

Comparative aspects of phytase and xylanase effects on performance, mineral digestibility, and ileal phytate degradation in broilers and turkeys

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ABSTRACT Two experiments were performed, using broilers or turkeys, each utilizing a 3 × 2 factorial arrangement, to compare their response to phytase and xylanase supplementation with growth performance, nutrient digestibility, and ileal phytate degradation as response criteria. For both experiments, 960 Ross 308 or 960 BUT 10 (0-day-old) birds were allocated to 6 treatments: (1) control diet, containing phytase at 500 FTU/kg; (2) the control diet with xylanase (16,000 BXU/kg); (3) the control diet supplemented on top with phytase (1,500 FTU/kg); (4) diet supplemented with 1,500 FTU/kg phytase and xylanase (16,000 BXU/kg); (5) the control diet supplemented with phytase (3,000 FTU/kg); and (6) diet supplemented with 3,000 FTU/kg phytase and xylanase (16,000 BXU/kg). Each treatment had 8 replicates of 20 birds each. Water and diets based on wheat, soybean meal, oilseed rape meal, and barley were available *ad libitum*. Body weight gain and feed intake were measured from 0 to 28 D, and feed conversion ratio (**FCR**) corrected for mortality was calculated. Ileal

digestibility for dry matter and minerals on day 7 and 28 were analyzed in addition to levels of inositol phosphate esters (InsP6-3) and *myo*-inositol. Statistical comparisons were performed using ANOVA. Xylanase supplementation improved 28D FCR in broilers and turkeys. Increasing doses of phytase reduced FI and improved FCR only in broilers. In broilers, the age × phytase interaction for phosphorous digestibility showed that increasing phytase dose was more visible on day 7, than on day 28. Mineral digestibility was lower in 28-day-old turkey compared with 7-day-old turkey. InsP6 disappearance increased with increasing phytase levels in both species, with lower levels analyzed in turkeys. InsP6 disappearance was greater in younger turkeys (day 7 compared with day 28). In conclusion, although broilers and turkeys shared several similarities in their growth and nutrient utilization responses, the outcomes of the 2 trials also differed in many aspects. Whether this is because of difference in diets (InsP or Ca level) or differences between species needs further investigation.

Key words: xylanase, phytase, ileal phytate degradation, broilers, turkeys

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INTRODUCTION

Primarily phytase, but also xylanase, is routinely used in poultry feed worldwide to address the issue of optimizing phosphorus (P) utilization and mitigating the negative impact of high dietary levels of nonstarch polysaccharides especially in younger birds. Studies abound, especially for broilers, on the application of phytase and

studying a wide array of factors influencing its efficacy (Adeola and Cowieson, 2011). There are comparatively much fewer studies on the use of phytase and/or xylanase for turkeys. And even fewer studies have directly compared the response of broilers and turkeys (Rodehutsord and Dieckmann, 2005; Pirgozliev et al., 2007; Adebiyi and Olukosi, 2015a, b, c; Ingelmann et al., 2019) to enzymes supplementation.

The reason for the disparity in the amount of studies done on the different species is not clear, but one possibility might be the view that broilers and turkeys are sufficiently close relatives such that observations made on one species could be directly applied, more or less, to the other. In view of the importance of P to the growth of all living things, response of all nonruminant animals

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to phytase supplementation of P-deficient diets is rather straight-forward, and improvement in growth performance and, nearly always, P utilization under such conditions are expected in virtually all species (Olukosi et al., 2007a, b; Adeola and Cowieson, 2011). But, it is unclear whether the same can be expected when the objective of using phytase, or in combination with other enzymes, goes further than improving growth and P utilization.

In addition, in recent years, a wider array of enzymes is used in combination in poultry feed, and the focus is on much more than the focal nutrient (e.g., phosphorus, carbohydrates, or amino acids). There is now considerable interest in understanding the role of higher levels of phytase supplementation than traditionally employed (Walk et al., 2013; Kim et al., 2018; Skřivan et al., 2018). This will have implications of wider scope than enhancing P utilization but also on more complete destruction of phytate, the release of lower inositol phosphate esters (InsPs), and production of *myo*-inositol (MI) in the digestive tract (Beeson et al., 2017; Sommerfeld et al., 2018; Walk and Rao, 2019). In addition, the use of other enzymes, for example xylanase, under such conditions of high levels of phytase supplementation is not clear.

It was the aim of the current comparative experiments to study the response of broiler chickens and turkey poults receiving diets supplemented with phytase and xylanase, individually or in combination. The responses of interest were growth performance, nutrient digestibility, and the hydrolysis of InsP6 to lower InsPs and MI. The responses were studied on day 7 (early) and day 28 (older) of age for elucidation of age-related differences. Direct species comparison also enabled clarification of species-dependent differences.

MATERIALS AND METHODS

The experimental procedures used in both experiments were approved by the Animal Experiment Committee of the Scotland's Rural College.

Birds and Housing

A total of 960 Ross 308 male broilers (Expt. 1) and 960 BUT 10 male turkey poults (Expt. 2) at 0-day-old were used for the studies. The 2 experiments were conducted within 4 wk of each other. Upon arrival, birds were placed immediately in 48 floor pens in an environmentally controlled rooms, with 20 birds per pen. Each pen for broilers was 2.1 m² in size, whereas the pens for turkeys were 1.7 m². All pens were equipped with a hopper feeder and a bell drinker, and white pine wood shavings were used as litter. Test diets and water were provided *ad libitum* throughout the trials. The rooms were preheated to 33°C 2 D before the commencement of the studies and kept at 33°C for the first 2 D. Then room temperature was gradually reduced to 23°C on day 21 and were kept at 22°C until the end of the trials. From day 0, the dark

hours were increased daily by 1 h from 24 h light until the light-dark cycle were 18 h light and 6 h dark daily.

Experimental Diets

Wheat, soybean meal, rapeseed, and barley were used as primary ingredients to formulate the experimental diets that met breeder recommendations for broilers and turkeys fed in one phase: from 0 to 28 D of age. The compositions of the experimental diets and the analyzed chemical composition are shown in Tables 1 and 2, respectively. For each species, one basal diet was made, then split equally into 6 subsamples each of which were supplemented with the experimental products: (1) control diet, containing the standard dose (500 FTU/kg) of phytase (Quantum Blue 5G; AB Vista, Marlborough, UK; 5,000 FTU/g); (2) the control diet with the standard dose (16,000 BXU/kg) of xylanase (Econase XT 25P; AB Vista, 160,000 BXU/g); (3) the control diet supplemented on top with 3-fold the standard dose of phytase (1,500 FTU/kg), also referred as superdosing; (4) the superdosed diet with xylanase; (5) the control diet supplemented with 6-fold the standard dose of phytase (3,000 FTU/kg), also referred to as megadosing; and

Table 1. Ingredient and calculated composition (%) of the experimental basal diets.

Items	Broilers	Turkeys
Wheat	62.53	50.00
Barley	5.00	5.00
Soybean meal	17.62	26.34
Rapeseed meal	10.00	11.77
Soya oil	0.50	1.03
Salt	0.22	0.18
Limestone	0.66	0.79
Dicalcium phosphate	1.01	2.45
Sodium bicarbonate	0.10	0.10
L-Tryptophan	0.05	0.00
Lysine HCl	0.55	0.65
DL-Methionine	0.33	0.33
L-Threonine	0.23	0.19
L-Valine	0.19	0.12
Trace mineral-vitamin premix ¹	0.50	0.50
Quantum Blue 5G ²	0.01	0.01
Titanium dioxide	0.5	0.5
Total	100	100
Calculated composition (% as fed)		
AME, kcal/kg	2,800	2,700
Crude protein	20.52	24.00
Ca	0.90	1.34
Available P	0.31	0.60
Fat	2.13	2.65
Crude fiber	3.33	3.58
D Met + Cys	0.92	1.01
D Lys	1.25	1.56
D Trp	0.26	0.25
D Thr	0.82	0.91
D Val	0.95	1.04

¹Vitamin/mineral premix supplied per kilogram of diet: vitamin A, 16,000 IU; vitamin D₃, 3000 IU; vitamin E, 25 IU; vitamin B₁, 3 mg; vitamin B₂, 10 mg; vitamin B₆, 3 mg; vitamin B₁₂, 15 µg; nicotinic acid, 60 mg; pantothenic acid, 14.7 mg; folic acid, 1.5 mg; biotin, 125 µg; choline chloride, 25 mg; Fe as iron sulfate, 20 mg; Cu as copper sulfate, 10 mg; Mn as manganese oxide, 100 mg; Co as cobalt oxide, 1.0 mg; Zn as zinc oxide, 82.222 mg; I as potassium iodide, 1 mg; Se as sodium selenite, 0.2 mg; and Mo as molybdenum oxide, 0.5 mg.

