li et al., 2015; Zeller et al., 2015a). Changes in the hydrolysis of InsP<sub>6</sub> from phytase-supplemented corn diets have been reflected in the pattern of InsPs in the lower ileum of broiler chickens (Zeller et al., 2015a). However, to the best of our knowledge, there are no studies on the effect of phytase supplementation on pc InsP<sub>6</sub> hydrolysis and the appearance of lower InsPs at the terminal ileum of turkeys.

The concentrations of total P and InsP<sub>6</sub>-P in corn can differ considerably between corn sources, ranging from 2.5 to 4.0 g/kg DM and from 2.2 to 3.5 g/kg DM, respectively (Eeckhout and De Paepe, 1994; Rodehutsord et al., 2016). However, studies systemically investigating the effect of the corn source on P digestibility in broilers and turkeys are rare. Hence, the first objective of the present study was to test whether diets based on corn sources differing in total and InsP<sub>6</sub>-P contents differ in pc InsP<sub>6</sub> hydrolysis and pc digestibility of P (pcdP). The second objective was to investigate the effect of phytase supplementation on the pcdP and pc InsP<sub>6</sub> hydrolysis from such diets and to analyze the appearance of lower InsPs at the terminal ileum of broilers and turkeys. As the literature indicates differences between species, we addressed these objectives in 2 separate experiments using broilers and turkeys.

## MATERIALS AND METHODS

The 4 corn sources used in this study represented genotypes no. 1, 2, 4, and 6 as denoted and characterized by Rodehutsord et al. (2016) and the supplementary tables of this publication. These corn genotypes differed in their contents of total P (2.8 to 4.0 g/kg DM), InsP<sub>6</sub> (6.7 to 11.0 g/kg DM), and ether extract (EE, 44 to 123 g/kg DM), and all exhibited very low phytase activity ( $\leq 190$  U/kg DM; Table 1).

### Experimental Diets

Both experiments involved a 2-factorial arrangement of treatments with 8 diets, 4 corn diets (CD1, CD2, CD4, CD6), each supplemented with phytase or non-supplemented. The diets were formulated to contain adequate levels of all nutrients accord-

**Table 1.** Concentration of total P, InsP<sub>6</sub>-P, ether extract (g/kg DM), and phytase activity (U/kg DM) in the corn sources used in the present work.

Corn source <sup>1</sup>	Total P	InsP <sub>6</sub> -P	InsP <sub>6</sub>	Ether extract	Phytase activity <sup>2</sup>
4	2.84	1.90	6.79	44	n.d.
1	2.83	1.94	6.93	69	150
6	3.81	2.01	7.18	101	n.d.
2	4.00	3.09	11.04	123	190

<sup>1</sup>Corn sources represent corn genotypes no. 4, 1, 6 and 2 used in the "GrainUp" project and described in detail in the supplementary tables of Rodehutsord et al. (2016).

<sup>2</sup>Determined at pH 5 and 45°C according to Greiner and Egli (2003). n.d. = not detectable.

ing to the recommendations of the Gesellschaft für Ernährungsphysiologie (GfE, 1999, 2004) for broilers and turkeys (Table 2), with the exception of Ca and P (Table 3). The diets used for broiler chickens were based on corn and potato protein, while those used for turkeys contained mainly corn and soybean meal (SBM). To formulate the 4 different diets, 434 g of corn sources no. 1, 2, 4, or 6 were incorporated, making the corn source the only ingredient causing variation in P content in these diets (Table 3). The corn was ground to pass through a 3-mm sieve screen using a hammer mill

**Table 2.** Composition of corn diets fed to broilers and turkeys between 20 and 28 d of age in experiments 1 and 2, respectively (g/kg of feed).<sup>1</sup>

	Experiment 1 (broiler)	Experiment 2 (turkey)
Ingredient		
Corn source	434	434
Corn	330	100.9
Soybean meal	12.5	341
Potato protein	180	69
Soybean oil	15	15
L-Lysine HCl	–	3.6
D,L-Methionine	0.7	4.9
L-Arginine	1.5	1.7
Limestone	12.8	12.9
Vitamin premix <sup>2</sup>	1.5	–
Mineral premix <sup>3</sup>	1	–
Premix turkey <sup>4</sup>	–	6
Sodium chloride	1	1
Choline chloride	2	2
Sodium bicarbonate	3	3
Titanium dioxide	5	5

<sup>1</sup>Corn diets supplemented with 43.4% of one of four corn sources. Diets remained non-supplemented or supplemented with phytase.

<sup>2</sup>Vitamin premix (Raiffeisenkraftfutterwerke Süd GmbH, Würzburg, Germany) provided per kilogram of complete diet: vitamin A, 9000 IU; vitamin D<sub>3</sub>, 2250 IU; vitamin E, 22.5 mg; vitamin B<sub>1</sub>, 2.25 mg; vitamin B<sub>2</sub>, 4.5 mg; vitamin B<sub>6</sub>, 4.5 mg; vitamin B<sub>12</sub>, 22.5 mg; vitamin K<sub>3</sub>, 1.8 mg; nicotinic acid, 37.5 mg; pantothenic acid, 10.5 mg; biotin, 75 µg; folic acid, 0.75 mg.

<sup>3</sup>Mineral premix (Gelamin SG 1, GFT mbH, Memmingen, Germany) provided per kilogram of complete diet: Mn, 120 mg; Fe, 90 mg; Zn, 80 mg; Cu, 15 mg; I, 1.6 mg; Co, 0.6 mg; Se, 0.5 mg.

<sup>4</sup>Premix turkey (BASU Mineralfutter GmbH, Bad Sulza, Germany) provided per kilogram of complete diet: Ca, 159.7 mg; P, 48.9 mg; Na, 1.8 mg; Mg, 16.2 mg; Cl, 4.8 mg; Fe, 80.0 mg; Mn, 128.0 mg; Zn, 96.0 mg; Cu, 16.0 mg; I, 1.60 mg; Se, 0.56 mg; Co, 0.40 mg; vitamin A, 19,200 IU; vitamin D, 6400 IU; vitamin E, 64.0 mg; vitamin K, 3.8 mg; vitamin B<sub>1</sub>, 3.4 mg; vitamin B<sub>2</sub>, 11.5 mg; vitamin B<sub>6</sub>, 6.4 mg; vitamin B<sub>12</sub>, 36 µg; nicotinic acid, 105.6 mg; pantothenic acid, 21.6 mg; folic acid, 2.4 mg; biotin, 0.28 mg; lysine, 28.8 mg; methionine, 11.4 mg; threonine, 24.6 mg; tryptophan, 10.2 mg; antioxidant, 120 mg.

