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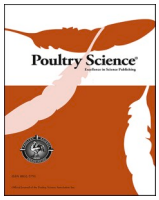

Effects of phytase supplementation on energy and nutrient availability, and phytate degradation in turkeys

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ABSTRACT

The study aimed to investigate the effect of graded levels of supplementary phytase (PHY) on energy and nutrient availability, and phytate (IP6) degradation of rapeseed meal (RSM) containing, wheat-based diets in turkeys. A control diet containing 6.8 g/kg available P (positive control; PC), a low-P diet containing 5.3 g/kg available P (negative control; NC) and a diet produced by mixing 810 g of the NC with 190 g industry produced RSM containing 5.6 g/kg available P (RSM diet) were produced. The NC and the RSM diets were then split in four parts each and PHY was added at 0, 500, 2500 and 12500 FTU/kg, respectively, resulting in nine diets in total. Feed intake (FI), weight gain (WG), and feed conversion ratio (FCR), from 27 to 35 d age, AMEn, retention coefficients for dry matter (DMR), nitrogen (NR), fat (FR), Ca (CaR), P (PR) and the profile of inositol phosphate esters (IP3-6) and myo-inositol (MYO) in excreta were determined. There was a positive quadratic relationship ($P < 0.05$) between dietary PHY activity and daily FI, as dosage of 2500 FTU was the optimum for FI. Feeding RSM reduced daily weight gain ($P < 0.05$) and feed efficiency ($P < 0.001$). Dietary AMEn increased linearly with PHY supplementation ($P < 0.05$) although feeding RSM reduced ($P < 0.001$) AMEn. Compared to NC, the PC had greater AMEn, DMR, CaR, PR, ($P < 0.001$) and NR ($P < 0.05$). Dietary CaR and PR linearly increased ($P < 0.001$) with PHY dosage which coincided with a decrease in IP5 and IP6 isomers ($P < 0.001$). The response to PHY followed curvilinear shape for IP4 ($P < 0.001$) and IP3 ($P = 0.001$) isomers.

Introduction

Oilseed rape (*Brassica napus*) is the third-largest source of vegetable oil in the world (Mielke, 2018), with the highest production quantities being in Europe and Canada (USDA, 2022). Rapeseed meal (RSM) is a by-product of oil production and due to its relatively high, well balanced protein content, it is used in poultry nutrition (Watts et al., 2021). Compared to soybean meal (SBM), RSM has a relatively low environmental footprint, thus its use in poultry diets could be a viable tool for reducing the negative impact on climate change (Grossi et al. 2022; Wilke et al., 2023). Although the majority of currently available RSM cultivars are registered as "double zero" (00) due to their low erucic acid and glucosinolate content (AHDB 2021)21, compared to SBM, RSM contains more fibre and less available P, which does not usually exceed 25% (Nwokolo and Bragg, 1980). Formulating poultry diets using RSM remains challenging as the metabolizable energy content and protein availability of RSM is considered lower and less consistent than that of SBM (Khajali, 2012).

Application of dietary enzymes, including phytase (PHY), protease, and carbohydrases, to facilitate phosphorus (P), protein, and energy utilization is a common approach to quality improvements of RSM (Olukosi et al., 2017; Bedford, 2018). Most studies to date have examined the use of carbohydrases in an attempt to improve carbohydrate digestibility and to eliminate any potential nutrient-encapsulating effect of cell wall non-starch polysaccharides (Józefiak et al., 2011; Khajali, 2012; Rutkowski et al., 2012). Supplementing RSM containing diets with proteases also improved protein digestibility when fed to broilers (Toghyani et al., 2017; Watts et al., 2020;Watts et al., 2020. Rutkowski et al. (1997) found that PHY supplementation improved dressing percentage and P availability of chickens fed RSM diets. Reports by Kong and Adeola (2011) showed that PHY supplementation improved the protein efficiency ratio of chicks fed diets containing RSM. Dietary PHY

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also modulated inositol phosphate (IP) isomers and myo-inositol (MYO) in excreta of broilers fed RSM containing diets, but there were differences in response between RSM samples (Pirgozliev et al., 2022). Although it is considered that supplementing diets with PHY is the most effective way of utilising minerals linked to phytate, there are different results regarding the exact dosage, dietary formulation, duration, type of PHY and species of birds (Sena et al., 2020; Cufadar et al., 2024; de Leo et al., 2025). To ensure most profitable PHY use in terms of P replacement, producers need to adapt their dosing strategy based on the ability of the PHY to liberate P and current inorganic P and PHY costs (Wealleans et al., 2016). Bedford and Rodehutscord (2024) reported that exogenous PHY can produce different results in chickens and turkeys, and information on the use of RSM in turkey rations or the effect of PHY on phytate degradation in RSM when fed to turkeys is scarce. In addition, little is known on the effect of super-dosing of exogenous PHY on metabolizable energy (ME), P availability and phytate degradation of RSM containing diet when fed to turkeys. The main objective of this experiment was to assess the impact of PHY superdosing on phytate degradation and P liberation/ retention in RSM containing diets when fed to young turkeys. Energy and nutrient availability, and growth performance variables, including feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) were also determined as baseline performance metrics.