²Quantum Blue 5G, AB Vista, Marlborough, UK; 5000 FTU/g.

Table 2. Analyzed composition (% , as fed) of the experimental diets.

Treatment	Broilers										
	DM	N	Ca	Na	Mg	Cu, ppm	Fe, pp,	Mn, ppm	Zn, ppm	K	P
1	89.3	3.37	0.74	0.11	0.16	16	122	115	98	0.86	0.54
2	89.2	3.20	0.74	0.11	0.16	14	125	123	104	0.80	0.52
3	89.6	3.28	0.79	0.12	0.17	14	149	126	109	0.83	0.58
4	89.5	3.29	0.80	0.13	0.18	16	136	124	102	0.89	0.58
5	89.0	3.24	0.70	0.11	0.16	15	121	119	96	0.79	0.53
6	89.2	3.14	0.78	0.13	0.16	18	134	124	104	0.81	0.56
	Turkeys										
1	89.3	3.89	1.05	0.10	0.17	16	130	107	103	0.84	0.72
2	89.2	3.90	1.11	0.10	0.18	15	124	108	104	0.96	0.77
3	89.6	3.79	1.02	0.08	0.17	19	122	100	94	0.88	0.73
4	89.5	3.83	1.16	0.11	0.18	18	125	126	106	0.94	0.78
5	89.0	3.86	1.08	0.10	0.23	17	123	109	101	0.90	0.75
6	89.2	3.87	1.07	0.10	0.17	16	115	102	98	0.90	0.75

(6) the megadosed diet with xylanase; resulting in 6 experimental treatments. Diets were presented in mash form and contained 0.5% titanium dioxide as an indigestible marker. Experimental diets did not contain any coccidiostat, antibiotic, or any other growth promoter.

Experimental Procedures

In each experiment, birds and feed were weighed on D 0, 7, and 28. After weighing birds on D 7, ten birds were randomly selected per pen, euthanized by cervical dislocation, and the digesta from the terminal ileum (distal half, and up to 5 cm before ileo-cecal junction) was collected for further analysis. The remaining birds continued in the respective treatments until D 28 when an additional 5 randomly selected birds per pen were euthanized for ileal digesta collection. Digesta were flushed with distilled water, pooled per pen, and immediately placed on dry ice before freezing at -20°C in preparation for further processing.

Chemical Analyses

The ileal digesta were freeze-dried before milling and chemical analysis. All the samples for each experiment were milled through 0.5 mm sieve before analysis. Diets and ileal digesta samples were subsequently analyzed for DM, nitrogen (N), minerals (calcium, Ca; sodium Na; magnesium, Mg; copper, Cu; iron, Fe; manganese, Mn; zinc, Zn; potassium, K; phosphorus, P), titanium, InsP6-3, and MI. In addition, diet samples were analyzed for phytase and xylanase activities (Engelen et al., 2001).

Dry matter was determined by drying the samples in a drying oven at 100°C for 24 h (Method 934.01, AOAC, 2006). Nitrogen was determined by the combustion method (Method 968.06, AOAC, 2006). Mineral content was determined using inductively coupled plasma-optical emission spectroscopy (AOAC, 2006) following digestion, in turn, in concentrated HNO_3 and HCl. Titanium analysis was done using the method of Short et al. (1996). Analysis for phytate (InsP6), phytate esters (InsP5, InsP4, InsP3), and MI was performed according to methods described

by Pirgozliev et al. (2019) and Madsen et al. (2019). Disappearance of InsP6 was used as a synonym for “digestibility” and hence used the same formula in use for calculation of nutrient digestibility using the index method. Xylanase activity and phytase activity were analyzed by product-specific, validated ELISA methods, using Quantiplate Kits for Quantum Blue and Econase XT, both supplied by Envirologix (AB Vista Laboratories, Innovation & Technology Centre, Ystrad Mynach, UK).

Statistical Analysis

Performance data were subjected to two-way analysis of variance using JMP 14 Pro (SAS), whereas the effect of age for nutrient digestibility and ileal phytate degradation was also evaluated following a 3-way analysis of variance. Pen was the experimental unit. The nonparametric Wilcoxon test was used for mortality rates comparison between experimental treatments. Means were separated only when the treatment *P*-value was significant and then by using the least significant difference test. Statements of significance were based on *P*-value of equal to or less than 0.05.

RESULTS

The nutrient profiles in the diets were met (Table 2), but the analyzed phytase activity in some of the diets was greater than expected, mostly in those diets formulated to contain 500 or 1,500 FTU/kg (Table 3). Dietary InsPs levels (Table 4), in spite of similar diet compositions, differed between broilers and turkey diets.

Growth Performance Response in Broilers and Turkeys to Supplementation of Phytase and Xylanase

Overall broilers mortality was 1.56% (data not shown), and no differences were observed between the experimental treatments ($P = 0.569$). The effects of experimental treatments on broilers growth performance on D 0 to 7 and D 0 to 28 are shown in Table 5. No interactions were observed for any of the performance

Table 3. Analyzed enzyme activities in feed samples.

Treatments ¹	Expected		Broilers		Turkeys	
	Phytase, FTU/kg	Xylanase, BXU/kg	Phytase, ² FTU/kg	Xylanase, ³ BXU/kg	Phytase, FTU/kg	Xylanase, BXU/kg
1	500	0	750	<2,000	954	<2,000
2	500	16,000	865	16,100	994	16,100
3	1,500	0	1,190	<2,000	2,880	<2,000
4	1,500	16,000	2,250	14,300	1,840	15,000
5	3,000	0	2,720	<2,000	3,790	<2,000
6	3,000	16,000	3,930	17,400	2,460	13,300

¹Diets consisted in 6 experimental treatments: (1) diet containing standard dose of phytase without xylanase; (2) diet containing standard dose of phytase with xylanase; (3) diet containing superdosing of phytase without xylanase; (4) diet containing superdosing with xylanase; (5) diet containing megadosing of phytase without xylanase; (6) diet containing megadosing with xylanase.

²One FTU is defined as the amount of enzyme required to release 1 μ mol of inorganic P per minute from sodium phytate at 37°C and pH 5.5.

³One BXU is defined as the amount of enzyme that produces 1 nmol reducing sugars from birchwood xylan in one second at 50°C and pH 5.3.

parameters or periods measured. Increasing doses of phytase reduced FI ($P < 0.01$) and improved feed conversion ratio (**FCR**) ($P < 0.01$) between 0 and 28 D. Xylanase supplementation improved FCR between 0 and 28 D ($P < 0.05$).

In Expt. 2, overall mortality was 4.48% (data not shown), and no differences were observed between the experimental treatments ($P = 0.788$). The effects of experimental treatments on growth performance of turkeys on D 0 to 7 and D 0 to 28 are shown in Table 6. No interactions were observed for any of the performance parameters. Birds receiving the diets with 1,500 FTU/kg phytase had lower ($P < 0.05$) 28 D BW and weight gain compared with those receiving 500 or 3,000 FTU/kg. During 0 to 7 D period, birds receiving diets with 3,000 FTU/kg phytase had better FCR ($P < 0.05$) compared with those receiving diets with the other doses; however, this was not observed on D 28. Xylanase supplementation improved FCR between 0 and 28 D ($P < 0.05$).

Ileal Digestibility of Nutrient in Turkeys and Broilers

The ileal DM, N, and mineral digestibility of broiler chickens are presented in Table 7. There was no three-way interaction for any of the digestibility parameters measured. A significant age \times phytase interaction ($P < 0.05$) was observed for ileal Mg and N digestibility. At 28 D of age, Mg and N digestibility was higher for birds receiving 500 and 1,500 FTU/kg phytase, but 3,000 FTU/kg reduced ($P < 0.05$) digestibility to levels similar to that

observed on D 7. Birds at 28 D of age had greater ($P < 0.05$) ileal digestibility of DM, Fe, and K. On the other hand, ileal digestibility of Ca, Mn, Zn, and P were greater ($P < 0.05$) in younger birds at 7 D of age. There was a trend ($P < 0.10$) for age \times phytase interaction for P digestibility with the effect of increasing phytase dose being more visible on D 7 than on D 28. A significant phytase \times xylanase interaction ($P < 0.05$) was observed for DM, Na, Cu, and Mn digestibility. Sodium and Cu digestibility in diets with 500 FTU/kg phytase was greater ($P < 0.05$) when xylanase was also supplemented, but the effect was inconsistent at other phytase levels.