**Table 3.** Analyzed phytase activity (FTU/kg DM), concentrations of Ti, Ca, total P, InsP<sub>6</sub>-P (g/kg DM), and lower inositol phosphate isomers ( $\mu\text{mol/g DM}$ ) in diets fed to broilers and turkeys in experiment 1 and 2, respectively.<sup>1</sup>

	CD4		CD1		CD6		CD2	
	0	500	0	500	0	500	0	500
<b>Experiment 1 (broiler)</b>								
Phytase activity <sup>2</sup>	<50	438	<50	407	<50	502	<50	410
Ti	3.10	3.13	3.14	3.15	3.12	3.21	3.09	3.11
Ca	4.99	5.04	5.13	5.15	5.47	5.43	5.64	5.55
Total P	3.20	3.28	3.30	3.24	3.61	3.64	3.75	3.79
InsP <sub>6</sub> -P	1.83	1.78	1.82	1.82	2.19	2.22	2.35	2.33
InsP <sub>6</sub>	9.8	9.6	9.8	9.8	11.8	11.9	12.6	12.5
Ins(1,2,4,5,6)P <sub>5</sub>	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Ins(1,2,3,4,5)P <sub>5</sub>	0.2	0.2	0.1	<LOQ	0.1	0.2	0.1	0.1
Ins(1,2,3,4,6)P <sub>5</sub>	<LOQ <sup>3</sup>	n.d. <sup>4</sup>	n.d.	n.d.	<LOQ	n.d.	n.d.	<LOQ
Ins(1,5,6)P <sub>3</sub>	n.d.	<LOQ	<LOQ	n.d.	<LOQ	<LOQ	<LOQ	<LOQ
<b>Experiment 2 (turkey)</b>								
Phytase activity	~85	476	~65	519	<50	475	~65	469
Ti	3.35	3.13	3.46	3.40	3.33	3.34	3.38	3.32
Ca	7.15	7.05	7.34	7.27	7.16	7.17	7.26	7.17
Total P	4.22	4.14	4.22	4.21	4.62	4.56	4.75	4.65
InsP <sub>6</sub> -P	2.38	2.38	2.38	2.34	2.64	2.68	2.64	2.73
InsP <sub>6</sub>	12.8	12.8	12.8	12.6	14.2	14.4	14.2	14.7
Ins(1,2,4,5,6)P <sub>5</sub>	0.8	0.8	0.7	0.8	0.9	0.8	0.8	0.8
Ins(1,2,3,4,5)P <sub>5</sub>	0.4	0.4	0.4	0.3	0.4	0.4	0.4	0.4
Ins(1,2,3,4,6)P <sub>5</sub>	<LOQ	<LOQ	<LOQ	n.d.	<LOQ	<LOQ	n.d.	<LOQ

<sup>1</sup>CD4, CD1, CD6, CD2 = diets supplemented with corn sources no. 4, 1, 6, or 2 as used in the "GrainUp" project (Rodehutschord et al., 2016); diets remained non-supplemented (0) or supplemented with phytase (500).

<sup>2</sup>Determined at pH 4.5 and 60°C.

<sup>3</sup>LOQ (limit of quantification) = not quantifiable in the majority of feed samples.

<sup>4</sup>n.d. = not detected in the majority of feed samples.

(SKIOLD A/S, Sæby, Denmark) before being added to the diets. To maintain a constant Ca:P ratio of 1.6:1 in all diets, limestone was added. Titanium dioxide was used as an indigestible marker (0.5%). All diets were fed to animals with or without supplementation of an *Escherichia coli*-derived, thermotolerant 6-phytase (Phy, Quantum Blue, intended activity 500 FTU/kg feed), provided by AB Vista, Marlborough, UK.

Diets were mixed in the certified feed mill facilities of the Hohenheim University's Agricultural Experiment Station, location Lindenhöfe, Eningen, Germany, and pelleted through a 3-mm die without the use of steam. The pellet temperature was immediately measured after pelleting, and ranged between 45 and 69°C in experiment 1 (broiler) and between 40 and 80°C in experiment 2 (turkey). Representative samples of the experimental diets were taken and pulverized using a laboratory disc mill (Siebtechnik GmbH, Mühlheim an der Ruhr, Germany) before being stored at 4°C until chemical analysis.

The analyzed activity of phytase was very low in non-supplemented diets, and ranged from 407 to 502 and 469 to 519 FTU/kg DM in phytase-supplemented broiler and turkey diets, respectively. The concentrations of total P varied between 3.20 and 3.79 g/kg DM in broiler diets, and between 4.14 and 4.75 g/kg DM in turkey diets, while the InsP<sub>6</sub>-P in broiler and turkey diets ranged from 1.78 to 2.35 g/kg DM, and 2.34 to 2.73 g/kg DM, respectively (Table 3). The average (SD)

Ca:P ratio was 1.5 (0.04) in broiler diets and 1.6 (0.09) in turkey diets.

### **Birds, Animal Management, and Sampling Procedure**

The 2 experiments were carried out at the Agricultural Experiment Station of Hohenheim University, location Lindenhöfe in Eningen (Germany). The broiler experiment was approved in accordance with German Animal Welfare legislation by the Animal Welfare Commissioner of the University (approval number T97/12 TE) and the turkey experiment by the Regierungspräsidium Tübingen (approval number HOH 28/13).

For experiment 1, 720 unsexed broiler hatchlings (Ross 308) were obtained from a commercial hatchery (Brütereie Süd, Regenstauf, Germany) and randomly allocated to 48 floor pens (124 × 115 cm), with 15 birds per pen. Birds were raised on chopped straw before being placed on plastic slats at 10 d of age. Birds were fed a pelleted, commercial starter diet (Korngold BMK XS PELL, Raiffeisen Kraftfutterwerke Süd GmbH, Würzburg, Germany) containing 22% CP, 12.6 MJ ME/kg, 0.9% Ca, 0.65% P, and 600 FTU 3-phytase/kg feed. Birds underwent routine vaccination against coccidiosis (via starter diets) and Newcastle disease on day 12.

The room temperature in the animal house was set at 34°C on days 1 and 2 before being gradually reduced

to 21°C until day 20. Artificial lighting was provided at an intensity of 10 lux. During the first 2 d, light was provided for 24 h; thereafter, provision of light was reduced to 18 h per day.