Materials and methods

Ethics statement

The study procedures were approved by Harper Adams University Research Ethics Committee (Project number 0197-201803-STAFF). The manuscript has been prepared in compliance with the ARRIVE 2.0 guidelines (Percie du Sert et al., 2020).

Experimental diets

A basal diet, with reduced available P (avP) by 0.15 (avP in diet is calculated to be 0.53 %) and Ca by 0.165 (Ca in diet is calculated to be 1.12 %) was mixed and used as a negative control (NC) (Table 1). Another diet was produced mixing 81 % of the NC diet and 19 % of industry produced RSM (Cargill) that contained 4.07 μmol/g total glucosinolates (Watts et al., 2020) (Table 1). The two diets were then split in four parts each and phytase enzyme (Quantum ^{TM} Blue, ABVista, UK) was added at 0, 500, 2500 and 12500 FTU per kg diet, respectively. An additional diet with adequate levels of P and Ca was also fed as a positive control (PC) (Table 1), producing 9 diets in total. The nutrient specification of the diets met the breeder's recommendation (Aviagen Turkeys Ltd.).

Metabolizable energy and nutrient retention turkey study

Dietary AMEn and nutrient availability were examined in a turkey poults experiment from 27 to 35 d age. Female BUT Premium turkeys were obtained from a commercial hatchery (Faccenda Foods Ltd., Dalton, UK) at day old and were placed in a single floor pen and fed on a proprietary wheat–soya bean turkey feed until 26 d of age which contained per kilogram 11.85 MJ AME, 265 g crude protein (CP), 15.6 g available lysine, 12.2 g methionine + cysteine, 14 g Ca and 7.8 g available P, respectively. At 27 d age two birds were randomly allocated to one of 54 raised-floor pens with 0.36 m^2 floor area and given the experimental diets. Each pen was equipped with a trough feeder and nipple drinker. Access to the feed and the water was *ad libitum*. Each diet was fed to 6 pens following randomisation. The experimental house was equipped with a negative pressure ventilation system to meet commercial recommendations. Standard temperature and lighting programmes for turkeys were used (Aviagen, Turkeys Ltd.). At 32 d of age, after 5 d given to adjust to the diets, the total excreta were collected for 4 days until 35 d age, freeze dried, milled and subjected to further analyses.

Table 1
Ingredient composition of the experimental positive control (PC), negative control (NC) and rapeseed meal-based NC (RSM).

Ingredients (g/kg)	PC	NC	RSM
Wheat	525.1	529.8	420.08
Prairie meal	25.0	25.0	20.0
Rye	20.0	20.0	16.0
Rape seed meal (RSM)	50.0	50.0	240.0
Soya ext hipro	295.0	295.0	236.0
L-Lysine HCl	3.5	3.5	2.8
DL-methionine	3.5	3.5	2.8
L-threonine	0.9	0.9	0.72
Soya oil	30.0	30.0	24.0
Limestone flour tru.270	10.0	10.0	8.0
Dicalcium phosphate flour	30.0	26.3	24.0
Salt	3.0	3.0	2.4
Turkey premix[1]	4.0	4.0	3.2
Calculated provisions (as fed basis)			
Oil (g/kg)	45.6	45.6	41.0
CP (g/kg)	241.2	241.2	262.2
ME (MJ/kg)	12.16	12.16	11.43
Lysine available (g/kg)	13.9	13.9	13.1
Methionine + Cysteine (g/kg)	10.8	10.8	13.2
Ca (g/kg)	12.8	11.2	10.7
Available P	6.8	5.3	5.6
Determined values[2]			
DM (g/kg)	885	885	883
GE (MJ/kg)	16.81	16.81	16.96
Oil (g/kg)	35.8	40.6	36.9
CP (g/kg)	215	215	234
Ca (g/kg)	19.1	12.8	11.8
Total P (g/kg)	11.0	7.9	8.4
Phytate P (g/kg)	3.1	3.0	4.3
Non-phytate P (g/kg)	7.9	4.9	4.1
IP3 (nmol/g)[3]	1979	1553	1403
IP4 (nmol/g)[3]	5847	5101	4446
IP5 (nmol/g)[3]	5607	9566	9568
IP6 (nmol/g)[3]	534	3499	12489
Inositol (nmol/g)[3]	2049	2584	2581

