METABOLISM AND NUTRITION

Effect of supplemental phytase and xylanase in wheat-based diets on prececal phosphorus digestibility and phytate degradation in young turkeys

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ABSTRACT This study aimed to investigate the effect of phytase and a combination of phytase and xylanase on the prececal phosphorus digestibility (pcdP) of wheat-based diets in turkeys. A low-P basal diet (BD) based on cornstarch and soybean meal, and 2 diets containing 43% of different wheat genotypes (genotype diets GD_6 or GD_7) were fed to turkeys from 20 to 27 d of age. Diets were fed either without enzyme supplementation or supplemented with phytase (500 FTU/kg) or a combination of phytase and xylanase (16,000 BXU/kg). At 27 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) ($InsP_6$) disappearance, and to analyze the concentrations of lower inositol phosphate isomers. Similar pcdP was observed in non-supplemented BD and GD ($\sim$36%). Phytase alone increased the pcdP in all diets by 8 to 12%, but a beneficial effect of xylanase was found only for BD. Similar results were found for pc $InsP_6$ disappearance, although xylanase addition compared to phytase alone decreased pc $InsP_6$ disappearance in GD_7 compared to phytase alone. Animals fed GD_7 performed better than those fed GD_6; however, these differences could not be linked to the pcdP. The pattern of lower inositol phosphates in digesta also changed with enzyme supplementation, resulting in lower proportions of $InsP_5$ and higher proportions of $InsP_4$. Phytase alone decreased $Ins(1,2,3,4,6)P_5$ but increased D-$Ins(1,2,3,4,5)P_5$ and D-$Ins(1,2,5,6)P_4$ concentrations. An additional increase in D-$Ins(1,2,3,4,5)P_5$ and D-$Ins(1,2,5,6)P_4$ concentrations was achieved with xylanase, although for the former isomer, this was observed only with GD. These results indicate that enzyme supplementation alters the pc degradation of $InsP_6$, and that combining both enzymes had a minor additional effect on the pcdP from wheat-based diets when compared to phytase alone.

Key words: turkey, prececal digestibility, phosphorus, wheat, inositol phosphates

INTRODUCTION

Phosphorus (**P**) is an element with high relevance for poultry feeding. In feeds of plant origin, the majority of P is bound as phytate, the salt of phytic acid [(*myo*-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate or **$InsP_6$**). $InsP_6$-P has to be cleaved in the gastrointestinal tract by phytases and other phosphatases prior to absorption, but insufficient secretion of endogenous enzymes in nonruminants limits phytate hydrolysis.

Wheat is an important grain used in poultry diets (Coskuntuna et al., 2008). Among cereals, wheat contains a moderate concentration of total P and $InsP_6$-P, while its intrinsic phytase activity is relatively high (Rodehutscord et al., 2016). Nevertheless, a substantial fraction of P remains non-digestible in wheat-based diets for poultry (van der Klis et al., 1995; Juin et al., 2001; Woyengo et al., 2008; Selle et al., 2009; Zeller et al., 2015a). Therefore, diets are usually supplemented with exogenous phytases of microbial origin. These exhibit higher activity within the gastrointestinal tract than intrinsic phytases of plants, because of their broader optimum pH range and higher resistance to proteases (Woyengo and Nyachoti, 2011). The beneficial effect of supplemental phytase on P digestibility from wheat-based diets for broilers has been confirmed in previous studies (Kiieskinen et al., 1994; Zyla et al., 2000; Wu et al., 2003; Afsharmanesh et al., 2008; Woyengo et al., 2008; Selle et al., 2009; Zeller et al., 2015a). Little is known about the effect of phytase on

Table 1. Concentration of total P, inositol phosphate P, total arabinoxylans, phytase activity, and extract viscosity in wheat genotypes used in the present work.

Genotype[1]	CP (g/kg DM)	Total P (g/kg DM)	$InsP_6$-P (g/kg DM)	Phytase activity (FTU/kg DM)	Total arabinoxylans (g/kg DM)	Extract viscosity[2] (mPa · s)
6	125	3.41	1.79	2640	60.9	1.01
7	152	3.92	1.86	2040	67.4	1.28

[1]Genotypes represent wheat genotypes no. 6 and 7 used in the "GrainUp" project (Rodehutscord et al., 2016).
[2]Calculated at an assumed shear rate of 380 s^{-1}.