On day 20 post-hatch, broiler chickens were weighed on a pen basis and randomly assigned to one of the 8 dietary treatments. Each treatment was assigned to 6 pens ( $n = 6$ ) according to a non-randomized complete block design. Throughout the experiment, animals had free access to feed and tap water. The experimental diets were fed to the animals for 8 d. The ADG and ADFI were recorded on a pen basis. On day 28, birds were euthanized via CO<sub>2</sub> asphyxiation. The abdominal cavity of animals was opened immediately, the digestive tract removed, and the ileum (section between Meckel's diverticulum and 2 cm anterior to the ileo-ceco-colonic junction) was dissected. The digesta of the distal half of the ileum was gently flushed out with double-distilled water (4°C) and pooled for all birds on a pen basis. Samples were immediately frozen at -18°C and freeze-dried (Type Delta 1-24, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany). Digesta samples were pulverized by using a laboratory disc mill (Siebtechnik GmbH, Mühlheim an der Ruhr, Germany) while samples from turkeys in experiment 2 were ground to pass through a 0.12-mm sieve screen at a speed of 6,000 rpm using an ultracentrifugal mill (Type: ZM 200, Retsch GmbH, Haan, Germany). Thereafter, samples were stored at 4°C until chemical analyses.

For experiment 2, 720 unsexed turkey hatchlings (B.U.T. Big 6) were obtained from a local hatchery (Gebrüder Böcker Putenbrüterei GmbH, Wallhausen, Germany) and randomly allocated to 48 floor pens (154 × 115 cm), with 15 birds per pen. Birds were raised on pine shavings until 10 d of age before being placed on plastic slats. Birds were fed a commercial, pelleted starter diet (Deutsche Tiernahrung Cremer GmbH & Co. KG, Mannheim, Germany) containing 1.25% Ca, 0.90% P, 26.0% CP, 11.6 MJ ME/kg, 750 FTU 6-phytase/kg, and 10 IU endo-1,4-β-xylanase/kg feed. During the first days of life, an additional starter feed containing 1.30% Ca, 0.90% P, 27.5% CP, 11.5 MJME/kg, 750 FTU 6-phytase/kg, and 10 IU endo-1,4-β-xylanase/kg was additionally offered in crumbled form. Birds underwent routine vaccination against coccidiosis (via starter diets) and Newcastle disease on day 12.

The room temperature in the animal house was set at 36°C on days 1 and 2 before being gradually reduced to 21°C until day 20. Artificial lightening was provided at an intensity of 100 lux. During the first 2 d light was provided for 24 h, thereafter the provision of light was reduced to 16 h per day.

In accordance with experiment 1, animals were weighed on day 20 post-hatch by pen, and randomly assigned to one of the 8 dietary treatments. Treatments were assigned to 6 pens ( $n = 6$ ) per treatment, and were arranged in a non-resolvable block design, with 8 incomplete blocks each with 6 treatments. The following

procedures regarding animal handling and sampling of ileal digesta were as described for broilers in experiment 1, with the exception that animals were first stunned with a mixture of 35% CO<sub>2</sub>, 35% N<sub>2</sub>, and 30% O<sub>2</sub>, before being euthanized with CO<sub>2</sub> asphyxiation.

## Chemical Analyses

The DM content of feed and digesta samples was analyzed according to the official German methods (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten [VDLUF 1976]; Method 3.1). Concentrations of Ca, P, and Ti in feed and digesta samples were analyzed using an inductively coupled plasma optical emission spectrometer following sulfuric and nitric acid wet digestion, with the specifications described by Zeller et al. (2015b). Concentrations of InsP<sub>6</sub> and lower InsPs in the diets and digesta samples were analyzed following EDTA extraction at pH 10 using high-performance ion chromatography as described by Zeller et al. (2015a). Using this methodology, separation of enantiomers is not possible. Hence, the presentation of results does not distinguish between D- and L-form. Feed samples were analyzed for phytase activity by Enzyme Services and Consultancy (Ystrad Mynach, UK) using the validated analytical methods of the producer. Phytase activity was determined at pH 4.5 and 60°C by analyzing P release from phytate, after which the result was converted to the commonly used FTU using a validated transfer factor.

## Calculations and Statistics

The ADG, ADFI, and feed-to-gain ratio were determined on a pen basis and adjusted for mortality, which was recorded daily. The pcdP, pcdCa, and pc disappearance of InsP<sub>6</sub> ( $y$ ) were calculated on a pen basis according to the following equation:

$$y (\%) = 100 - 100 \times \left( \frac{\text{Ti in the diet (g/kg DM)}}{\text{Ti in the digesta (g/kg DM)}} \right) \times \left( \frac{\text{InsP}_6 \text{ or P or Ca in the digesta (g/kg DM)}}{\text{InsP}_6 \text{ or P or Ca in the diet (g/kg DM)}} \right) \quad (1)$$

Statistical analyses were performed using the software package SAS for Windows (Version 9.3, SAS Institute, Cary, NC, USA). A mixed models approach (procedure PROC MIXED) was used for both experiments, considering the effects of treatment factors "diet" (CD1, CD2, CD4, or CD4), "phytase" (0 or 500), and their interactions, as fixed and "block" effects as random. The model was as follows:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + b_k + e_{ijk}, \quad (2)$$

where  $y_{ijk}$  =  $k$ th observation of the  $i$ th diet and  $j$ th inclusion level of enzyme,  $\mu$  = general effect,  $\alpha_i$  = effect of the  $i$ th diet,  $\beta_j$  = effect of the  $j$ th inclusion level of phytase,  $(\alpha\beta)_{ij}$  = interaction effect between the  $i$ th diet and  $j$ th inclusion level of phytase,  $b_k$  = effect of the  $k$ th block, and  $e_{ijk}$  = error term associated with  $y_{ijk}$ . The assumptions of normality and variance homogeneity of residuals were checked graphically, and if necessary, subjected to arcsine square-root, square-root or log transformation. Least-square means from the analysis were back-transformed for presentation only. In this case, standard errors were back-transformed using the Delta method. Furthermore, the pooled SEM was calculated as the square root of the average variance of least-square means. If the F-test was significant, the multiple t-test was used for treatment comparison. Its results were presented via letter display. The level of significance was set at  $\alpha = 0.05$ . Additionally, Pearson correlations were calculated by correlation observations of the corresponding traits.