[1] Contained vitamins and trace elements to meet breeder's recommendation (Aviagen, Turkeys Ltd, UK) and provided per kg diet: 50 mg nicotinic acid, 34 mg α-tocopherol, 15 mg pantothenic acid, 7 mg riboflavin, 5 mg pyridoxine, 3.6 mg retinol, 3 mg menadione, 2 mg thiamine, 1 mg folic acid, 200 μg biotin, 125 μg cholecalciferol, 15 μg cobalamin, 100 mg manganese, 80 mg iron, 80 mg zinc, 10 mg copper, 1 mg iodine, 0.5 mg cobalt, 0.5 mg molybdenum and 0.2 mg selenium.
[2] Analyses were performed in duplicate.
[3] IP3-6, inositol phosphate esters.

Laboratory analysis

Dry matter (DM) in feed and excreta samples was determined by drying of samples in a forced draft oven at 105°C to a constant weight (AOAC, 2006; method 934.01). Crude protein (6.25 × *N*) in samples was determined by the combustion method (AOAC, 2006; method 990.03) using a LECO FP-528 N (Leco Corp., St. Joseph, MI). Oil (as ether extract) was extracted with diethyl ether by the ether extraction method (AOAC, 2006; method 945.16) using a Soxtec system (Foss Ltd., Warrington, UK). The gross energy (GE) value of feed and excreta samples was determined in a bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL) as previously described (Pirgozliev et al., 2006). Phosphorus and Ca in feed and excreta samples were determined by inductively coupled plasma emission spectrometry as described elsewhere (Whiting et al., 2022). Phytate-P was predicted by NIR (ESC Standard Analytical Method, SAM120; AB Vista). Non-phytate P was calculated by subtracting phytate-P from total P. Phytase was analyzed by ELISA specific for Quantum Blue (ESC Standard Analytical Method, SAM099; AB Vista), in a method similar to that described by Engelen et al. (2001). One unit of phytase is defined as "the quantity of enzyme that will liberate 1 mol inorganic orthophosphate per minute under the

conditions of the assay" (Engelen et al., 2001). Titanium in feed and excreta was determined as explained by Short et al. (1999).

Inositol and inositol phosphates (IP6, IP5, IP4, IP3) in feed and excreta were determined as previously described (Madsen et al., 2019). In brief, samples of feed and ileal digesta (100 mg) were extracted in 5 mL of 20 mM EDTA, 100 mM NaF, pH 10, on a rotary shaker for 15 min followed by sonication in an ice bath sonicator for 15 min. The samples were held at 4°C for 2 h before centrifugation at 14,000 x g for 15 min. The supernatant was filtered through a 13 mm x 45 μm pore PTFE filter, before analysis (20 μL injection) by HPLC-PAD.

Calculations

Dietary nutrient retention / and disappearance coefficients were calculated using the following equation:

$$Nutrient\ retention = 1 - \frac{exnut/exti}{dietnut/dietti}$$

where *exnut* is the concentration of the respective nutrient in the excreta, *exti* is the concentration of titanium dioxide in the excreta, *dietnut* is the concentration of the respective nutrient in the diet and *dietti* is the concentration of titanium in the diet.

The AMEn value of the experimental diets was determined following the method of Hill and Anderson (1958).

$$AMEn = GE\ diet - \frac{(GE\ ex\ X\ dietti)}{exti} - 34.39\ X\ N\ retained$$

where AMEn (MJ/kg) = *N*-corrected apparent metabolizable energy content of the diet; GE diet and GE ex (MJ/kg) = GE of the diet and excreta, respectively; *dietti* and *exti* (%) = titanium in the diet and excreta, respectively; 34.39 (MJ/kg) = energy value of uric acid; and *N retained* (g/kg) is the N retained by the birds per kilogram of diet consumed. The retained N was calculated as

$$N\ Retained = N\ diet - \frac{N\ ex\ X\ dietti}{exti}$$

where N Diet and N *ex* (%) = *N* contents of the diet and excreta, respectively.