the digestibility of P from wheat-based diets in turkeys, although the P availability from different P sources seems to differ between poultry species (Rodehutscord and Dieckmann, 2005). Juin et al. (2001) reported a 15% increase in P retention in young turkeys following the addition of 500 U phytase to a low-P wheat-soybean meal (**SBM**)-based diet. However, the maximum P retention achieved was 61%, indicating that a high proportion of phytate-bound P remained undegraded. Similar results were obtained with broilers in the aforementioned studies of Wu et al. (2003), Afsharmanesh et al. (2008) and Selle et al. (2009). Thus, several attempts have been made to further improve phytate hydrolysis. One approach is the use of non-starch-polysaccharide (**NSP**)-degrading enzymes, such as xylanases (Adeola and Cowieson, 2011; Woyengo and Nyachoti, 2011), which hydrolyze arabinoxylans. These indigestible NSP constitute the major components of cell walls in the aleurone layer (Bacic and Stone, 1981), which is the main site of phytate storage in wheat (O'Dell, 1972). Xylanases may increase the permeability of the aleurone layer (Parkkonen et al., 1997), and those with an affinity for soluble and insoluble arabinoxylans can decrease digesta viscosity (Adeola and Cowieson, 2011). This may facilitate the accessibility of phytase to phytate (Adeola and Cowieson, 2011; Woyengo and Nyachoti, 2011) as previously indicated in vitro (Zyla et al., 1999). In studies using growing broilers, Selle et al. (2009) found that combining phytase and xylanase had a positive effect on feed efficiency and prececal (**pc**) digestibility of amino acids, nitrogen, and energy from a wheat-based diet. However, compared with phytase alone, no additionally beneficial effect of xylanase was detected on the pc digestibility of P (**pcdP**) in their study. Further studies also have reported that no synergistic interaction exists between phytase and xylanase on the P digestibility (Peng et al., 2003; Juanpere et al., 2005; Olukosi and Adeola, 2008; Woyengo et al., 2008; Zeller et al., 2015a) or pc $InsP_6$ degradation (Kühn et al., 2017) in broilers fed wheat-based diets. Nevertheless, Zeller et al. (2015a) showed that $InsP_6$ and most of the detected lower inositol phosphates (**InsPs**) tended to be less concentrated in the ileal digesta of broilers when both enzymes were added in combination than with phytase alone. However, to the best of our knowledge, no studies investigating the effect of xylanase alone or in combination with phytase on the pcdP in turkeys are available.

Therefore, the objective of the research reported herein was to examine the effect of phytase, alone or in combination with xylanase, on the pcdP, the pc $InsP_6$ disappearance, and the appearance of InsPs in the lower ileum of young turkeys fed wheat-based diets. As synergism between phytase and xylanase depends on factors such as dietary NSP concentration, NSP composition, and the intrinsic phytase activity (Woyengo and Nyachoti, 2011), we used 2 different wheat genotypes, which differed in their physical and chemical characteristics.

MATERIALS AND METHODS

The 2 wheat genotypes used in this study represent genotypes no. 6 and 7 as denoted and characterized by Rodehutscord et al. (2016). These genotypes were selected based on their P and arabinoxylan content, as well as their pcdP as demonstrated in a previous P digestibility study with broilers (Witzig et al., 2018). Whereas wheat genotype no. 6 had a low P and arabinoxylan content (Table 1) and showed a low pcdP in broilers, genotype no. 7 contained a relatively high P and arabinoxylan content and had a higher pcdP in broilers than did 7 other genotypes (Witzig et al., 2018).

Experimental Diets

The experiment involved testing of 9 treatments. Three diets, a basal diet (**BD**) and 2 genotype diets (**GD**), were tested at 3 different enzyme combinations. While this is a 3 × 3 factorial arrangement of treatments, in the model, the first factor (diet) is split into the factors diet type and diet. The latter has the advantage to distinguish between differences between the 2 genotypes and differences between BD and GD.

The BD, based on cornstarch and SBM, was formulated to contain adequate levels of all nutrients according to the recommendations of the Gesellschaft für Ernährungsphysiologie (GfE, 2004), with the exception of P and calcium (**Ca**) (Table 2). To formulate the 2 GD, 43.4% of genotype no. 6 or 7 was included at the expense of cornstarch, making the wheat genotype the only source of P variation in these diets (Table 2). The wheat was ground to pass through a 2-mm sieve screen before being added to the diet. To maintain a constant Ca: P ratio in all diets, additional

Table 2. Composition of the basal diet and genotype diet used between 20 and 27 d of age in the experiment (g/kg).[1]

	BD	GD
Ingredient		
Wheat genotype	–	434
Wheat	74	74
Cornstarch	438.9	–
Soybean meal	352.9	352.9
Potato protein	88.8	88.8
Soybean oil	15	15
L-Lysine HCL	2	2
D,L-Methionine	3.5	3.5
Limestone	7.9	12.8
Premix[2]	6	6
Sodium chloride	1	1
Choline chloride	2	2
Sodium bicarbonate	3	3
Titanium dioxide	5	5
Calculated concentrations		
CP, g/kg DM	262	281
ME, MJ/kg	13.4	13.2

[1]BD = basal diet; GD = genotype diets supplemented with 2 different wheat genotypes as characterized in Table 1.

[2]Premix turkey (BASU Mineralfutter GmbH, Bad Sulza, Germany) provided per kilogram of complete diet: Ca, 30 mg; P, 53 mg; Na, 1.8 mg; Mg, 16 mg; Cl, 4.8 mg; Fe, 80 mg; Mn, 128 mg; Zn, 96 mg; Cu, 16 mg; I, 1.6 mg; Se, 0.56 mg; Co, 0.4 mg; vitamin A, 19,200 IU; vitamin D, 6,400 IU; vitamin E, 64 mg; vitamin K, 3.8 mg; vitamin B_1, 3.4 mg; vitamin B_2, 11.5 mg; vitamin B_6, 6.4 mg; vitamin B_{12}, 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, 1020 mg.

limestone was added at the expense of cornstarch. Titanium dioxide was used as an indigestible marker (0.5%). Diets were fed to animals with or without supplementation with an *Escherichia coli*-derived thermotolerant 6-phytase (Phy, Quantum™ Blue, intended activity 500 FTU/kg feed), alone or in combination with a commercial *Trichoderma reesei*-derived thermostable endo-1,4-β-xylanase (X, Econase® XT). The xylanase was supplemented to achieve an activity of 16,000 BXU/kg feed, which is the recommendation of the supplier for wheat-based diets. Both enzymes were provided by AB Vista, Marlborough, United Kingdom. Diets were mixed in the certified feed mill facilities of Hohenheim University's Agricultural Experiment Station, location Lindenhöfe in Eningen, Germany, and pelleted through a 3-mm die without the use of steam. The pellet temperature immediately measured after pelleting ranged between 55 and 78°C. Representative samples of the 9 experimental diets were taken and pulverized using a laboratory disc mill (Siebtechnik GmbH, Mühlheim an der Ruhr, Germany) and stored at 4°C until chemical analysis.