## RESULTS

### Experiment 1

Broiler growth and feed:gain was affected by diet and phytase addition ( $P < 0.01$ ), and the best results were achieved for birds fed CD1 and those fed phytase-supplemented diets (Table 4). The pcdP was also affected by diet and phytase ( $P < 0.001$ ). The pcdP for CD4 and CD1 was around 60%, while that for CD6 and CD2 was at around 52%. The addition of phytase increased the pcdP by around 15%, from 56 to 71%, irrespective of diet. There was no difference in the concentration of pcdP (g/kg DM) between diets, which increased with phytase addition by around 0.55 ( $\pm 0.07$ ) g/kg DM for all diets ( $P < 0.001$ ).

For the pcdCa, an interaction between phytase and diet was detected ( $P = 0.002$ ). While both non-supplemented and phytase-supplemented CD4 and CD1 showed similar pcdCa values at around 69%, phytase addition decreased the pcdCa values compared to non-supplemented CD6 and CD2 by 9 and 11%, respectively.

The effect of diet on the pc InsP<sub>6</sub> disappearance was consistent with the pcdP data, with higher disappearance found for CD4 and CD1 (~85%) than for CD6 and CD2 (~76%) ( $P < 0.001$ ) (Table 5). The addition of phytase increased the pc InsP<sub>6</sub> disappearance by around 20%, from an average of 70% to an average of 90% ( $P < 0.001$ ).

The concentration of Ins(1,2,4,5,6)P<sub>5</sub> in the ileal digesta was significantly affected by the interaction between phytase and diet ( $P = 0.017$ ). While in CD1, the concentration of Ins(1,2,4,5,6)P<sub>5</sub> seemed to decrease with phytase, the opposite was seen in CD2, and no differences were found between supplemented and non-

supplemented diets for CD4 and CD6. Similar interaction effects between phytase and diet were observed for the concentration of Ins(1,2,3,4,5)P<sub>5</sub> ( $P = 0.011$ ). The isomer Ins(1,2,3,4,6)P<sub>5</sub> was only detected when diets were not supplemented with phytase. Its concentration in the ileal digesta of broilers fed CD4 and CD1 was lower than in those fed CD6 and CD2 (0.14 vs. 0.26  $\mu\text{mol/g DM}$ ) ( $P < 0.001$ ).

The InsP<sub>4</sub> isomers Ins(1,2,5,6)P<sub>4</sub> and Ins(1,2,3,4)P<sub>4</sub> were only detected in phytase-supplemented CD6 and CD2. The concentration of the isomer Ins(1,5,6)P<sub>3</sub> was affected by diet ( $P = 0.038$ ), but not by phytase.

### Experiment 2

The effect of diet on turkey performance was restricted to the feed-to-gain ratio that was lower ( $P = 0.008$ ) in turkeys fed CD2 than the other diets (Table 4). Supplementation of phytase increased the ADG of animals from 56 to 59 g/d ( $P = 0.017$ ), while the effect on BW ( $P = 0.064$ ), ADFI ( $P = 0.076$ ), and feed-to-gain ratio ( $P = 0.063$ ) was slightly above the significance level. There was no interaction effect between phytase and diet on turkey performance or pcdCa. The pcdCa was not affected by diet, but increased by phytase ( $P < 0.001$ ).

The pcdP values showed a significant interaction between diet and phytase ( $P = 0.016$ ). Among the non-supplemented diets, CD4 was the one with the highest pcdP. Among the phytase-supplemented diets, the highest value was observed with CD2 and the pcdP was increased by phytase to a greater extent in CD1, CD6, and CD2 (~16%) than in CD4 (~9%). Similarly, the amount of pcdP in turkeys fed CD4 increased by only 0.3 g/kg DM upon phytase supplementation, while for the other diets an increase of  $\geq 0.68$  g/kg DM was found.

Consistent with the results for the pcdP, InsP<sub>6</sub> disappearance was affected by an interaction of diet and phytase ( $P = 0.012$ ) (Table 6). The greatest pc InsP<sub>6</sub> disappearance among non-supplemented diets was observed for turkeys fed CD4 (15 vs. ~5.8% for CD1, CD6, and CD2). However, upon phytase supplementation, the pc InsP<sub>6</sub> disappearance ranged from 32 to 38%, and was not significantly different between diets. Thus, the phytase-induced increase in the pc disappearance of InsP<sub>6</sub> was lower for CD4 (17%) than for the other diets (~29%). The concentrations of InsP<sub>6</sub> in ileal digesta samples of turkeys was not affected by an interaction between phytase and diet, but its concentration decreased with phytase supplementation from 37 to 27  $\mu\text{mol/g DM}$  ( $P < 0.001$ ). Moreover, InsP<sub>6</sub> concentrations were lower ( $P = 0.011$ ) in digesta samples of turkeys fed CD1 (30  $\mu\text{mol/g DM}$ ) compared with those fed CD6 and CD2 (34  $\mu\text{mol/g DM}$ ).

The concentration of the InsP<sub>5</sub> isomer Ins(1,2,4,5,6)P<sub>5</sub> in digesta samples was neither affected by diet nor by phytase and was on average

**Table 4.** BW at 28 d of age, ADG, ADFI, feed:gain ratio, pcdP, and pcCa of broilers and turkeys from 20 to 28 d of age.<sup>1</sup>