The ileal DM and mineral digestibility for turkeys are presented in Table 8. No three- or two-way interactions were observed for digestibility of any of the nutrients measured ($P > 0.05$). The 28-day-old turkeys had greater ($P < 0.01$) DM digestibility than younger poults. However, digestibility of all minerals was lower ($P < 0.01$) in 28-day-old turkeys compared with 7-day-old turkeys, with the exception of Cu for which there was no age effect. As observed in broilers, increasing dietary phytase levels led to higher P digestibility ($P < 0.01$), whereas xylanase supplementation tended ($P = 0.054$) to improve N digestibility.

Ileal Concentration of Inositol Phosphate Esters, Myo-Inositol, and Inositol-6-Phosphate Disappearance

Inositol phosphate concentration in ileal digesta and disappearance of InsP6 of broiler chickens are presented

Table 4. Analyzed dietary levels (nmol/g DM) of inositol and inositol phosphates.

Treatments ¹	Phytase, FTU/kg	Xylanase, BXU/kg	Broiler diets				Turkeys diets				
			InsP6	InsP5	InsP4	Inositol	InsP6	InsP5	InsP4	InsP3	Inositol
1	500	0	13,733	2,179	1,542	1,521	8,037	6,759	2,100	794	800
2	500	16,000	15,570	2,705	846	1,518	8,098	7,067	2,113	854	931
3	1,500	0	13,183	2,769	619	1,422	7,651	6,992	2,124	819	766
4	1,500	16,000	13,639	2,854	603	1,373	8,503	7,335	2,235	900	960
5	3,000	0	13,605	3,451	751	1,549	8,396	7,017	2,285	873	794
6	3,000	16,000	10,581	2,379	567	1,058	7,824	6,924	2,383	844	776

¹Diets consisted in 6 experimental treatments: (1) diet containing standard dose of phytase without xylanase; (2) diet containing standard dose of phytase with xylanase; (3) diet containing superdosing of phytase without xylanase; (4) diet containing superdosing with xylanase; (5) diet containing megadosing of phytase without xylanase; (6) diet containing megadosing with xylanase.

Table 5. Growth performance, at different ages, of the broiler chickens receiving phytase and xylanase individually or in combination¹.

Phytase, FTU/ kg	Xylanase, BXU/kg	Body weight (g)			0–7 D			0–28 D		
		Day 0	Day 7	Day 28	BWG (g/ bird)	FI (g/ bird)	FCR (g/g)	BWG (g/bird)	FI (g/bird)	FCR (g/g)
Main effects for phytase										
500		40.46	124.7	1,068	84.3	141.5	1.684	1,028	2,416 ^a	2.368 ^a
1,500		40.64	124.4	1,098	83.8	145.6	1.742	1,058	2,291 ^{a,b}	2.173 ^b
3,000		40.49	124.1	1,097	83.6	144.5	1.738	1,057	2,153 ^b	2.070 ^b
Least significant difference		0.3	4.6	73	4.65	5.1	0.081	73	141	0.183
Main effect for xylanase										
	0	40.55	123.2	1,066	82.7	142.3	1.726	1,025	2,331	2.294 ^a
	16,000	40.51	125.6	1,110	85.1	145.4	1.716	1,070	2,242	2.113 ^b
Least significant difference		0.24	3.8	60	3.8	4.2	0.066	60	115	0.15
Interaction effects										
500	0	40.6	125.4	1,048	84.9	142.0	1.676	1,007	2,504	2.498
500	16,000	40.3	124.0	1,088	83.7	141.0	1.691	1,048	2,329	2.237
1,500	0	40.7	122.5	1,108	81.9	141.5	1.731	1,068	2,327	2.189
1,500	16,000	40.6	126.3	1,088	85.7	149.8	1.754	1,048	2,255	2.157
3,000	0	40.3	121.6	1,040	81.3	143.5	1.772	1,000	2,163	2.195
3,000	16,000	40.6	126.5	1,153	85.9	145.4	1.703	1,113	2,142	1.945
Least significant difference		0.42	6.5	103	6.58	7.2	0.114	103	199	0.259
<i>P</i> -values for main effects and interaction										
Phytase		0.401	0.960	0.642	0.952	0.252	0.275	0.644	0.002	0.007
Xylanase		0.783	0.206	0.141	0.201	0.148	0.760	0.140	0.125	0.019
Phytase × Xylanase		0.134	0.341	0.197	0.406	0.180	0.451	0.199	0.541	0.373

^{a-c}Values in the same column with different letters are significantly different ($P < 0.05$).

Abbreviations: BWG, body weight gain; FCR, feed conversion ratio.

¹Data are means of 8 pens with 20 birds in the first period (day 0–7) and with 10 birds per pen in the second period (day 0–28).

in Table 9. A 3-way age × phytase × xylanase interaction was observed for InsP4 and InsP3 ($P < 0.05$). The concentration of InsP4 and InsP3 in 7-day-old broilers was largely uninfluenced by phytase and xylanase

supplementation. However, on D 28, birds receiving 1,500 FTU/kg phytase without xylanase had greater ($P < 0.05$) InsP4 and InsP3 concentration in ileal digesta compared with when xylanase was

Table 6. Growth performance, at different ages, of the turkey poulters receiving phytase and xylanase individually or in combination¹.

Phytase, FTU/ kg	Xylanase, BXU/kg	BW (g)			0–7 D			0–28 D		
		Day 0	Day 7	Day 28	BWG (g/ bird)	FI (g/ bird)	FCR (g/g)	BWG (g/bird)	FI (g/bird)	FCR (g/g)
Means for the main effects for phytase										
500		51.6	128	1,034 ^a	76.3	118	1.548 ^a	983 ^a	1,830	1.864
1,500		51.9	129	999 ^b	76.7	119	1.548 ^a	947 ^b	1,793	1.896
3,000		51.5	131	1,048 ^a	79.8	116	1.449 ^b	997 ^a	1,858	1.866
Least significant difference		0.30	5	34	4	7	0.078	34	93	0.097
Means for the main effect for xylanase										
	0	51.5	129	1,021	77.8	119	1.532	970	1,863	1.924 ^a
	16,000	51.8	129	1,033	77.5	116	1.498	981	1,791	1.827 ^b
Least significant difference		0.24	4	28	4	6	0.063	28	76	0.079
Phytase × xylanase interaction										
500	0	51.6	128	1,017	76.7	120	1.562	965	1,844	1.912
500	16,000	51.6	128	1,052	76.0	116	1.534	1,001	1,815	1.816
1,500	0	51.7	129	997	77.1	119	1.547	945	1,846	1.956
1,500	16,000	52.1	128	1,001	76.4	118	1.549	949	1,739	1.836
3,000	0	51.3	131	1,051	79.8	119	1.487	999	1,899	1.904
3,000	16,000	51.6	132	1,046	79.9	113	1.411	995	1,818	1.828
Least significant difference		0.42	6	49	6	10	0.110	49	131	0.137
<i>P</i> -values for main effects and interaction										
Phytase			0.296	0.017	0.236	0.669	0.019	0.015	0.367	0.754
Xylanase			0.948	0.404	0.835	0.231	0.288	0.414	0.060	0.017
Interaction			0.971	0.469	0.977	0.814	0.592	0.462	0.690	0.898

^{a-c}Values in the same column with different letters are significantly different ($P < 0.05$).

¹Data are means of 8 pens with 20 birds in the first period (day 0–7) and with 10 birds in the second period (day 0–28).

Abbreviations: BWG, body weight gain; FCR, feed conversion ratio.

Table 7. Effect of phytase and xylanase supplementation individually or in combination on ileal digestibility of nutrients at different ages in broiler chickens¹.