The analyzed activity of phytase and xylanase was very low in non-supplemented diets, and ranged from 376 to 521 FTU and from 14,400 to 18,900 BXU/kg feed, respectively, in enzyme-supplemented diets (Table 3). The concentrations of total P and InsP$_6$-P in the diets ranged from 3.24 to 4.94, and from 1.58

to 2.42 g/kg DM, respectively. The average Ca: P ratio was (SD) 1.5 (0.05).

Birds, Animal Management, and Sampling Procedure

The animal experiment was performed at the Agricultural Experiment Station of Hohenheim University, location Lindenhöfe in Eningen (Germany), in accordance with German Animal Welfare legislation. All procedures regarding animal handling and treatments were approved by the Animal Welfare Commissioner of the University.

Turkey hatchlings (B.U.T. Big 6; unsexed), were obtained from a local hatchery (Gebrüder Böcker Putenbrüterei GmbH, Wallhausen, Germany) and randomly allocated to 72 floor pens (154 × 115 cm), with 15 birds per pen. Birds were raised on wood shavings before being placed on plastic slats at 10 d of age. They were fed a commercial starter diet (Deutsche Tiernahrung Cremer GmbH & Co. KG, Düsseldorf, Germany) containing 1.25% Ca, 0.90% P, 26.0 % CP, 11.6 MJ ME/kg, 750 FTU 6-phytase (EC.3.1.3.26, 4a1641[i])/kg, and 10 IU endo-1,4-β-xylanase EC3.2.1.8 (E 1606)/kg in pelleted form. During the first d of life, an additional starter feed containing 1.30% Ca, 0.90% P, 27.5% CP, 11.5 MJ ME/kg, 750 FTU 6-phytase (EC.3.1.3.26, 4a1641[i])/kg, and 10 IU endo-1,4-β-xylanase EC3.2.1.8 (E 1606)/kg was offered in crumbled form. Birds underwent routine vaccination against coccidiosis (via starter diets) and Newcastle disease on d 12.

The room temperature was set at 36°C on d 1 and 2 before being gradually reduced to 21°C until d 21. Light intensity was 100 lx. During the first 2 d, light was provided for 24 h; thereafter, the provision of light was reduced to 18 h per day.

Turkeys were weighted at 20 d of age on a pen basis and randomly assigned to one of 9 dietary treatments using a non-resolvable incomplete block design within the animal house. The design included 18 incomplete blocks each with 4 pens, as 4 pens formed a row within the animal house. All treatments were tested in 8 (n = 8) pens and therefore in 8 out of 18 blocks. Throughout the experiment, animals had free access to feed and tap water. The experimental diets were fed to the animals for 7 d and ADFI as well as ADG were recorded. On d 27, birds were stunned with a mixture of 35% CO_2, 35% N_2, and 30% O_2, and euthanized via CO_2 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. According to the method described for determination of the pcdP by the World's Poultry Science Association (WPSA, 2013), the digesta of the distal half of the ileum were 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, freeze-dried (Type Delta

Table 3. Analyzed phytase and xylanase activity, and concentrations of Ti, Ca, total P, $InsP_6$-P, and lower (D-) inositol phosphates in experimental diets.[1]

	BD			GD_6			GD_7		
	0	Phy	PhyX	0	Phy	PhyX	0	Phy	PhyX
Phytase activity (FTU/kg)[2]	<100[3]	402	376	1200[3]	484	521	654[3]	462	500
Xylanase activity (BXU/kg)	<2000	<2000	14,400	<2000	<2000	18,300	<2000	<2000	15,900
Ti (g/kg DM)	3.42	3.40	3.37	3.38	3.41	3.48	3.36	3.41	3.44
Ca (g/kg DM)	5.13	5.01	5.10	7.43	7.55	7.67	7.38	7.67	7.47
Total P (g/kg DM)	3.24	3.51	3.33	4.73	4.78	4.90	4.90	4.94	4.94
$InsP_6$-P (g/kg DM)	1.58	1.64	1.58	2.25	2.32	2.30	2.38	2.42	2.38
$InsP_6$ (μmol/g DM)	8.50	8.80	8.50	12.10	12.50	12.40	12.80	13.00	12.80
Ins(1,2,4,5,6)P_5 (μmol/g DM)	0.60	0.70	0.60	0.60	0.60	0.60	0.70	0.60	0.60
Ins(1,2,3,4,5)P_5, (μmol/g DM)	<LOQ[4]	0.30	0.30	0.30	0.30	0.30	0.20	0.30	0.30
Ins(1,2,3,4,6)P_5 (μmol/g DM)	n.d.[5]	<LOQ	n.d.	n.d.	<LOQ	n.d.	<LOQ	n.d.	n.d.

[1]BD = basal diet; GD_6 = genotype diet supplemented with wheat genotype no. 6 as used in the "GrainUp" project; GD_7 = genotype diet supplemented with wheat genotype no. 7 as used in the "GrainUp" project (Rodehutscord et al., 2016); diets remained non-supplemented (0) or were supplemented either with phytase (Phy) or with phytase and xylanase (PhyX).

[2]Determined at pH 4.5 and 60°C.

[3]Determined at pH 5 and 45°C according to Greiner and Egli (2003), U/kg.