	CD4		CD1		CD6		CD2		Pooled SEM	P value <sup>2</sup>		
	0	500	0	500	0	500	0	500		Diet	Phy <sup>3</sup>	Diet × Phy
<b>Experiment 1 (broiler)</b>												
BW (g)	1,319	1,360	1,371	1,386	1,289	1,351	1,313	1,350	20.7	0.009	0.003	0.545
ADG (g/d)	58	65	64	69	53	61	57	62	2.1	<0.001	<0.001	0.431
ADFI (g/d)	114	120	119	121	115	120	118	122	2.6	0.442	0.006	0.820
Feed:gain (g/g)	1.97	1.84	1.85	1.76	2.16	1.96	2.05	1.97	0.040	<0.001	<0.001	0.307
pcdP (%)	59.8	74.7	59.0	74.2	52.5	69.3	51.3	65.9	1.80	<0.001	<0.001	0.926
pcdP (g/kg DM)	1.91	2.45	1.95	2.40	1.90	2.52	1.92	2.49	0.057	0.919	<0.001	0.531
pcdCa (%) <sup>4</sup>	70.0 <sup>a</sup>	67.4 <sup>a,b</sup>	68.2 <sup>a,b</sup>	69.3 <sup>a</sup>	56.2 <sup>d</sup>	62.9 <sup>c</sup>	65.2 <sup>b,c</sup>	52.6 <sup>d</sup>	1.50	<0.001	<0.001	0.002
<b>Experiment 2 (turkeys)</b>												
BW (g)	1,121	1,118	1,109	1,116	1,091	1,140	1,129	1,167	17.2	0.162	0.064	0.375
ADG (g/d)	55.5	57.4	55.5	57.1	55.0	58.9	57.9	62.2	1.64	0.098	0.017	0.800
ADFI (g/d)	84.8	85.3	84.4	85.1	82.5	87.7	84.2	86.1	1.63	0.997	0.076	0.457
Feed:gain (g/g)	1.53	1.49	1.52	1.49	1.50	1.49	1.45	1.39	0.028	0.008	0.063	0.793
pcdP (%) <sup>4</sup>	28.2 <sup>c</sup>	36.7 <sup>b</sup>	21.9 <sup>d</sup>	38.5 <sup>a,b</sup>	23.3 <sup>d</sup>	38.4 <sup>a,b</sup>	24.4 <sup>c,d</sup>	41.7 <sup>a</sup>	1.46	0.173	<0.001	0.016
pcdP (g/kg DM) <sup>4</sup>	1.19 <sup>d</sup>	1.52 <sup>c</sup>	0.92 <sup>e</sup>	1.62 <sup>b,c</sup>	1.07 <sup>d,e</sup>	1.75 <sup>a,b</sup>	1.16 <sup>d</sup>	1.94 <sup>a</sup>	0.065	0.002	<0.001	0.008
pcdCa (%)	37.1	37.3	33.9	38.7	29.5	38.8	30.3	41.8	2.19	0.510	<0.001	0.081

<sup>1</sup>CD4, CD1, CD6, CD2 = corn diets supplemented with corn sources no. 4, 1, 6, or 2 as used in the “GrainUp” project (Rodehutschord et al., 2016); diets remained non-supplemented (0) or were supplemented with phytase (500). Data are given as LS means or back-transformed LS means; n = 6 pens per treatment with 15 birds per pen.

<sup>2</sup>P value of an F test.

<sup>3</sup>Phy = phytase; pcdP = prececal digestibility of phosphorus; pcdCa = prececal digestibility of calcium.

<sup>4</sup>Estimates within a row not sharing a common superscript differ significantly (multiple *t*-tests in case of interactions between Phy and Diet), *P* ≤ 0.05.

**Table 5.** Concentrations of different inositol phosphates (μmol/g DM), pc InsP<sub>6</sub> disappearance (%), and the amount of pc disappeared InsP<sub>6</sub> (g/kg DM) in the digesta of the lower ileum of broiler chickens (experiment 1)<sup>1</sup>

	CD4		CD1		CD6		CD2		Pooled SEM	P value <sup>2</sup>		
	0	500	0	500	0	500	0	500		Diet	Phy <sup>3</sup>	Diet × Phy
InsP <sub>3x</sub> <sup>4</sup>	n.d. <sup>5</sup>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.12	0.040	-	-	-
Ins(1,5,6)P <sub>3</sub>	0.17	0.18	0.22	0.22	0.20	0.19	0.18	0.24	0.019	0.038	0.207	0.210
Ins(1,2,3,4)P <sub>4</sub>	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.18	0.047	-	-	-
Ins(1,2,5,6)P <sub>4</sub>	n.d.	n.d.	n.d.	n.d.	n.d.	0.30	n.d.	0.58	0.173	-	-	-
Ins(1,2,3,4,6)P <sub>5</sub>	0.16	n.d.	0.13	n.d.	0.25	n.d.	0.27	n.d.	0.025	<0.001	-	-
Ins(1,2,3,4,5)P <sub>5</sub> <sup>6</sup>	0.25 <sup>c-e</sup>	0.16 <sup>e,f</sup>	0.20 <sup>d,f</sup>	0.12 <sup>f</sup>	0.35 <sup>b-d</sup>	0.61 <sup>a,b</sup>	0.40 <sup>b,c</sup>	0.94 <sup>a</sup>	0.115	<0.001	0.491	0.011
Ins(1,2,4,5,6)P <sub>5</sub> <sup>6</sup>	0.06 <sup>d</sup>	0.05 <sup>d</sup>	0.06 <sup>c,d</sup>	n.d.	0.13 <sup>b,c</sup>	0.18 <sup>a,b</sup>	0.09 <sup>c,d</sup>	0.28 <sup>a</sup>	0.028	<0.001	0.008	0.017
InsP <sub>6</sub>	9.75	2.50	8.63	2.34	14.23	4.83	16.08	7.05	1.411	<0.001	<0.001	0.234
pc InsP <sub>6</sub> disappearance	74.8	93.1	76.3	93.3	65.5	88.4	63.8	83.8	2.34	<0.001	<0.001	0.886
pc InsP <sub>6</sub> disappeared	4.84	5.87	4.90	6.00	5.08	6.94	5.32	6.88	0.179	<0.001	<0.001	0.083

<sup>1</sup>CD4, CD1, CD6, CD2 = corn diets supplemented with corn sources no. 4, 1, 6, or 2 as used in the “GrainUp” project (Rodehutschord et al., 2016); diets remained non-supplemented (0) or supplemented with phytase (500). Data are given as LS means or back-transformed LS means; n = 6 pens per treatment with 15 birds per pen.

<sup>2</sup>P value of an F test.

<sup>3</sup>Phy = phytase; pc = prececal.

<sup>4</sup>At least one out of the following InsP<sub>3</sub> isomers: Ins(1,2,6)P<sub>3</sub>, Ins(1,4,5)P<sub>3</sub>, Ins(2,4,5)P<sub>3</sub>.

<sup>5</sup>n.d. = not detected in the majority of digesta samples.

<sup>6</sup>Estimates within a row not sharing a common superscript differ significantly (multiple *t*-tests in case of interactions between Phy and Diet), *P* ≤ 0.05.

1.1 (SD 0.07) μmol/g DM (Table 6). In contrast, the concentration of Ins(1,2,3,4,5)P<sub>5</sub> was increased by phytase (*P* < 0.001) from 1.3 to 3.2 μmol/g DM, but was not affected by diet. An opposite effect of phytase supplementation was observed for Ins(1,2,3,4,6)P<sub>5</sub> the concentration of which was decreased from 0.5 to 0.2 μmol/g DM (*P* < 0.001). The only InsP<sub>4</sub> isomer that was detectable in turkey ileal digesta when phytase was supplemented was Ins(1,2,5,6)P<sub>4</sub>. No InsP<sub>3</sub> isomer was detected in the ileal digesta.