Age, D	Phytase, FTU/kg	Xylanase, BXU/kg	DM	Ca	Na	Mg	Cu	Fe	Mn	Zn	K	P	N
Means for age × phytase interaction													
7	500		68.1	69.8	-4.8	24.1 ^c	29.8	22.9	30.4	31.3	72.9	72.0	78.5 ^d
	1,500		68.7	68.5	10.3	26.4 ^c	23.0	21.5	28.4	30.4	72.8	78.4	79.6 ^{c,d}
	3,000		68.9	70.8	19.8	28.1b ^c	30.6	24.0	31.9	32.2	74.7	83.6	80.8 ^c
28	500		73.3	57.1	-2.7	34.1 ^a	28.4	31.1	20.6	31.8	80.1	67.8	84.4 ^a
	1,500		72.8	55.6	-1.1	32.6 ^{a,b}	31.9	26.3	18.9	27.3	81.1	70.6	83.9 ^{a,b}
	3,000		70.6	53.8	1.2	26.1 ^c	23.8	25.8	18.1	25.8	78.3	73.3	82.7 ^b
Least significant difference			2.9	5.6	17.1	5.5	12.6	5.2	5.0	4.3	3.4	3.6	1.7
Means for phytase × xylanase interaction													
	500	0	69.9 ^{a,b}	62.7	-17.4 ^b	28.2	20.8 ^b	26.3	23.6 ^{b,c}	30.9	75.2	69.8	80.8
	500	16,000	71.5 ^a	64.3	10.0 ^a	30.0	37.4 ^a	27.8	27.4 ^{a,b}	32.3	77.9	70.0	82.1
	1,500	0	71.2 ^a	59.7	5.9 ^a	31.2	22.3 ^b	23.3	22.7 ^{b,c}	29.2	75.9	73.6	81.7
	1,500	16,000	70.2 ^a	64.5	3.3 ^a	27.8	32.6 ^{a,b}	24.5	24.6 ^{a,c}	28.6	77.9	75.4	81.8
	3,000	0	67.2 ^b	64.1	8.6 ^a	24.1	31.9 ^{a,b}	25.0	28.7 ^a	29.8	73.4	80.0	80.7
	3,000	16,000	72.3 ^a	60.6	12.4 ^a	30.1	22.5 ^b	24.8	21.3 ^c	28.2	79.6	76.9	82.8
Least significant difference			2.9	5.6	17.1	5.5	12.6	5.2	5.0	4.3	3.4	3.6	1.7
Means for the simple effect of age × phytase × xylanase													
7	500	0	66.7	69.4	-22.2	22.3	15.1	23.5	29.7	31.6	71.0	71.8	77.8
	500	16,000	69.5	70.2	12.5	25.8	44.6	22.4	31.1	31.0	74.9	72.3	79.3
	1,500	0	67.9	66.2	10.8	27.1	15.1	19.2	28.8	31.1	70.6	76.3	78.9
	1,500	16,000	69.4	70.9	9.8	25.7	30.9	23.9	28.1	29.7	74.9	80.5	80.3
	3,000	0	66.9	72.4	17.0	27.2	34.3	23.0	34.8	32.8	73.0	85.8	79.6
	3,000	16,000	71.0	69.3	22.7	29.0	27.0	25.0	29.1	31.6	76.4	81.5	82.0
28	500	0	73.1	55.9	-12.7	34.0	26.6	29.1	17.5	30.1	79.4	67.9	83.9
	500	16,000	73.5	58.3	7.4	34.2	30.2	33.2	23.6	33.5	80.8	67.7	84.9
	1,500	0	74.6	53.1	1.0	35.3	29.4	27.5	16.7	27.2	81.3	70.9	84.4
	1,500	16,000	70.9	58.0	-3.2	29.8	34.4	25.1	21.1	27.5	80.9	70.3	83.3
	3,000	0	67.6	55.7	0.3	21.1	29.5	27.0	22.7	26.7	73.9	74.3	81.8
	3,000	16,000	73.7	51.8	2.1	31.2	18.0	24.6	13.5	24.9	82.7	72.3	83.6
Least significant difference			4.0	7.9	24.1	7.8	17.8	7.4	7.1	6.0	4.8	5.2	2.4
<i>P</i> -values for main effects and interactions ²													
Age			<0.001	<0.001	0.066	0.004	0.960	0.002	<0.001	0.019	<0.001	<0.001	<0.001
Phytase			0.592	0.755	0.070	0.442	0.902	0.243	0.565	0.144	0.930	<0.001	0.875
Xylanase			0.025	0.556	0.059	0.365	0.121	0.596	0.683	0.845	0.001	0.708	0.019
Age × phytase			0.213	0.479	0.231	0.011	0.214	0.246	0.434	0.091	0.137	0.069	0.005
Age × xylanase			0.282	0.916	0.468	0.923	0.071	0.505	0.472	0.505	0.772	0.619	0.230
Phytase × xylanase			0.013	0.118	0.039	0.065	0.016	0.896	0.006	0.630	0.191	0.179	0.269
Age × phytase × xylanase			0.205	0.952	0.871	0.227	0.487	0.232	0.424	0.762	0.097	0.414	0.620

^{a-c}Values in the same column with different letters are significantly different ($P < 0.05$).

¹Data are means of 10 birds (day 7) and 5 birds (day 28) per pen with 8 pens per treatment.

²For clarity in the table, the means for some main effects and 2-way interactions were not presented in the table but could be computed from the data on 3-way interaction.

supplemented. In contrast, birds supplemented with 3,000 FTU/kg phytase plus xylanase had greater ($P < 0.01$) InsP4 and InsP3 concentration compared with the same diet without xylanase. Age × phytase interaction was significant ($P < 0.01$) for InsP6 and InsP5. Increasing doses of phytase reduced ($P < 0.01$) the concentration of InsP6 and InsP5 on D 7 and 28, with the exception of InsP5 which was only marginally decreased at 3,000 FTU/kg phytase on D 7. *Myo*-inositol concentration was lower in older birds ($P < 0.01$) but increased ($P < 0.01$) with increasing levels of dietary phytase. InsP6 disappearance increased ($P < 0.01$) with increasing phytase and a trend ($P = 0.055$) for phytase × xylanase interaction for InsP6 disappearance was shown by no effect of xylanase supplementation in diets with 500 or 1,500 FTU/kg phytase. On the other hand, there was lower ($P < 0.05$) InsP6 disappearance in diet containing 3,000 FTU/kg phytase plus xylanase, compared with when no xylanase was supplemented.

Inositol phosphate concentration in ileal digesta and disappearance of InsP6 for the turkeys are presented in Table 10. There were no age × phytase × xylanase, phytase × xylanase, or age × xylanase interactions nor main effect of xylanase supplementation. Age × phytase interaction was observed ($P < 0.05$) for InsP6 and InsP5 concentrations. At both D 7 and 28, phytase supplementation reduced ($P < 0.01$) InsP6 and InsP5 concentrations, although the reduction was more evident between 500 and 1,500 FTU/kg levels. InsP4 and InsP3 concentrations were greater, whereas MI concentration was lower in older birds ($P < 0.05$). In addition, InsP6 disappearance was greater ($P < 0.01$) in younger birds and increased ($P < 0.01$) in response to increasing phytase level.

DISCUSSION

The objective of the current experiment was to study the influence of supplementation of phytase and

Table 8. Effect of phytase and xylanase supplementation individually or in combination on ileal digestibility of nutrients at different ages in turkey poult¹.