[4]LOQ = limit of quantification (InsP isomer was not quantifiable in the sample).

[5]n.d. = not detectable (InsP isomer was not detectable in the majority of samples).

1–24, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany), 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), and stored at 4°C until chemical analyses.

Chemical Analyses

The DM content of feed and digesta samples was analyzed according to the official German methods (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten [VDLUFA], 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 specifications described by Zeller et al. (2015b). Concentrations of $InsP_6$ 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. (2015c). Phytase and xylanase activities of the experimental diets were measured as described in detail by Zeller et al. (2015a). In brief, the intrinsic phytase activity of diets without enzyme supplementation was analyzed by the direct incubation method (quantification of liberated inorganic P), as described by Greiner and Egli (2003). Activity of the supplemented phytase and xylanase product in diets was assayed according to the manufacturer's protocol (U at pH 4.5 and 60°C transferred to commonly used FTU by a validated factor and BXU at pH 5.3 and 50°C). One FTU is the amount of enzyme that liberates 1 μmol of inorganic phosphate per min from a sodium phytate substrate at pH 5.5 and 37 °C. One unit of xylanase (BXU) is the amount of enzyme that liberates 1 nmol reducing sugars as xylose from birch xylan per s at pH 5,3 and 50 °C.

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 $InsP_6$ (y) were calculated on a pen basis according to the following equation:

$$y(\%) = 100 - 100 \times \left(\frac{\text{Ti in the diet (g/kg DM)}}{\text{Ti in the digesta (g/kg DM)}} \right)$$
$$\times \left(\frac{InsP_6 \text{ or P or Ca in the digesta (g/kg DM)}}{InsP_6 \text{ or P or Ca in the diet (g/kg DM)}} \right) (1)$$

Statistical evaluation of the data was carried out with the software package SAS for Windows (Version 9.3, SAS Institute, Cary, NC). A mixed models approach (procedure PROC MIXED) was used, considering the effects of treatment factors "diet type" (BD or GD), "diet" (BD, GD_6, or GD_7), "enzyme" (0, phytase, phytase/xylanase), and interaction between these factors, as fixed and "block" effects as random. The model can be described by the following equation:

$$y_{ijkl} = \mu + \alpha_i + \beta_{ij} + \gamma_k + (\alpha\gamma)_{ik}$$
$$+ (\beta\gamma)_{ijk} + b_1 + e_{ijkl}, \quad (2)$$

Where: y_{ijkl} = lth observation of the ith diet type, jth diet, and kth inclusion level of enzymes, μ = general mean, α_i = effect of the ith diet type, β_{ij} = effect of the jth diet within ith diet type, γ_k = effect of the kth inclusion level of enzymes, $(\alpha\gamma)_{jk}$ = interaction effect between the ith diet type and kth inclusion level of enzymes, $(\beta\gamma)_{ijk}$ = interaction effect of the jth diet and the kth inclusion level of enzymes within the diet type, b_l = effect of the lth incomplete block, and e_{ijkl} = error term associated with y_{ijkl}. Prior to statistical analyses, data were graphically checked for normal distribution and variance homogeneity, and if necessary

Table 4. BW at 27 d of age, ADG, ADFI, feed: gain ratio, pcdP, and pcCa of broilers between 20 and 27 d of age.[1]

Diet	BD			GD$_6$			GD$_7$			Pooled SEM	P-value[2] DT[3]	D[4](DT)	E[5]	E × DT	E × D(DT)
	0	Phy	PhyX	0	Phy	PhyX	0	Phy	PhyX						
BW (g)	969	985	989	1030	1073	1086	1041	1094	1084	14.3	<0.001	0.361	0.002	0.384	0.722
ADG (g/d)	48	50	52	57	63	63	60	66	64	1.6	<0.001	0.035	<0.001	0.468	0.698
ADFI (g/d)	80	80	79	89	95	94	91	96	93	1.8	<0.001	0.356	0.056	0.099	0.778
Feed:gain (g/g)	1.66	1.58	1.54	1.59	1.51	1.49	1.51	1.47	1.45	0.024	<0.001	0.022	<0.001	0.611	0.551
pcdP (%)[6]	36^a	48^c	53^d	35^{a*}	45^{b*}	45^{b*}	36^{a*}	44^{b*}	42^{b*}	1.0	<0.001	0.398	<0.001	0.287	<0.001
pcdCa (%)	40	44	49	34	38	39	37	40	38	1.7	<0.001	0.274	<0.001	0.515	0.107

[1]BD = basal diet; GD$_6$ = genotype diet supplemented with wheat genotype no. 6 as used in the "GrainUp" project; GD$_7$ = genotype diet supplemented with wheat genotype no. 7 as used in the "GrainUp" project (Rodehutscord et al., 2016); diets remained non-supplemented (0) or were supplemented either with phytase (Phy) or with phytase and xylanase (PhyX). Data are given as LS means or back-transformed LS means; n = 8 (BD0: pcdP n = 7) pens per treatment with 15 birds per pen.
[2]P-value of an F test testing for difference between levels of the according effect.
[3]DT = Diet type = BD vs. GD.
[4]D = Diet.
[5]E = Enzyme.
[6]Estimates within a row not sharing a common superscript differ significantly (multiple t tests in case of interaction), $P \leq 0.05$. *Lower cases for GD$_6$ and GD$_7$ are identical, as both diets did not differ. $^{a-d}$Different superscripts indicate differences of LS means between BD and GD in the case of an interaction detected for DT and E.