## DISCUSSION

### Degradation of InsP<sub>6</sub> and Prececal Digestibility of P in Response to Corn Source and Phytase Supplementation in Broilers

The high levels of pc InsP<sub>6</sub> disappearance and pcdP from diets without phytase supplementation in broilers in the present study are consistent with previous findings (Zeller et al., 2015a,b; Shastak et al., 2014), thus

**Table 6.** Concentrations of different inositol phosphates ( $\mu\text{mol/g DM}$ ), pc InsP<sub>6</sub> disappearance (%) and the amount of pc disappeared InsP<sub>6</sub> (g/kg DM) in the digesta of the lower ileum of turkeys (experiment 2)<sup>1</sup>

	CD4		CD1		CD6		CD2		Pooled SEM	<i>P</i> value <sup>2</sup>		
	0	500	0	500	0	500	0	500		Diet	Phy <sup>3</sup>	Diet × Phy
Ins(1,2,5,6)P <sub>4</sub>	<LOQ <sup>4</sup>	1.08	<LOQ	1.21	<LOQ	1.19	<LOQ	1.18	0.102	0.795	-	-
Ins(1,2,3,4,6)P <sub>5</sub>	0.51	0.25	0.53	0.18	0.53	0.20	0.60	0.23	0.040	0.393	<0.001	0.504
Ins(1,2,3,4,5)P <sub>5</sub>	1.30	3.25	1.27	3.13	1.27	3.41	1.36	3.21	0.137	0.777	<0.001	0.688
Ins(1,2,4,5,6)P <sub>5</sub>	1.21	1.16	1.15	1.09	1.06	1.06	1.09	0.99	0.066	0.095	0.256	0.885
InsP <sub>6</sub>	35.7	28.2	35.6	25.2	37.9	29.0	40.9	27.1	1.14	0.011	<0.001	0.062
pc InsP <sub>6</sub> disappearance <sup>5</sup>	15.3 <sup>b</sup>	32.4 <sup>a</sup>	5.5 <sup>c</sup>	33.9 <sup>a</sup>	6.3 <sup>c</sup>	32.9 <sup>a</sup>	5.5 <sup>c</sup>	38.3 <sup>a</sup>	2.56	0.105	<0.001	0.012
pc InsP <sub>6</sub> disappeared <sup>5</sup>	1.30 <sup>c</sup>	2.74 <sup>b</sup>	0.53 <sup>d</sup>	2.84 <sup>b</sup>	0.63 <sup>d</sup>	3.15 <sup>a,b</sup>	0.65 <sup>c,d</sup>	3.72 <sup>a</sup>	0.217	0.155	<0.001	0.006

<sup>1</sup>CD4, CD1, CD6, CD2 = corn diets supplemented with corn sources no. 4, 1, 6, or 2 as used in the “GrainUp” project (Rodehutsord et al., 2016); diets remained non-supplemented (0) or supplemented with phytase (500). Data are given as LS means or back-transformed LS means; n = 6 pens per treatment with 15 birds per pen.

<sup>2</sup>*P* value of an F-test.

<sup>3</sup>Phy = phytase; pc = prececal.

<sup>4</sup>LOQ = not quantifiable in the majority of digesta samples.

<sup>5</sup>Estimates within a row not sharing a common superscript differ significantly (multiple *t*-tests in case of interactions between Phy and Diet), *P* ≤ 0.05.

confirming the ability of broilers, or their microbiome, to hydrolyze InsP<sub>6</sub> in the upper GIT upon feeding of low P/Ca diets (Sommerfeld et al., 2018b). However, pc InsP<sub>6</sub> degradation and pcdP were negatively correlated with the InsP<sub>6</sub>-P concentration of the corn source (Pearson *r* = -0.66 and *r* = -0.70, *P* < 0.001, respectively). This is consistent with the results of Kasim and Edwards (2000), who found a negative relationship between the phytate content of corn samples and the P retention of young broilers. Dietary phytate has been shown to non-competitively inhibit the phytase activity of the intestinal mucosa (Onyango and Adeola, 2009). However, this does not explain the observations made in the present study; compared with diets with lower phytate contents, higher levels of InsP<sub>6</sub> were degraded from CD6 and CD2, which showed the highest InsP<sub>6</sub> contents. Effects of P, Ca, and phytase on digestive tract microbiota composition can occur (Witzig et al., 2015; Borda-Molina et al., 2016), and it thus can be speculated that microbiota-associated phytase activity compensated for the limited mucosal phytase activity with increased levels of dietary InsP<sub>6</sub>. Phytate-degrading bacteria with high in vitro phytase activity have been isolated from the upper GIT of chickens (Palacios et al., 2008). Nevertheless, data suggest that the amount of InsP<sub>6</sub> that can be prececaly hydrolyzed in broilers fed corn-SBM-based diets is limited, thus confirming the results reported by Rodehutsord et al. (2017). The short retention time of digesta in the pc digestive tract may also limit the degradation of InsP<sub>6</sub> from high-phytate diets.

The lower pc InsP<sub>6</sub> degradation and pcdP (%) also coincided with higher EE contents in corn samples (*r* = -0.61 and *r* = -0.68, *P* < 0.001, respectively). A negative effect of dietary fats added to wheat-based diets on the retention of phytate P in young broilers was shown by Matyka et al. (1990). As any negative effects

of Ca could be excluded herein, the authors assumed that the activity of microbial phytases in the upper GIT was depressed by the fat. In corn, oil and phytate are co-localized in the germ (O'Dell, 1972; Lambert, 2001). Thus, a negative impact of the high oil content in CD6 and CD2 diets on microbial phytate degradation in relation to the amount of available InsP<sub>6</sub> seems possible.

As expected, supplementation of phytase increased the pc InsP<sub>6</sub> degradation and pcdP in broilers by 20 and 15%, respectively. The smaller effect of the same phytase product on pc InsP<sub>6</sub> degradation and pcdP reported by Zeller et al. (2015a,b) for broilers fed corn-SBM-based diets might be explained by differences in diet composition and dietary P and Ca levels. Although the amount of additional degraded InsP<sub>6</sub> with phytase was greater in CD6 and CD2 (1.86 and 1.56 g/kg DM) than in CD4 and CD1 (~1 g/kg DM), the incremental pcdP (g/kg DM) was similar among diets (0.55 g/kg DM). Thus, the effect of phytase on pcdP was not affected by corn source; however, a more efficient use of P from lower InsPs with CD4 and CD1 than with CD6 and CD2 seems evident. The stoichiometry suggests that a 0.55-g/kg DM increment in pcdP on phytase use in the diets could not have derived from the breakdown of phytate alone, as this would require the additional hydrolysis of 1.96 g InsP<sub>6</sub> to inositol (phytate being 28% P). However, the subsequent accumulation of InsP<sub>5</sub> isomers, especially of Ins(1,2,3,4,5)P<sub>5</sub>, was much lower than the decrease in InsP<sub>6</sub> concentration in digesta samples. This suggests that the further degradation of lower InsPs and complete hydrolysis were affected by phytase addition, as recently shown by Sommerfeld et al. (2018a). The additional use of P from lower InsPs found in diets may have also contributed to the additional amount of pcdP with phytase.