Statistical analysis

Statistical comparisons were performed using the general ANOVA procedure of Genstat 23rd edition (VSN International Ltd) in a 2 × 4 factorial arrangement testing for the main effects of PHY, RSM and the interaction term. Additionally, the PC and NC were compared with a single contrast comparison test, and PHY level was tested for linear and quadratic responses using polynomial contrast comparisons, within the factorial ANOVA model. All data were checked for normality and homogeneity of residuals prior to ANOVA.

Results

The analyzed PHY activity in the treatments was variable but close to the expected 0, 500, 2500 and 12500 FTU/kg (Table 2). There were no mortalities during the experiment. The effects of experimental treatments on turkey growth performance, AMEn and nutrient retention coefficients are shown in Table 3. There was a positive quadratic relationship ($P < 0.05$) between dietary PHY level and daily FI, and a dosage of 2500 FTU was the optimum for FI. There was a similar tendency between PHY activity and daily WG ($P = 0.053$), but no response for FCR ($P > 0.05$) was observed. Dietary RSM reduced daily WG by 5.7 % ($P < 0.05$), increased FCR (reduced feed efficiency) by 7.3 % ($P < 0.001$) but did not affect daily FI ($P > 0.05$). There were no differences ($P > 0.05$) between the PC and NC for the growth performance variables. There were no ($P > 0.05$) PHY by RSM interactions for the growth performance variables in Table 3. Dietary AMEn, DMR and NR increased linearly ($P < 0.05$) with increased PHY dosage. Dietary FR responded in a quadratic manner ($P < 0.05$) to PHY activity. Dietary RSM reduced AMEn, DMR and NR with 7.3, 7.9 and 7.8, respectively ($P < 0.001$) but did not affect FR ($P > 0.05$). Compared to the NC, the PC had greater AMEn ($P < 0.001$), DMR ($P < 0.001$) and NR ($P < 0.05$), CaR ($P < 0.001$) and PR ($P < 0.001$) by 2.8, 2.6 and 4.8 %, respectively. Dietary FR between PC and NC did not differ ($P > 0.05$). There were no ($P > 0.05$) PHY by RSM interactions for the studied variables in Table 3.

Table 2
Analysis of phytase (PHY) activity in the experimental diets[1].

	Expected Phytase	Analyzed Phytase[2]
Treatments[1]	PHY, FTU / kg	PHY, FTU / kg
1	0	< 50
2	0	< 50
3	500	1200
4	2500	2820
5	12500	17100
6	0	< 50
7	500	350
8	2500	4070
9	12500	15600

[1] Diets consisted in 9 experimental treatments: (1) Diet with adequate levels of P and Ca without PHY supplementation was fed as a positive control (PC); (2) Diet, with reduced levels of available (non-phytate) P (4.9 g/kg) and Ca (12.8 g/kg) without PHY supplementation was fed as a negative control (NC); (3) Diet 2 supplemented with 500 FTU/kg; (4) Diet 2 supplemented with 2500 FTU/kg; (5) Diet 2 supplemented with 12500 FTU/kg; (6) Another diet was produced mixing 81 % of the NC diet and 19 % of industry produced RSM with reduced levels of available (non-phytate) P (4.1 g/kg) and Ca (11.8 g/kg) without PHY supplementation; (7) Diet 6 supplemented with 500 FTU/kg; (8) Diet 6 supplemented with 2500 FTU/kg; (9) Diet 6 supplemented with 12500 FTU/kg.

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

The responses of the IP isomers, MYO concentration in excreta, CaR and PR to the experimental treatments is shown in Table 4. The shape of the response for the IP isomers was curvilinear ($P < 0.001$), i.e. linear and quadratic, and there were no significant ($P > 0.05$) deviations from this relationship. MYO in excreta was not changed ($P > 0.05$) by dietary PHY. The RSM inclusion did not change ($P > 0.05$) the concentration of the IP isomers and the MYO, although IP5 tended ($P = 0.058$) to increase with RSM diet. There were no differences ($P > 0.05$) between PC and NC for the IP isomers and inositol in excreta. There was a linear increase with an increase of PHY dosage for CaR and PR ($P < 0.001$). Dietary RSM reduced PR with 7.4 % ($P < 0.001$) but did not affect CaR ($P > 0.05$). Compared to the NC, the PC had greater CaR ($P < 0.001$) and PR ($P < 0.001$) by 19.6 and 7.0 %, respectively. There were no ($P > 0.05$) PHY by RSM interactions for the mineral retention coefficients.