Table 5. Concentrations of different (D-) inositol phosphates (μmol/g DM) and InsP$_6$ disappearance (%) in the digesta of the lower ileum of turkeys.[1]

Diet	BD			GD$_6$			GD$_7$			Pooled SEM	P-value[2] DT[3]	D[4](DT)	E[5]	E × DT	E × D(DT)
	0	Phy	PhyX	0	Phy	PhyX	0	Phy	PhyX						
Ins(1,2,3,4)P$_4$	n.d.[6]	n.d.	n.d.	n.d.	0.15	0.20	n.d.	0.13	0.18	0.035	.	0.498	0.153	.	0.952
Ins(1,2,5,6)P$_4$	n.d.	0.21	0.25	0.33	0.88	1.06	0.23	0.83	1.01	0.047	<0.001	0.052	<0.001	0.352	0.384
Ins(1,2,3,4,6)P$_5$[7]	0.30^b	0.20^a	n.d.	0.45^{c*}	0.23^{a*}	0.20^{a*}	0.47^{c*}	0.20^{a*}	0.23^{a*}	0.013	<0.001	0.503	<0.001	<0.001	0.056
Ins(1,2,3,4,5)P$_5$[7]	0.65^a	1.16^b	1.04^b	1.35^{b*}	2.24^{c*}	2.41^{d*}	1.20^{b*}	2.23^{c*}	2.58^{d*}	0.098	<0.001	0.970	<0.001	<0.001	0.277
Ins(1,2,4,5,6)P$_5$	0.54	0.58	0.46	0.75	0.77	0.75	0.71	0.75	0.82	0.041	<0.001	0.836	0.436	0.085	0.407
ΣInsP$_{3 \text{ to } 5}$[7]	1.5^a	2.2^b	1.75^{a,b}	2.9^{e*}	4.3^{d*}	4.8^{e*}	2.6^{c*}	4.2^{d*}	5.0^{e*}	0.18	<0.001	0.634	<0.001	<0.001	0.435
InsP$_6$[7]	21.2^c	18.7^b	16.1^a	26.8^{e*}	21.9^{c*}	23.3^{d*}	27.8^{e*}	22.2^{c*}	24.7^{d*}	0.70	<0.001	0.104	<0.001	0.002	0.766
InsP$_6$ disappearance[7]	35^b	47^e	55^f	29^a	44^{d,e}	40^{c,d}	29^a	45^{d,e}	38^{b,c}	1.9	<0.001	0.776	<0.001	0.587	<0.001

[1]BD = basal diet; GD$_6$ = genotype diet supplemented with wheat genotype no. 6 as used in the "GrainUp" project; GD$_7$ = genotype diet supplemented with wheat genotype no. 7 as used in the "GrainUp" project (Rodehutscord et al., 2016); diets remained non-supplemented (0) or were supplemented either with phytase (Phy) or with phytase and xylanase (PhyX). Data are given as LS means or back-transformed LS means; n = 8 (BD0, BDPhyX: InsP$_6$ disappearance n = 7) pens per treatment with 15 birds per pen.
[2]P-value of an F test testing for difference between levels of the according effect.
[3]DT = Diet type = BD vs. GD.
[4]D = Diet.
[5]E = Enzyme.
[6]n.d. = not detectable (InsP isomer was not detectable in the majority of samples).
[7]Estimates within a row not sharing a common superscript differ significantly (multiple t tests in the case of interaction), $P \leq 0.05$. *Lower cases for GD$_6$ and GD$_7$ are identical, as both diets did not differ. $^{a-f}$Different superscripts indicate differences of LS means between BD and GD in the case of an interaction detected for DT and E.

(percentage data), subjected to arcsine square-root transformation. Least square means from the analysis were back-transformed for presentation only. If the F-test was significant, the multiple t test was used for treatment comparison. The level of significance was set at $\alpha = 0.05$.

RESULTS

Diet type significantly affected all response traits (Tables 4 and 5). As indicated by the increase in ADG and the decrease in the feed: gain ratio, birds fed the GD performed better than those fed the BD. The pcdCa of GD was, on average, 38% and lower than that of BD (45%). Enzyme supplementation had a significant effect on all traits, except on ADFI ($p = 0.056$). The BW at 27 d of age, the ADG, and pcdCa were increased, and the feed: gain ratio was decreased following the addition of phytase to the diets. However, additional supplementation with xylanase did not further increase the performance of turkeys or the pcdCa of diets. The pcdP of diets was affected by an interaction between diet and enzyme supplementation. Thus, in birds fed the BD, phytase alone increased the pcdP of diets from 36 to 48%, while a further increase of 5% was observed with additional xylanase supplementation. In GD$_6$ and GD$_7$, supplementation with phytase also increased the pcdP from 35 and 36% to 45 and 44%, respectively, but no further increase was achieved with the addition of xylanase.

The significant effect of diet within diet type was restricted to the ADG and the feed: gain ratio of animals. The results indicated a significantly higher ADG, as well as a lower feed: gain ratio, in animals fed GD$_7$ than in those fed GD$_6$.