### **Presence of Lower Inositol Phosphate Isomers in Response to Corn Source and Phytase Supplementation in Broilers**

Consistent with previous studies on broiler chickens fed corn-SBM-based diets (Shastak et al., 2014; Zeller et al., 2015a,b), Ins(1,2,3,4,5)P<sub>5</sub> was the dominant InsP<sub>5</sub> in the ileal digesta of broilers in the present study, but not in feed. This InsP<sub>5</sub> may derive from the activity of microbial 6-phytases (Greiner et al., 2000; Haros et al., 2009); however, it may also be co-eluted with (but not separated from) Ins(1,2,3,5,6)P<sub>5</sub> in the analysis, which is a product of 4-phytases from cereal grains (Greiner and Alminger, 2001; Wu et al., 2015). As the intrinsic phytase activity in corn and SBM is low, it seems reasonable that microbiota-associated phytases contributed considerably to the presence of Ins(1,2,3,4,5)P<sub>5</sub> in digesta samples, even without phytase supplementation. The high concentration of this isomer in the digesta of birds fed CD6 and CD2 indicates a certain limitation in the further degradation of this InsP<sub>5</sub> in the upper GIT. The results obtained with phytase addition confirm this assumption; the concentration of Ins(1,2,3,4,5)P<sub>5</sub> increased substantially in animals fed CD6 and CD2 (to approximately 13% of the residual ileal InsP<sub>6</sub> level), while values of only 2.5% were achieved for all diets without phytase. Even in CD4 and CD1, Ins(1,2,3,4,5)P<sub>5</sub> increased relative to the level of residual InsP<sub>6</sub> (5 to 6% of the residual ileal InsP<sub>6</sub> level), even though the absolute concentrations tended to decrease. This interaction suggests that the rate of Ins(1,2,3,4,5)P<sub>5</sub> formation and hydrolysis following phytase addition depends on the corn source and likewise its InsP<sub>6</sub> content. The limitation in degradation of Ins(1,2,3,4,5)P<sub>5</sub> may thus contribute to the less efficient use of P from lower InsPs in CD6 and CD2 in the case of phytase supplementation.

The main product of Ins(1,2,3,4,5)P<sub>5</sub> hydrolysis by *E. coli*-derived phytases is Ins(2,3,4,5)P<sub>4</sub> (Greiner et al., 2000), which co-elutes with Ins(1,2,5,6)P<sub>4</sub>. This explains the detection of the latter InsP<sub>4</sub> in broilers fed with phytase-supplemented CD6 and CD2. Its accumulation in the lower ileum of broilers fed the same *E. coli*-derived phytase was shown by Zeller et al. (2015a,b) for corn-SBM-based diets of  $\geq 2.7$  g InsP<sub>6</sub>-P/kg DM. The CD6 and CD2 diets contained  $\sim 2.2$  g InsP<sub>6</sub>-P/kg DM, while CD4 and CD1 contained much less InsP<sub>6</sub>-P. The dietary InsP<sub>6</sub>-P content and the amount of degraded InsP<sub>6</sub> via Ins(1,2,3,4,5)P<sub>5</sub> may have been relevant for the pattern of diet-dependent degradation observed in the present study. The isomer Ins(1,2,3,4)P<sub>4</sub> (co-eluted with Ins(1,2,3,6)P<sub>4</sub>) can be formed by 5- or 6-phytases; however, its sole occurrence in the digesta from birds fed phytase-supplemented CD2 suggests this isomer relies on the presence of both the exogenous phytase and the endogenous phytases that exist in the CD2 diet, which can further degrade Ins(1,2,3,4,5)P<sub>5</sub>.

The presence of Ins(1,2,4,5,6)P<sub>5</sub> may have resulted from 3-phytase activity of soybean (Phillippy and Bland, 1988) or microbial origin (Konietzny and Greiner, 2002). Moreover, it was the most abundant lower InsP detected in the diets. The lower concentrations of Ins(1,2,4,5,6)P<sub>5</sub> found in digesta compared with the diets indicate a significant degree of degradation of this isomer in the upper GIT of chickens. The high concentrations of this isomer in digesta samples obtained from phytase-supplemented CD6 and CD2 may indicate a less marked degradation or the further formation of this InsP<sub>5</sub> by the lower ileum from these diets compared with the other treatments.

### **Degradation of InsP<sub>6</sub> and Prececal Digestibility of P in Response to Corn Source and Phytase Supplemented in Turkeys**

Values reported for the pcdP and P retention from low-P-low-Ca corn-SBM-based diets in young turkeys range from 35 to 59% (Qian et al., 1996; Applegate et al., 2003; Charbeneau and Roberson, 2004; Esteve-Garcia et al., 2005; Pirgozliev et al., 2011; Wealleans et al., 2016), which are higher than the values for pcdP (%) obtained for non-supplemented diets in the present study. Charbeneau and Roberson (2004) reported that phytate P retention in 4-wk-old turkeys was also higher (35 to 48%) than the pc InsP<sub>6</sub> degradation observed for turkeys in experiment 2. However, in most of those studies, the diets contained mineral phosphates and higher proportions of SBM than were used in the present study, the latter resulting in a higher dietary phytate level. Our results confirm the limited capability of phytate degradation in turkeys, as previously described by Lescoat et al. (2005), and Rodehutschord and Dieckmann (2005). Nevertheless, there was no correlation between the InsP<sub>6</sub>-P content and the pc InsP<sub>6</sub> degradation or pcdP (%) from diets. However, as was found with broilers, there was a reduction in pc InsP<sub>6</sub> degradation (%) with increasing EE contents ( $r = -0.51$ ;  $P = 0.018$ ). This suggests that high oil contents may impair phytate access or microbial phytase activity.