Age, D	Phytase, FTU/kg	Xylanase, BXU/kg	DM	Ca	Na	Mg	Cu	Fe	Mn	Zn	K	P	N
Means for main effect of age, D													
7			63.5 ^b	58.9 ^a	26.3 ^a	39.3 ^a	34.4	20.1 ^a	20.5 ^a	31.7 ^a	66.8 ^a	68.8 ^a	84.5 ^a
28			68.4 ^a	51.0 ^b	-78.2 ^b	33.3 ^b	34.0	10.2 ^b	4.8 ^b	17.8 ^b	54.4 ^b	62.2 ^b	82.4 ^b
Least significant difference			1.6	3.5	16.0	3.4	13.6	7.3	4.1	3.9	2.9	3.1	0.9
Means for main effect of phytase													
	500		66.0	53.7	-22.8	36.0	37.7	13.0	12.7	24.1	61.1	62.4 ^b	83.6
	1,500		66.6	55.0	-26.2	36.0	26.0	17.4	13.3	24.5	59.3	65.7 ^{a,b}	83.5
	3,000		65.3	56.2	-28.8	36.9	38.9	15.2	11.9	25.6	61.4	68.5 ^a	83.2
Least significant difference			1.9	4.3	19.6	4.2	16.6	9.0	5.0	4.8	3.5	3.8	1.1
Means for main effect of xylanase													
		0	65.7	53.8	-32.9	35.5	33.5	12.9	12.3	23.7	60.1	64.9	83.0
		16,000	66.2	56.1	-19.0	37.0	34.9	17.4	13.0	25.8	61.1	66.1	83.9
Least significant difference			1.6	3.5	16.0	3.4	13.6	7.3	4.1	3.9	2.9	3.1	0.9
Means for the simple effect of age × phytase × xylanase													
7	500	0	62.3	55.5	29.8	36.9	41.5	14.1	17.7	28.3	64.3	63.6	84.0
	500	16,000	64.8	55.8	21.4	40.0	39.7	21.7	20.2	33.3	67.5	65.2	84.7
	1,500	0	64.7	58.9	25.4	40.5	19.6	24.0	23.1	30.0	67.4	68.6	84.0
	1,500	16,000	64.2	59.4	28.8	38.8	17.5	22.9	20.6	31.0	66.0	69.5	84.8
	3,000	0	61.2	58.9	26.2	37.1	41.5	13.1	19.5	30.3	65.1	71.0	83.5
28	3,000	16,000	63.7	65.1	26.3	42.5	46.9	24.8	21.6	37.5	70.4	75.1	85.8
	500	0	67.6	49.3	-85.7	30.3	29.5	0.8	4.6	15.3	56.2	59.1	82.6
	500	16,000	69.2	53.9	-56.9	36.7	40.4	15.3	8.5	19.7	56.6	61.6	83.2
	1,500	0	69.4	49.2	-104.2	31.2	33.4	11.4	4.3	17.8	51.0	61.9	82.0
	1,500	16,000	68.1	52.6	-54.7	33.4	33.5	11.2	5.3	19.4	52.7	62.7	83.2
	3,000	0	68.9	51.1	-88.9	37.2	35.4	14.2	4.5	20.8	56.6	65.1	81.9
	3,000	16,000	67.3	49.9	-78.7	30.8	31.8	8.6	1.8	13.8	53.6	62.7	81.7
	Least significant difference			3.8	8.5	39.2	8.4	33.2	18.0	10.1	9.7	7.1	7.5
<i>P</i> -values for main effects and interactions ²													
Age			<0.001	<0.001	<0.001	0.001	0.950	0.010	<0.001	<0.001	<0.001	<0.001	<0.001
Phytase			0.401	0.497	0.847	0.895	0.243	0.627	0.850	0.829	0.440	0.010	0.737
Xylanase			0.504	0.197	0.100	0.403	0.833	0.235	0.742	0.314	0.490	0.431	0.054
Age × phytase			0.784	0.234	0.801	0.846	0.281	0.888	0.569	0.623	0.340	0.440	0.399
Age × xylanase			0.206	0.985	0.066	0.668	0.888	0.671	1.000	0.240	0.376	0.543	0.445
Phytase × xylanase			0.316	0.988	0.556	0.420	0.945	0.423	0.706	0.626	0.907	0.942	0.930
Age × phytase × xylanase			0.605	0.344	0.668	0.136	0.818	0.398	0.704	0.264	0.298	0.593	0.384

^{a-c}Values in the same column with different letters are significantly different ($P < 0.05$).

¹Data are means of 10 birds (day 7) and 5 birds (day 28) per pen with 8 pens per treatment.

²For clarity in the table, the means for 2-way interactions were not presented in the table but could be computed from the data on 3-way interaction.

xylanase, at different rates of phytase supplementation, on the growth performance, nutrient utilization, and InsPs characteristics in the digestive tract of broiler and turkeys. Because the aim was to compare responses in the 2 species, it would have been ideally preferable to run the experiment with both species reared together and subjected to the same environment as done in a similar experiment (Pirgozliev et al., 2007). However, the recommended management procedures (temperature and light regimes) were different for BUT 10 poult and Ross 308 broilers, such that it was not advisable to rear them together. Thus, the approach used in the current experiment is similar to what we and other authors have used (Rodehutscord and Dieckmann, 2005; Adebisi et al., 2015a; b; c) in similar comparative studies. Consequently, this needs to be borne in mind regarding the comparative aspects of the data.

There is a plethora of data on the effect of phytase and xylanase supplementation in broilers (Olukosi et al., 2007a, 2010; Tiwari et al., 2010; Zeller et al., 2015) but much less information about turkeys (Ingelmann et al.,

2018, 2019). In addition, the traditional conventional phytase levels in diets ranges between 500 and 1000 FTU/kg (Adeola and Cowieson, 2011). However, with the possibility of using much higher phytase levels (superdosing or megadosing as defined in this experiment) with the objective of attaining more complete phytate destruction (Beeson et al., 2017; Gautier et al., 2018; Sommerfeld et al., 2018), research into the possible impact of xylanase supplementation at those levels is also warranted.

Comparative Growth Performance Response to Phytase and Xylanase Supplementation in Diets for Broilers and Turkeys

Growth performance was evaluated in both species at 7 D of age to ascertain early age response to the dietary interventions. In general, performance for broilers remained below the breeder's targets throughout the

Table 9. Inositol phosphate concentration (nmol/g DM) in ileal digesta and disappearance (%) of InsP6 of broiler chickens¹.

Age, D	Phytase, FTU/kg	Xylanase, BXU/kg	InsP6	InsP5	InsP4	InsP3	Myo- inositol	InsP6 disappearance
Means for age × phytase interaction								
7	500		18,636 ^b	3,162 ^{b,c}	904	660	13,958	60.2
	1,500		13,193 ^c	2,830 ^{b,c}	1,177	708	18,120	70.3
	3,000		9,000 ^{c,d}	2,218 ^c	1,248	745	19,563	76.7
28	500		27,755 ^a	7,066 ^a	4,800	1,556	11,393	50.9
	1,500		13,247 ^c	4,082 ^b	6,609	2,773	14,473	73.6
	3,000		7,275 ^d	2,427 ^c	5,025	2,093	18,042	82.6
Least significant difference			5,034	1,312	1,517	434	3,152	10
Means for the phytase × xylanase interaction								
	500	0	21,867	5,051	3,088	1,186	13,105	54.4
	500	16,000	24,524	5,177	2,617	1,030	12,245	56.8
	1,500	0	13,585	3,737	4,839	1,969	15,924	72.1
	1,500	16,000	12,855	3,175	2,948	1,512	16,669	71.8
	3,000	0	5,628	1,474	2,100	1,213	19,994	86.8
	3,000	16,000	10,647	3,171	4,174	1,624	17,610	72.4
Least significant difference			5,034	1,312	1,517	434	3,152	10
Means for the simple effect of age × phytase × xylanase								
7	500	0	17,546	2,955	884 ^e	713 ^e	15,166	58.9
	500	16,000	19,726	3,369	924 ^e	608 ^e	12,751	61.5
	1,500	0	13,561	2,818	1,037 ^e	655 ^e	17,821	68.9
	1,500	16,000	12,824	2,842	1,318 ^{d,e}	761 ^e	18,419	71.6
	3,000	0	5,920	1,382	798 ^e	663 ^e	19,917	86.0
	3,000	16,000	12,080	3,055	1,699 ^{d,e}	827 ^e	19,208	67.4
28	500	0	26,187	7,146	5,292 ^{b,c}	1,659 ^{c,d}	11,045	49.8
	500	16,000	29,322	6,986	4,309 ^c	1,452 ^d	11,740	52.1
	1,500	0	13,609	4,656	8,641 ^a	3,282 ^a	14,028	75.3
	1,500	16,000	12,885	3,507	4,578 ^{b,c}	2,263 ^{b,c}	14,919	72.0
	3,000	0	5,336	1,566	3,402 ^{c,d}	1,764 ^{b-d}	20,071	87.6
	3,000	16,000	9,214	3,287	6,649 ^{a,b}	2,422 ^b	16,012	77.5
Least significant difference			7,120	1,856	2,145	614	4,458	15
<i>P</i> -values for main effects and interactions ²								
Age			0.093	<0.001	<0.001	<0.001	0.006	1.000
Phytase			<0.001	<0.001	0.144	0.001	<0.001	<0.001
Xylanase			0.117	0.273	0.829	0.599	0.365	0.177
Age × phytase			0.007	0.001	0.239	0.001	0.640	0.093
Age × xylanase			0.881	0.460	0.259	0.342	0.993	0.908
Phytase × xylanase			0.277	0.051	0.002	0.024	0.382	0.055
Age × phytase × xylanase			0.898	0.808	0.011	0.038	0.357	0.613

^{a-c}Values in the same column with different letters are significantly different ($P < 0.05$).

¹Data are means of 10 birds (day 7) and 5 birds (day 28) per pen with 8 pens per treatment.

²For clarity in the table, the means for some main effects and 2-way interactions were not presented in the table but could be computed from the data on 3-way interaction.

study. This could be partly because of the higher dietary phytate content of InsP6 in broilers diets compared with turkeys, diets. This also might have influenced the age effects to some extent as high dietary content from oilseed rape meal might have impacted nutrient digestibility in young birds. Whereas turkeys had improved FCR with increasing phytase levels, no such response was observed for broilers on D 7. However, there was improvement in FCR for both turkeys and broiler chickens on D 28 in the birds receiving xylanase-supplemented diets, whereas positive phytase effect on FCR at D 28 was only observed in broiler chickens.