Table 5 shows the concentrations of the different InsPs detected in digesta samples of the lower ileum and the pc $InsP_6$ disappearance in turkeys. Concentrations of $InsP_6$ in digesta samples were not affected by diet, or by interactions between diet and enzyme supplementation; however, significant interactions were observed between diet type and enzymes. Irrespective of diet type, enzyme supplementation decreased the concentration of $InsP_6$ in the ileal digesta. However, in animals fed the BD, the lowest $InsP_6$ concentration was found following supplementation with both enzymes, whereas for GD, the lowest $InsP_6$ concentrations were achieved with phytase alone. Moreover, the concentrations of $InsP_6$ in birds fed the BD were lower than those in animals fed the GD. The pc $InsP_6$ disappearance was significantly affected by interaction effects between diet and enzymes. Phytase supplementation increased the pc $InsP_6$ disappearance irrespective of diet type, but a further increase with xylanase was achieved only for the BD. In animals fed GD, xylanase reduced the pc $InsP_6$ disappearance, and with GD_6 and GD_7, a numerically lower and significantly lower pc $InsP_6$ disappearance was observed, respectively, than with phytase alone.

The concentration of $D\text{-}Ins(1,2,4,5,6)P_5$ was affected only by diet type, with lower values found for BD than for GD. Concentrations of $D\text{-}Ins(1,2,3,4,5)P_5$ and $Ins(1,2,3,4,6)P_5$ were also lower in BD than with GD; however, these $InsP_5$ isomers also were affected by an interaction between diet type and enzymes. In digesta samples of animals fed BD, supplementation with phytase alone or combined with xylanase led to a similar increase in the concentration of $D\text{-}Ins(1,2,3,4,5)P_5$. In birds fed GD with xylanase, there was an additional increase in the concentrations of this isomer. In contrast, concentrations of $Ins(1,2,3,4,6)P_5$ in digesta samples decreased with enzyme supplementation. Upon feeding with BD, the concentration of $Ins(1,2,3,4,6)P_5$ decreased below the limit of detection when both enzymes were supplemented, whereas with GD, the concentrations did not differ between enzyme treatments. The $InsP_5$ isomer $D\text{-}Ins(1,3,4,5,6)P_5$ could not be detected in digesta samples.

The $InsP_4$ isomer $D\text{-}Ins(1,2,3,4)P_4$ could be detected only in digesta samples of animals fed the enzyme-supplemented GD, with no obvious differences between GD_6 and GD_7, or between enzyme treatments. Concentrations of $D\text{-}Ins(1,2,5,6)P_4$ were lower in animals fed BD than those fed GD, and higher with enzyme supplementation (phytase/xylanase>phytase>0), thus indicating an effect of diet type and enzymes, whereas no interaction effects were observed.

The detection of $InsP_3$ isomers was restricted to digesta samples from animals fed GD_6 and GD_7 supplemented with phytase and xylanase. One or more of the $InsP_3$ isomers $D\text{-}Ins(1,2,6)P_3$, $D\text{-}Ins(1,4,5)P_3$, and $D\text{-}Ins(2,4,5)P_3$ were found at concentrations of 0.16 and 0.15 $\mu mol/g$ DM in samples obtained from treatments GD_6 and GD_7, respectively. As in most samples, $InsP_3$ isomers were not detected; therefore,

these values were not used for subsequent statistical evaluation.

DISCUSSION

Disappearance of $InsP_6$ and Prececal Digestibility of P and Ca in Response to Wheat Genotypes and Supplemented Enzymes

Although the intrinsic phytase activity in GD_6 was higher than that in GD_7, there was no difference in the pc $InsP_6$ disappearance or pcdP. In studies with broilers, diets containing 20 or 40% of wheat genotype no. 7 exhibited an even higher pcdP than those including genotype no. 6 (Witzig et al., 2018). These results confirm those of former studies on broilers, which indicated a minor role of the intrinsic phytase activity in wheat-based diets on pcdP and pc $InsP_6$ disappearance (Shastak et al., 2014; Zeller et al., 2015a). Moreover, differences in the total of arabinoxylan and NSP contents, or in extract viscosity between both genotypes, seemed to be of minor importance in the present study. In contrast, former studies on broilers have reported a reduced pcdP with increased intraluminal viscosity induced by feeding high- rather than low-viscosity wheat cultivars (van der Klis et al., 1995). However, those authors used wheat varieties that differed more in extract viscosity than the wheat genotypes used in the present study.

In turkeys, beneficial effects of phytase supplementation on P digestibility often have been reported (Lescoat et al., 2005; Kozłowski et al., 2010). However, in most studies, fungal phytases were used, whereas only a few studies have used *E. coli* phytases (Applegate et al., 2003; Kozłowski et al., 2010; Adebiyi and Olukosi, 2015; Tatara et al., 2015). Our findings confirm those of Applegate et al. (2003), who reported 9% higher P retention in 3-week-old turkeys fed a corn-SBM-based diet supplemented with 500 FTU/kg of an *E. coli* phytase than in birds fed a non-supplemented diet. Tatara et al. (2015) noted positive effects of an *E. coli* phytase (500 FTU/kg) added to a diet containing corn, SBM, and wheat, on skeletal properties in 16-week-old turkeys. Under similar conditions, Kozłowski et al. (2010) achieved an 8% increase in the pcdP with 500 FTU and a significant increase of 16% with 1,000 FTU. However, in young turkeys fed semi-synthetic diets containing 20 to 60% wheat distillers' dried grains with soluble, 1,000 FTU of *E. coli* phytase did not affect the pcdP (%) (Adebiyi and Olukosi, 2015). Those authors explained the lack of a phytase effect by the low phytate P content of the diets.