The beneficial effects of phytase supplementation on P digestibility and phytate P retention in turkeys are well known (Lescoat et al., 2005; Kozłowski et al., 2010), but only a few studies are available on the effect of *E. coli*-derived phytases. By supplementing corn-SBM-based diets with 500 FTU/kg feed of an *E. coli*-derived phytase, Applegate et al. (2003) and Pirgozliev et al. (2011) achieved 9 and 22% higher P retention in young turkeys compared with those fed non-supplemented diets, respectively, while Kozłowski et al. (2010) reported a numerical increase in the pcdP of 8% in 112-day-old turkeys. Thus, our findings of a 9 to 17% increment in



pcdP confirm those from previous studies on turkeys. Phytase efficiency was greatest in turkeys fed diets with high-oil corn sources and high InsP<sub>6</sub> contents. Phytase equilibrated the differences in the pc InsP<sub>6</sub> degradation found between non-supplemented diets, thus resulting in higher amounts of pcdP (g/kg DM) for diets containing high InsP<sub>6</sub> contents (CD6, CD2).

Regardless of diet or phytase supplementation, turkeys in the present study showed much lower pc InsP<sub>6</sub> degradation and pcdP than broiler chickens fed low-P diets based on corn (experiment 1, Rodehutschord and Rosenfelder, 2016). These observations are consistent with those from comparative studies on P retention and pcdP from low-P corn-based diets in young broilers and turkeys (Rodehutschord and Dieckmann, 2005; Adebisi and Olukosi, 2015). Since the diets fed in the pre-feeding period were quite similar, a pre-feeding effect seems unlikely. The differences noted between turkeys and broilers in their capacity for pc InsP<sub>6</sub> hydrolysis and pcdP may be the result of differences in small intestine maturity (Adebisi and Olukosi, 2015), endogenous P loss, pH along the GIT, passage rate (Rodehutschord and Dieckmann, 2005; Adebisi and Olukosi, 2015), and associated differences in the microbiota (Pan and Yu, 2014). The latter may also be due to differences in crop development (Zeller et al., 2016). Furthermore, differences in diet composition (higher SBM and Ca level in turkey diets) in the current study may well have compromised InsPs degradation in turkeys.

### **Presence of Lower Inositol Phosphate Isomers in Response to Corn Source and Supplemented Phytase in Turkeys**

This is the first report to describe the presence of lower InsPs in the upper GIT of turkeys. Thus, any comparison with other studies is restricted to broiler data (Shastak et al., 2014; Zeller et al., 2015a,b; experiment 1). This comparison revealed the same dominance of Ins(1,2,3,4,5)P<sub>5</sub> in the ileal digesta of turkeys as found in broilers fed corn-SBM-based diets. However, irrespective of phytase supplementation Ins(1,2,3,4,5)P<sub>5</sub> concentrations were much higher in turkeys than in broilers, thus indicating the more limited hydrolysis of this isomer in the upper GIT of turkeys. This, in addition to lower InsP<sub>6</sub> degradation, is likely to have contributed to the lower pcdP (g/kg DM) in turkeys. The addition of phytase caused a similar increase in Ins(1,2,3,4,5)P<sub>5</sub> concentrations in ileal digesta for all diets, although the increase in InsP<sub>6</sub> degradation was again greater for CD6 and CD2 compared with CD4 and CD1. This suggests a higher flow of InsP<sub>6</sub> through Ins(1,2,3,4,5)P<sub>5</sub> to lower InsPs for CD6 and CD2 compared with the low-phytate diets.

As found in broilers, ileal Ins(1,2,3,4,6)P<sub>5</sub> concentrations decreased in turkeys when phytase was added to diets. However, no differences in the concentration

of this InsP<sub>5</sub> were observed among diets, as found for broilers. This may indicate a different response of the microbiota to corn sources in turkeys and a different expression of microbial 5-phytases; nonetheless, other effects, such as differences in the retention time, cannot be ruled out.

Ins(1,2,4,5,6)P<sub>5</sub> is typically found in soybeans (Phillippy and Bland, 1988), thus explaining its higher concentration in turkey diets compared to those formulated for broilers (Table 3). In contrast to broilers, concentrations of Ins(1,2,4,5,6)P<sub>5</sub> in digesta samples of turkeys were higher compared with those in the diets. As the InsP<sub>6</sub> degradation was much lower in turkeys than in broilers, this may rather indicate a lower capability of turkeys to preceally degrade this isomer instead of a higher 3-phytase activity of microbial or plant origin.

The accumulation of Ins(1,2,5,6)P<sub>4</sub> in the ileal digesta of turkeys upon phytase addition is a typical effect of *E. coli*-derived 6-phytases. An increase of this InsP<sub>4</sub> with phytase addition was also found in experiment 1 for CD6 and CD2, and also in the studies of Zeller et al. (2015a,b) and Sommerfeld et al. (2018b). However, the higher accumulation of Ins(1,2,5,6)P<sub>4</sub> in turkeys than in broilers indicates a more limited hydrolysis of this InsP<sub>4</sub> or an impact of dietary differences, such as the Ca level. The inability to detect InsP<sub>3</sub> in turkey ileal digesta suggests the rapid degradation of these isomers in the upper GIT, or alternatively, its incapacity to produce this isomer.

The differences between diets with respect to phytase-induced pc InsP<sub>6</sub> degradation were not reflected by the concentrations of lower InsPs in digesta samples of turkeys, probably due to diet-dependent differences in the degradation rate of lower InsPs.

In conclusion, our results indicate a negative effect of high-oil corn with high InsP<sub>6</sub> contents on the pcdP and pc InsP<sub>6</sub> degradation in broilers. Data from turkeys also suggest a negative impact of high-oil contents in corn on the percentage InsP<sub>6</sub> degradation. However, the total amount of InsP<sub>6</sub> degraded up to the terminal ileum was the same or higher in birds fed high-oil corn. It remains unclear whether the rate of digesta passage limited the amount of phytate degradation in such samples, or whether the fat has a direct negative effect on phytate degradation. The origin of the corn source and the differences between species affect the pcdP in both species. The poorer efficacy of phytase in turkeys should be considered in diet formulation. The reduced ability of animals to hydrolyze phytate and lower InsPs seems to account for the lower pcdP in turkeys than in broilers. Thus, further research should focus on factors influencing phytate degradation in turkeys, taking different diet compositions, growth phases, and crop development into consideration, to improve the efficacy of phytase and P digestibility from feedstuffs of plant origin.

## ACKNOWLEDGMENTS

The project was supported by funds from the Federal Ministry of Food, Agriculture, and Consumer Protection (BMELV) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support program.

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