Different species have different rates of growth of the body components which is reflected in their gain to feed ratio. There are very limited studies that directly compare the growth response of broilers and turkeys in phosphorus-associated or enzyme-associated studies. Rodehutsord and Dieckmann (2005) reported FCR of 1.3 and 1.7 for turkeys and broilers, respectively, when the birds received the same diet. Pirgozliev et al. (2007)

reported FCR of 1.7 and 1.2 for turkeys and broilers receiving phytase-supplemented diets when reared together. Differences observed between the 2 studies are very likely because of various factors, 2 of which are mentioned below. In the Rodehutsord and Dieckmann (2005) study, the species were fed the same diet which, therefore, met the requirement of one of the species but not the other, and this could have influenced the response of the different species to inorganic P supplementation. In the Pirgozliev et al. (2007) study, the diets were specifically targeted to each of the species, but rearing the birds under the same environmental condition could have influenced their response because the recommended rearing temperatures and lighting differ for turkeys and broilers.

The lack of effect of increasing phytase level in the current study should be understood against the backdrop of the fact that all the diets were supplemented with phytase. Consequently, the observation of no growth performance effect in response to phytase cannot be related to P

Table 10. Inositol phosphate concentration (nmol/g DM) in ileal digesta and disappearance (%) of InsP6 of turkeys^{1,2}.

Age, D	Phytase, FTU/kg	Xylanase, BXU/kg	InsP6	InsP5	InsP4	InsP3	Myo- inositol	InsP6 disappearance
Means for the main effects for age								
7			11,255	3,078	4,490 ^b	2,354 ^b	4,177 ^a	49.0 ^a
28			16,997	5,225	9,868 ^a	2,873 ^a	2,991 ^b	34.7 ^b
Least significant difference			2,501	692	1,567	399	392	9
Means for the main effect of phytase								
	500		23,375	6,192	6,251	2,124 ^b	2,712 ^c	3.1 ^c
	1,500		12,339	4,057	7,951	2,859 ^a	3,600 ^b	50.1 ^b
	3,000		6,664	2,206	7,335	2,857 ^a	4,441 ^a	72.4 ^a
Least significant difference			3,063	848	1,919	489	481	12
Age × phytase Interaction effects								
7	500		18,234 ^b	4,452 ^b	3,897	2,102	3,158	16.2
	1,500		10,489 ^{c,d}	3,171 ^c	5,149	2,565	4,156	54.2
	3,000		5,041 ^e	1,611 ^d	4,424	2,396	5,217	76.6
28	500		28,516 ^a	7,932 ^a	8,606	2,146	2,265	-10.1
	1,500		14,190 ^{b,c}	4,944 ^b	10,753	3,154	3,045	46.0
	3,000		8,287 ^{d,e}	2,800 ^{c,d}	10,246	3,318	3,665	68.3
Least significant difference			4,331	1,199	2,713	691	680	16
Age × phytase × xylanase								
7	500	0	18,383	4,527	3,856	2,064	3,023	10.8
	500	16,000	18,086	4,377	3,938	2,140	3,294	21.7
	1,500	0	9,913	2,940	4,763	2,588	4,137	54.7
	1,500	16,000	11,064	3,401	5,534	2,541	4,175	53.7
	3,000	0	5,464	1,678	4,601	2,570	5,350	74.8
	3,000	16,000	4,618	1,544	4,247	2,221	5,083	78.4
28	500	0	29,915	8,537	8,386	1,993	2,208	-19.1
	500	16,000	27,118	7,327	8,825	2,299	2,322	-1.1
	1,500	0	14,076	4,731	10,504	3,214	3,298	45.7
	1,500	16,000	14,303	5,157	11,002	3,095	2,791	46.3
	3,000	0	8,388	2,851	10,504	3,429	3,825	69.7
	3,000	16,000	8,185	2,750	9,987	3,208	3,505	66.8
Least significant difference			6,125	1,696	3,837	977	961	23
<i>P</i> -values for the main effects and interactions								
Age			<0.001	<0.001	<0.001	0.012	<0.001	0.004
Phytase			<0.001	<0.001	0.210	0.004	<0.001	<0.001
Xylanase			0.715	0.735	0.846	0.769	0.572	0.306
Age × phytase			0.043	0.024	0.830	0.202	0.385	0.208
Age × xylanase			0.713	0.612	0.987	0.814	0.525	0.933
Phytase × xylanase			0.769	0.424	0.854	0.625	0.551	0.365
Age × phytase × xylanase			0.878	0.773	0.985	0.953	0.866	0.842

^{a-c}Values in the same column with different letters are significantly different ($P < 0.05$).

¹Data are means of 10 birds (day 7) and 5 birds (day 28) per pen with 8 pens per treatment.

²For clarity in the table, the means for some main effects and 2-way interactions were not presented in the table but could be computed from the data on 3-way interaction.

availability because, notionally, 500 FTU/kg phytase should release sufficient P to meet the needs for the growth of the birds. By the same token, the improvement in FCR observed on day 7 for turkeys was also likely not driven by enhancement of P availability in as much as all the diets probably met P requirement because of phytase supplementation. Apparently, the improvement in FCR for the turkeys at 7 D old was driven both by a marginal increase in weight gain and simultaneous decrease in feed intake, which was not observed in the broiler chickens. A closer look at the data showed that the improvement in FCR for turkeys was primarily driven by the drop in feed intake in the xylanase-supplemented diets, and a marginally greater weight gain at the highest level of phytase supplementation.

On the other hand, xylanase supplementation improved FCR in both broiler chickens and turkeys on D 28. The improvement in FCR in both species was

driven primarily by a reduction in feed intake but also an increase in weight gain in response to xylanase supplementation. The lack of interaction indicated that the response to xylanase was not influenced by the level of phytase supplementation and vice versa. Some have suggested that complementarity of enzyme activities might help to maximize response to enzyme supplementation (Meng et al., 2005), but the response of poultry species to combination of enzymes with different activities can be additive (Cowieson and Adeola, 2005; Olukosi et al., 2010) or not (Karimi et al., 2013). These observations are inextricably linked with differences in nutritional profile of the diets (i.e., what the limiting nutrients are), the enzymes involved, or a combination of these factors (Cowieson, 2010). If enzymes, via microbial changes in the intestine, influence growth, it also cannot be excluded that longer feeding periods are needed to see such combination effects.

The observed decrease in feed intake in the current experiment is possibly an indication of optimum use of nutrients because it was accompanied by an increase in weight gain. This likely resulted from an enhancement of digestible nutrient intake, driven by an increase in nutrient digestibility. There are clear differences in the growth and development of digestive organs in broilers and turkeys. [Uni et al. \(1995; 1999\)](#) showed the digestive tract of turkey poults matures at a slower rate compared with broiler chickens. In addition, the authors observed that the activities of specific digestive enzymes were lower in turkeys compared with broilers.

Comparative Nutrient Utilization Response to Phytase and Xylanase Supplementation in Diets for Broilers and Turkeys

Unlike DM, the apparent digestibility of N, Ca, and P were generally lower on D 28 compared with D 7 in both broilers and turkeys. An exception was that, for broilers, N digestibility was greater on D 28 compared with D 7. For turkeys, there was no interaction between age and the enzymes effects on nutrient digestibility, but there were limited interactions of age with enzymes supplementation for broilers. The major effect was phytase and age interaction on N digestibility. Generally, phytase supplementation increased N digestibility in a somewhat stepwise fashion on D 7, but not on D 28. Various studies have reported an age effect on enzyme supplementation efficacy ([Olukosi et al., 2007a; Gracia et al., 2009](#)). Most studies suggest that the marginal effect of enzyme supplementation diminishes as birds grow older, when potentially the birds have a more physiologically matured digestive tract. However, the difference between the current study and the ones previous was that all the diets in the current study were supplemented with phytase. This follows the commercial practice of having phytase added to all diets. Consequently, the lack of response to phytase level should be translated in the context of all diets having added phytase, albeit, at different rates.

Further calculations to compare the response of each species to phytase supplementation, relative to the control diet, revealed that on D 7, turkeys had greater positive response with regards to Ca, Na, and Zn digestibility, whereas broilers had greater response in N digestibility. On D 28, turkeys had greater positive response for DM and Mg digestibility compared with broilers. A combination of these observations is an indication that turkeys seemed to benefit more from phytase supplementation in comparison with broilers at the same age. This will concur with observation of different rate of maturation of the digestive tract in broilers and turkeys ([Uni et al., 1995; 1999](#)). It is reasonable to suggest that species with more matured digestive tract may be more efficient at utilizing nutrient and hence have lower marginal benefit from enzyme supplementation.