The 15% increase in pc $InsP_6$ disappearance in GD with phytase addition is consistent with previous reports on broiler chickens fed wheat-based diets supplemented with the same phytase product (Zeller et al., 2015a). However, in BD, in which SBM was the main

source of P, a smaller increase in pc $InsP_6$ disappearance was achieved with phytase than in GD, while the opposite was found for the pcdP (%). As $InsP_6$-P: total P ratios were similar in all diets, this indicates a more efficient utilization of P from lower InsPs in SBM, than from wheat following the addition of phytase. The opposite was observed for non-supplemented diets; despite lower pc $InsP_6$ disappearance in GD, the pcdP (%) did not differ between BD and GD. This demonstrates that the pcdP is not necessarily correlated to the pc $InsP_6$ disappearance, probably due to differences in the degradation of lower InsPs.

The decreased pc $InsP_6$ disappearance in the non-supplemented GD with increased concentrations of $InsP_6$ underlines the limited capacity of animals to hydrolyze $InsP_6$, as was previously shown in broilers fed corn-SBM-based diets (Rodehutscord et al., 2017) and as we also observed in turkeys (unpublished). Moreover, these differences between BD and GD confirm that intrinsic phytase activity in wheat has only a minor role with respect to the degradation of $InsP_6$.

In addition to phytase, the response of turkeys to xylanase addition differed between animals fed BD and GD. Beneficial effects of xylanase on pcdP and pc $InsP_6$ disappearance were restricted to animals fed BD with SBM as the main P source. SBM contains a much higher NSP content than wheat, but arabinoxylans are less abundant (Choct, 2015). While xylanase was shown to increase the release of total sugars from SBM in vitro (Narasimha et al., 2013), this effect may depend on the specific enzyme used. For the xylanase used in the present work, xylose was not detected to be a relevant degradation product (AB Vista, personal communication). This suggests an alternate mode of action of xylanases is likely playing a role such as prebiotic release resulting in changes in microbial metabolites (Singh et al 2012; Lee et al., 2017). However, because the enzyme was fed for only a relatively short time period, it is unclear whether one of the main xylanase effects—the production of arabinoxylooligosaccharides—in the digestive tract (Courtin et al., 2008) could have evolved microbial volatile fatty acid production. Such effects seem to need a longer application period to change the microbiome and by this increase in fatty acid production (Lee et al., 2017).

Woyengo and Nyachoti (2011) noted that xylanase can interact synergistically with phytase in wheat-based diets for poultry only if the wheat has a NSP concentration higher than 10%. The genotypes used in the present study contained NSP concentrations close to 10% (9.6 and 10.5%), which under the given feeding conditions, were probably not sufficient for the animals to achieve a beneficial response to xylanase. Instead, the pc disappearance of $InsP_6$ decreased somewhat with the addition of xylanase in GD. The fact that the xylanase effect on pc $InsP_6$ disappearance was greater in the BD diet suggests that the NSP substrate content is not the only relevant factor in explaining xylanase effects. Zeller et al. (2015a) also reported no beneficial effect of xylanase when added in combination with phytase to wheat-based diets, on pcdP or pc $InsP_6$ disappearance in broilers. Those authors hypothesized that the accessibility of the remaining phytate would either not be restricted by arabinoxylans, or that other structures or the short retention time would not permit the sufficient degradation of the thick cell walls of the aleurone layer in wheat. Moreover, xylanase inhibitors in wheat are suspected to negatively affect the performance of exogenous xylanase (Smeets et al., 2014). However, as not all exogenous xylanases are inhibited, it is difficult to assess their role in the present study.

The results regarding the pcdCa are consistent with those of previous studies in turkeys, in which the pcdCa significantly increased in diets supplemented with 500 FTU/kg feed of an *E. coli* phytase (Kozłowski et al., 2010). The positive effect of phytase on the pcdCa in nonruminants is well known, and may be explained by reduced formation of insoluble Ca-phytate complexes in the small intestine due to lower proportions of $InsP_{3-6}$ entering this section (Adeola and Cowieson, 2011) or by the up-regulated absorption of Ca in response to increased P availability. This also may explain the higher numerical increase in the pcdCa with xylanase for BD than for GD. Moreover, differences in the pcdCa between BD compared to GD seems to be a result of the differences in the Ca level of those diet types.

Regardless of the diet used or the enzyme supplementation, the turkeys in the present study showed much lower pc $InsP_6$ disappearance (29 vs. 69%) and pcdP (36 vs. 55%) than did broiler chickens fed low-P diets based on wheat (Zeller et al., 2015a). In broiler studies using the same wheat genotypes fed to turkeys in the present study (Witzig et al., 2018), the pcdP of the respective GD was also 11 to 16% higher than that observed for the non-supplemented GD_6 and GD_7 in the present study. These observations are consistent with those from comparative studies on P retention and pcdP from low-P corn-based diets in 3- to 4-week-old broiler chickens and turkeys (Rodehutscord and Dieckmann 2005; Adebiyi and Olukosi, 2015). Reasons for the different capacity of pc $InsP_6$ hydrolysis and P digestion in young turkeys and broilers may include differences in the maturity of the small intestine (Adebiyi and Olukosi, 2015), endogenous P loss, pH along the gastrointestinal tract, and the passage rate (Rodehutscord and Dieckmann, 2005; Adebiyi and Olukosi, 2015).