Phosphorus digestibility was generally lower on D 28 compared with D 7 in both broilers and turkeys.

Phosphorus digestibility was generally lower in turkeys compared with broilers at the same age. These differences obviously reflected differences in the ability of the different species to utilize P, and differences in their intestinal milieu (pH, microbial composition, etc.) which will likely influence P utilization efficiency as well as efficiency of phytase supplementation. On the other hand, the age effect on P digestibility might be because of the fact that the same diet (hence the same dietary P level) was used throughout the experiment and that at younger age, high P digestibility was essential to help meet a greater P requirement at that age. All the diets in the current study were supplemented with phytase, and therefore, the measurement of background P digestibility without phytase supplementation was not possible. However, previous observations ([Rodehutsord and Dieckmann 2005; Ingelmann et al., 2019](#)) showed that turkeys had lower P digestibility in comparison with broilers when both received diets deficient in P. Moreover, lower true P digestibility and total tract retention in turkeys (76 and 71%, respectively) compared with broilers (94 and 92% for ileal digestibility and total tract retention, respectively) when fed wheat distillers' dried grains with solubles were observed in previous studies ([Adebiyi and Olukosi 2015a](#)). In that study, the species differences in P digestibility narrowed with phytase supplementation, and although phytase effect on P digestibility was marginal in both species, turkeys clearly benefited more in terms of enhanced P digestibility because of phytase compared with the broilers.

Comparative Inositol Phosphate Esters Level in the Ileal Digesta in Response to Phytase and Xylanase Supplementation in Broilers and Turkeys Diets

Surprisingly, feed InsP6 level was higher in broilers, compared with turkeys, diets even though phytate-containing raw material levels did not differ hugely between diets. Because the Ca level in turkeys diets were higher which impacts phytate degradation ([Sommerfeld et al., 2018](#)), the reduced InsP6 degradation seen in turkeys compared with broilers partly might be because of the dietary Ca level and not species differences. The increase in InsPs concentration in the ileal digesta of turkeys and broilers at 28 D compared with 7-day-old birds possibly reflected the effect of increased feed intake or higher passage rate with reduced retention time in the upper intestinal tract where phytate is degraded. This trend for greater concentration of InsPs in older birds was species-dependent, except for InsP6, which was largely uninfluenced by age of broilers. This observation was also confirmed in the calculation of InsP6 disappearance, which was not influenced by age of broilers but was lower for turkeys on D 28, compared with D 7. Because the calculation of disappearance rate depends on the concentrations in both the diet and the digesta, the lower disappearance rate observed for

turkeys on D 28 can be attributed to greater InsP6 concentration in the digesta on D 28, because the dietary level of InsP6 was unchanged between D 7 and D 28.

The effect of bird age on InsPs digesta concentration was observed mainly for InsP6 and InsP5 and was similar for both turkeys and broilers. However, the effect of phytase in reducing InsP6 and InsP5 was more evident on D 28, compared with D 7, primarily for broilers but less so for turkeys. For example, on D 7 and 28, phytase supplementation at highest level reduced Σ InsP6+InsP5 concentration in broilers by approximately 50 and 74%, respectively. Equivalent values for turkeys on D 7 and 28 were 72 and 70%, respectively. Notably, the ileal InsP6 and InsP5 concentrations for turkeys and broilers in the birds receiving the control diets were comparable for both species at both ages. These observations show that whereas the highest phytase level achieved comparable reduction in digesta for InsP6 and InsP5 concentrations on D 28 in both species, the phytase effect on these InsPs was more pronounced on D 7 in turkeys. This suggests that turkeys showed comparatively greater response to increasing phytase level at younger age than broilers at similar age possibly in response to limited capacity of turkeys at the younger age (7-day-old in this experiment) to liberate phytate P. In addition, turkeys diets were higher in Ca, which inhibits phytate degradation less so when superdosed phytase levels are supplemented (Sommerfeld et al., 2018); thus, a Ca effect cannot be excluded. The reduction in InsP6 and InsP5 by phytase supplementation has been widely reported for turkeys (Gautier et al., 2018; Ingelmann et al., 2019) and broilers (Zeller et al., 2015; Beeson et al., 2017; Ingelmann et al., 2018; Sommerfeld et al., 2018; Walk et al., 2018).

The only effects observed for InsP3 in turkeys was a marginally greater concentration of InsP3 in older, compared with younger, turkeys as well as with increasing phytase concentration. A comparison for the 2 species on D 7 showed that InsP4 and InsP3 concentrations in the ileal digesta of turkeys were in excess of 4-fold and 2-fold, respectively, of what was observed in ileal digesta of broilers. On D 28 however, InsP4 concentration in digesta for turkeys was approximately twice what was observed in broilers, whereas InsP3 concentration was comparable for both species. Even though it needs to be kept in mind that the referenced DM digestibility was somewhat higher in broilers, the observation suggests that these lower inositol phosphates, especially in younger birds were either (1) more recalcitrant to hydrolysis in turkeys, compared with broilers; (2) that the intestinal conditions, for example, pH profile, microbial, or animals phosphatases, differ sufficiently for both species; or (3) that the higher dietary Ca and P contents in turkey diets reduced the efficacy of the supplemental phytase as had been previously observed in broilers (Zeller et al., 2015; Li et al., 2017; Sommerfeld et al., 2018) and turkeys (Qian et al., 1997; Atia et al., 2000). Ingelmann et al. (2018) similarly observed that turkeys rapidly accumulated InsP5 following InsP6 hydrolysis but a much slower hydrolysis of InsP5 to lower InsPs similar to the current observation. This may also have accounted for why the

MI concentration in the digesta of broilers was more than 4 times the concentration observed in turkeys on D 7 and 28.

The observation that substantial quantity of wheat seed phytate resides in the aleurone layer, which in turn might have been hydrolyzed by xylanase, makes it intuitive that combination of phytase and xylanase may produce greater hydrolysis of InsPs than with the use of phytase alone (Woyengo and Nyachoti, 2011). No such interactive effect was observed for P digestibility in the current study in either broilers and turkeys. Zeller et al. (2015) similarly reported, for broilers, that P net absorption and InsP6 hydrolysis were primarily driven by phytase supplementation, with no further effects observed with xylanase supplementation. This is in agreement with other studies (Olukosi and Adeola, 2008; Olukosi et al., 2008; Tiwari et al., 2010). On the other hand, Ingelmann et al. (2018) found higher InsP6 degradation in turkeys fed basal diets when xylanase was added on top of phytase but no additional effect in other treatment diets. Phytase by xylanase interactions for digesta concentration of InsP3 and InsP4 that were observed in the current study did not translate to greater InsP6 hydrolysis or ileal MI concentration. There is the possibility that the use of phytase in all the diets made the effect of xylanase less apparent in as much as most of the InsPs hydrolysis was arguably driven by phytase, or it could be that a longer application of xylanase is needed if the effect is more indirect, for example by influencing retention time or microbial enzyme production. It is noteworthy that such interactions were only observed in broilers and not for turkeys.

In conclusion, the current studies indicate that broiler chickens and turkey poults shared several similarities in their growth and nutrient utilization responses, but there are differences in their responses in terms of nutrient utilization and InsPs hydrolysis. Whether these are driven by differences between species or influenced, in addition, by unavoidable differences in dietary requirements of each species or by differences in their development curve, need further investigation.

REFERENCES

- Adebiyi, A. O., and O. A. Olukosi. 2015a. Determination in broilers and turkeys of true phosphorus digestibility and retention in wheat distillers dried grains with solubles without or with phytase supplementation. *Anim. Feed Sci. Technol.* 207:112–119.
- Adebiyi, A. O., and O. A. Olukosi. 2015b. Apparent and standardised ileal amino acids digestibility of wheat distillers dried grains with solubles with- or without exogenous protease in broilers and turkeys. *Br. Poult. Sci.* 56:239–246.
- Adebiyi, A. O., and O. A. Olukosi. 2015c. Metabolizable energy content of wheat distillers' dried grains with solubles supplemented with or without a mixture of carbohydrases and protease for broilers and turkeys. *Poult. Sci.* 94:1270–1276.
- Adeola, O., and A. J. Cowieson. 2011. Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. *J. Anim. Sci.* 89:3189–3218.
- AOAC. 2006. *Official Methods of Analysis of AOAC International*, 18th ed. AOAC, Arlington, VA.
- Atia, F. A., P. E. Waibel, I. Hermes, C. W. Carlson, and M. M. Walser. 2000. Effect of dietary phosphorus, calcium,

- and phytase on performance of growing turkeys. *Poult. Sci.* 79:231–239.
- Beeson, L. A., C. L. Walk, M. R. Bedford, and O. A. Olukosi. 2017. Hydrolysis of phytate to its lower esters can influence the growth performance and nutrient utilization of broilers with regular or super doses of phytase. *Poult. Sci.* 96:2243–2253.
- Cowieson, A. J. 2010. Strategic selection of exogenous enzymes for corn/soy-based poultry diets. *J. Poult. Sci.* 47:1–7.
- Cowieson, A. J., and O. Adeola. 2005. Carbohydrases, protease, and phytase have an additive beneficial effect in nutritionally marginal diets for broiler chicks. *Poult. Sci.* 84:1860–1867.
- Engelen, A. J., F. C. van der Heeft, P. H. G. Randsdorp, and W. A. C. Somers. 2001. Determination of phytase activity in feed by colorimetric enzymatic method: Collaborative interlaboratory study. *J. AOAC Int.* 84:629–633.
- Gautier, A. E., C. L. Walk, and R. N. Dilger. 2018. Effects of a high level of phytase on broiler performance, bone ash, phosphorus utilization, and phytate dephosphorylation to inositol. *Poult. Sci.* 97:211–218.
- Gracia, M. I., R. Lázaro, M. A. Latorre, P. Medel, M. J. Aranibar, E. Jimenez-Moreno, and G. G. Mateos. 2009. Influence of enzyme supplementation of diets and cooking–flaking of maize on digestive traits and growth performance of broilers from 1 to 21 d of age. *Anim. Feed Sci. Technol.* 150:303–315.
- Ingelmann, C. J., M. Witzig, J. Möhring, M. Schollenberger, I. Kühn, and M. Rodehutschord. 2018. Effect of supplemental phytase and xylanase in wheat-based diets on prececal phosphorus digestibility and phytate degradation in young turkeys. *Poult. Sci.* 97:2011–2020.
- Ingelmann, C. J., M. Witzig, J. Möhring, M. Schollenberger, I. Kühn, and M. Rodehutschord. 2019. Phytate degradation and phosphorus digestibility in broilers and turkeys fed different corn sources with and without added phytase. *Poult. Sci.* 98:912–922.
- Karimi, A., Y. Min, C. Lu, C. Coto, M. R. Bedford, and P. W. Waldroup. 2013. Assessment of potential enhancing effects of a carbohydrase mixture on phytase efficacy in male broiler chicks fed phosphorus-deficient diets from 1 to 18 days of age. *Poult. Sci.* 92:192–198.
- Kim, J. C., P. Wilcock, and M. R. Bedford. 2018. Iron status of piglets and impact of phytase superdosing on iron physiology: a review. *Anim. Feed Sci. Technol.* 235:8–14.
- Li, W., R. Angel, S. W. Kim, K. Brady, S. Yu, and P. W. Plumstead. 2017. Impacts of dietary calcium, phytate, and phytase on inositol hexakisphosphate degradation and inositol phosphate release in different segments of digestive tract of broilers. *Poult. Sci.* 96:3626–3637.
- Madsen, C. K., C. A. Brearley, and H. Brinch-Pedersen. 2019. Lab-scale preparation and QC of phytase assay substrate from rice bran. *Anal. Biochem.* 578:7–12.
- Meng, X., B. A. Slominski, C. M. Nyachoti, L. D. Campbell, and W. Guenter. 2005. Degradation of cell wall polysaccharides by combinations of carbohydrase enzymes and their effect on nutrient utilization and broiler chicken performance. *Poult. Sci.* 84:37–47.
- Olukosi, O. A., and O. Adeola. 2008. Whole body nutrient accretion, growth performance and total tract nutrient retention responses of broilers to supplementation of xylanase and phytase individually or in combination in wheat-soybean meal based diets. *J. Poult. Sci.* 45:192–198.
- Olukosi, O. A., A. J. Cowieson, and O. Adeola. 2008. Influence of enzyme supplementation of maize-soyabean meal diets on carcass composition, whole-body nutrient accretion and total tract nutrient retention of broilers. *Br. Poult. Sci.* 49:436–445.
- Olukosi, O. A., A. J. Cowieson, and O. Adeola. 2010. Broiler responses to supplementation of phytase and admixture of carbohydrases and protease in maize-soyabean meal diets with or without maize Distillers' Dried Grain with Solubles. *Br. Poult. Sci.* 51:434–443.
- Olukosi, O. A., A. J. Cowieson, and O. Adeola. 2007a. Age-related influence of a cocktail of xylanase, amylase and protease or phytase individually or in combination in broilers. *Poult. Sci.* 86:77–86.
- Olukosi, O. A., J. S. Sands, and O. Adeola. 2007b. Supplementation of carbohydrases or phytase individually or in combination to diets for weanling and growing-finishing pigs. *J. Anim. Sci.* 85:1702–1711.
- Pirgozliev, V., O. Oduguwa, T. Acamovic, and M. R. Bedford. 2007. Diets containing *Escherichia coli*-derived phytase on young chickens and turkeys: effects on performance, metabolizable energy, endogenous secretions, and intestinal morphology. *Poult. Sci.* 86:705–713.
- Pirgozliev, V., C. A. Brearley, S. P. Rose, and S. C. Mansbridge. 2019. Manipulation of plasma myo-inositol in broiler chickens: effect on growth performance, dietary energy, nutrient availability, and hepatic function. *Poult. Sci.* 98:260–268.
- Qian, H., E. T. Kornegay, and D. M. Denbow. 1997. Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol, and the calcium: total phosphorus ratio in broiler diets. *Poult. Sci.* 76:37–46.
- Rodehutschord, M., and A. Dieckmann. 2005. Comparative studies with three-week-old chickens, turkeys, ducks, and quails on the response in phosphorus utilization to a supplementation of mono-basic calcium phosphate. *Poult. Sci.* 84:1252–1260.
- Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. *Anim. Feed Sci. Tech.* 59:215–221.
- Skřivan, M., M. Enghmaierová, and V. Skřivanová. 2018. Negative effect of phytase superdosing in laying hens. *Czech J. Anim. Sci.* 63:182–187.
- Sommerfeld, V., M. Schollenberger, I. Kühn, and M. Rodehutschord. 2018. Interactive effects of phosphorus, calcium, and phytase supplements on products of phytate degradation in the digestive tract of broiler chickens. *Poult. Sci.* 97:1177–1188.
- Tiwari, S. P., M. K. Gendley, A. K. Pathak, and R. Gupta. 2010. Influence of an enzyme cocktail and phytase individually or in combination in Ven Cobb broiler chickens. *Br. Poult. Sci.* 51:92–100.
- Uni, Z., Y. Noy, and D. Sklan. 1995. Posthatch changes in morphology and functions of the small intestines in heavy- and light-strain chicks. *Poult. Sci.* 74:1622–1629.
- Uni, Z., Y. Noy, and D. Sklan. 1999. Posthatch development of small intestinal function in the poult. *Poult. Sci.* 78:215–222.
- Walk, C. L., and S. V. R. Rao. 2018. High doses of phytase on growth performance and apparent ileal amino acid digestibility of broilers fed diets with graded concentrations of digestible lysine. *J. Anim. Sci.* 97:698–713.
- Walk, C. L., M. R. Bedford, and O. A. Olukosi. 2018. Effect of phytase on growth performance, phytate degradation and gene expression of myo-inositol transporters in the small intestine, liver and kidney of 21 day old broilers. *Poult. Sci.* 97:1155–1162.
- Walk, C. L., M. R. Bedford, T. S. Santos, D. Paiva, J. R. Bradley, H. Wladecki, C. Honaker, and A. P. McElroy. 2013. Extra-phosphoric effects of superdoses of a novel microbial phytase. *Poult. Sci.* 92:719–725.
- Woyengo, T. A., and C. M. Nyachoti. 2011. Review: supplementation of phytase and carbohydrases to diets for poultry. *Can. J. Anim. Sci.* 91:177–192.
- Zeller, E., M. Schollenberger, M. Witzig, Y. Shastak, I. Kühn, L. E. Hoelzle, and M. Rodehutschord. 2015. Interactions between supplemented mineral phosphorus and phytase on phytate hydrolysis and inositol phosphates in the small intestine of broilers. *Poult. Sci.* 94:1018–1029.