Appearance of Lower Inositol Phosphate Isomers in Response to Wheat Genotypes and Supplemented Enzymes

In line with previous in vitro experiments (Sommerfeld et al., 2017) and broiler studies (Zeller et al., 2015a) on wheat-based diets, in the present study, D-Ins(1,2,3,4,5)P_5 represented the major $InsP_5$ in digesta

samples of turkeys. The corresponding enantiomer D-Ins(1,2,3,5,6)P$_5$ may be co-eluted with (but not separated from) D-Ins(1,2,3,4,5)P$_5$, and is known to be the main product of 6-phytases in wheat, which preferentially initiate phytate degradation at the L-6 (D-4) position (Nakano et al., 2000; Wu et al., 2015). Additionally, 3-phytases from wheat cereals are known to target the D-3 position, thus producing D-Ins(1,2,4,5,6)P$_5$ (Wu et al., 2015). The additional main route of degradation from both InsP$_5$ isomers is via D-Ins(1,2,5,6)P$_4$ (Nakano et al., 2000; Wu et al., 2015). In addition to the higher concentration of InsP$_6$ in GD, the increased intrinsic phytase activity in GD from wheat may thus explain the higher concentrations of D-Ins(1,2,3,4,5)P$_5$, D-Ins(1,2,4,5,6)P$_5$, and D-Ins(1,2,5,6)P$_4$ than with BD when diets were not supplemented with enzymes. Differences in the intrinsic phytase activity also may explain the effect of diet on InsP$_4$. Despite being of minor importance for most of the other InsPs or the pcdP, the higher intrinsic phytase activity in GD$_6$ may have resulted in the higher D-Ins(1,2,5,6)P$_4$ concentration than with GD$_7$.

As expected, supplementation with *E. coli* 6-phytase, which initiates dephosphorylation at the D-6 (L-4) position (Greiner et al., 2000), resulted in an increased concentration of D-Ins(1,2,3,4,5)P$_5$ in digesta samples. The additional main route of InsP$_5$ degradation by *E. coli* phytase is via D-Ins(2,3,4,5)P$_4$. The latter may be co-eluted with D-Ins(1,2,5,6)P$_4$, the concentration of which also was found to be increased with phytase supplementation. D-InsP(1,2,3,4)P$_4$ is a minor product of D-Ins(1,2,3,4,5)P$_5$ hydrolysis by *E.coli* phytase (Greiner et al., 2000), thus explaining the slight increase in its concentration in GD with phytase supplementation. These results confirm those reported from in vitro studies with wheat- or corn-based diets, in which the same phytase product was used (Sommerfeld et al., 2017). Similar effects of this *E. coli* phytase on InsP concentrations have been observed in digesta samples from the duodenum/jejunum section (Zeller et al., 2015a) or the lower ileum (Zeller et al., 2015b, 2015c) of broilers fed wheat or corn and SBM based diets.

Decreased Ins(1,2,3,4,6)P$_5$ concentrations in digesta samples with *E. coli* phytase supplementation were observed for different diets and sections of the upper gastrointestinal tract of broilers at several time points (Zeller et al., 2015a, 2015b, 2015c; Zeller et al., 2016). Zeller et al. (2015b, 2015c) speculated that the lower 5-phytase activity of microbial origin may explain these findings, as 5-phytases are known only from bacteria (Puhl et al., 2008; Haros et al., 2009) and lily pollen (Barrientos et al., 1994). Structural and functional changes of the microbiota in the gastrointestinal tract of broilers in response to phytase addition have previously been shown (Ptak et al., 2015; Witzig et al., 2015; Borda-Molina et al., 2016; Tilocca et al., 2016). Consistent with other reports (Zeller et al., 2015a), the concentration of D-Ins(1,2,4,5,6)P$_5$, which is a product of 3-phytases of plant or microbial origin, was not reduced in the presence of phytase.

Compared with phytase alone, supplementation with xylanase increased the InsP$_6$ concentration in digesta samples of GD. Data on lower InsPs indicated an accumulation of D-Ins(1,2,3,4,5)P$_5$ and a less rapid hydrolysis of InsP$_5$ to InsP$_4$ with GD than with BD, for which beneficial effects of xylanase were observed. Although the supplemented xylanase product possessed hydrolytic activity against insoluble and soluble arabinoxylans, it is possible that the release of high levels of soluble from insoluble NSP in wheat by xylanase increased the viscosity of digesta, and thus reduced the efficiency of phytase in GD. However, despite the decrease in pc InsP$_6$ disappearance, xylanase did not negatively affect the pcdP in GD, which contained more NSP substrate. Thus, an increase in digesta viscosity seems unlikely. The concentrations of InsP$_6$ and lower InsPs did not differ in the lower ileum of broiler chickens fed wheat-based diets supplemented either with phytase alone or with a combination of phytase and xylanase (Zeller et al., 2015a). Thus, differences in the pc hydrolysis of InsP$_6$ seem to exist between broilers and young turkeys when xylanase is added to wheat-based diets in combination with phytase.

To conclude, the results of the present study confirm the positive effect of supplementing wheat-based diets with an *E. coli* phytase on the pcdP in turkeys, as previously reported with fungal phytases by Juin et al. (2001). For the first time, these data report on the appearance of lower InsPs in the ileal digesta of turkeys fed wheat-based diets. These results revealed similar effects of *E. coli* phytase on the pattern of InsPs in the lower ileum, as previously reported for broilers; however, in combination with xylanase, a different response was observed in turkeys. Synergistic effects of both enzymes were restricted to the pc degradation of InsP$_6$ and pcdP of the cornstarch-SBM-based BD, and were not found for wheat-based diets. Thus, synergism between the enzymes seems to depend on the composition of the diet. The wheat genotype significantly affected animal performance, but the differences were not linked with the pcdP, pcdCa, or pc InsP$_6$ disappearance. Nevertheless, these results need to be considered in the context of the relatively short application period of the treatments employed. Moreover, our data suggest that intrinsic phytase activity in wheat is of only minor relevance to pcdP and pc InsP$_6$ degradation in turkeys.

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