Discussion

Apart from the lower available P in NC and RSM diets, the rest of the dietary requirements for turkeys at this age were met (Aviagen Turkeys Ltd.). The reduced growth performance in RSM fed birds agrees with previous report with turkeys fed similar dietary RSM inclusion (Mikulski et al., 2012; Plesch et al., 2014). The fibre content in the RSM is relatively high and correlates negatively to AMEn (Watts et al., 2020; Pirgozliev et al., 2024), thus explaining the reduced AMEn and nutrient retention coefficient in RSM diet. This can also explain the reduced weight gain and feed efficiency, i.e. increased FCR, of the turkeys fed RSM diet. Dietary glucosinolate content can also impact birds growth performance (Plesch et al. 2014). Feeding 15 % RSM with glucosinolate contents of 7.69 μMol/g, Plesch et al. (2014) found no adverse effects on performance and health status of turkeys. In the current study, however, the glucosinolate concentration in RSM was 4.07 μMol/g, the high RSM

Table 3
Selected productivity variables of broiler chickens, dietary metabolizable energy and nutrient retention coefficients.

Treatment		FI[1] (g/b/d)	WG[2] (g/b/d)	FCR[3] (g:g)	AMEn[4] (MJ/kg)	DMR[5]	NR[6]	FR[7]
QB (FTU/kg)[8]								
0		109	66	1.672	11.73	0.633	0.562[a]	0.797
500		113	69	1.648	11.77	0.637	0.563[a]	0.782
2500		114	69	1.662	11.91	0.646	0.584[a]	0.748
12500		111	68	1.630	12.04	0.658	0.596[b]	0.793
SEM[9]		1.4	1.1	0.0208	0.103	0.0071	0.0092	0.0173
RSM[10]								
-		111	70	1.595	12.31	0.670	0.600	0.783
+		113	66	1.711	11.41	0.617	0.553	0.777
SEM		0.97	0.8	0.0147	0.073	0.0050	0.0065	0.0122
QB (FTU/kg)	RSM							
0 (PC)[11]	no	109	66	1.664	12.61	0.684	0.620	0.791
0 (NC)[12]	no	109	68	1.603	12.27	0.666	0.590	0.816
500	no	112	69	1.620	12.29	0.667	0.592	0.799
2500	no	114	72	1.597	12.30	0.670	0.601	0.719
12500	no	109	70	1.559	12.38	0.678	0.616	0.796
0	yes	109	63	1.742	11.20	0.600	0.534	0.778
500	yes	115	69	1.675	11.24	0.607	0.535	0.764
2500	yes	114	66	1.727	11.52	0.622	0.566	0.777
12500	yes	113	67	1.701	11.69	0.638	0.577	0.789
SEM		1.9	1.6	0.0294	0.145	0.0100	0.0130	0.0244
Probabilities								
QB (FTU/kg)		0.071	0.115	0.516	0.158	0.078	0.033	0.196
L[13]		0.702	0.237	0.205	0.027	0.010	0.005	0.930
Q[14]		0.011	0.053	0.919	0.832	0.867	0.726	0.043
Deviations		0.530	0.341	0.428	0.684	0.799	0.407	0.470
RSM		0.292	0.002	<0.001	<0.001	<0.001	<0.001	0.755
QB x RSM		0.588	0.408	0.416	0.475	0.585	0.775	0.197
PC vs NC		0.935	0.879	0.317	<0.001	<0.001	0.037	0.188

[a, b] Means within the same column with different superscript letters differ statistically.
[1] FI, feed intake per bird.
[2] WG, weight gain per bird.
[3] FCR, feed conversion ratio.
[4] AMEn, nitrogen corrected apparent metabolizable energy.
[5] DMR, coefficient of dry matter retention.
[6] NR, coefficient of nitrogen retention.
[7] FR, coefficient of fat retention.
[8] QB, exogenous phytase enzyme.
[9] SEM, standard error of the mean.
[10] RSM, rapeseed meal.
[11] PC, positive control.
[12] NC, negative control.
[13] L, linear effects.
[14] Q, quadratic effects.

inclusion, may bring a cocktail of antinutrients that young turkeys may not be able to deal with.

As expected, feeding PHY improved most of the studied variables, although it was not well pronounced in growth performance. The short period of time over which these diets were fed perhaps limited the scope for the efficacy of the PHY to manifest itself in significant changes in performance, although numerical improvements were recorded here. In some studies the level of non-phytate dietary P has been shown as important for the efficacy of PHY in turkey feed. Applegate et al. (2003) did not find growth performance response of 21d old turkeys to *E. coli* phytase when fed diets containing over 4.7 g/kg non-phytate P. Reports with lower levels of non-phytate P, e.g. less than 4 g/kg, found an increase in FI and WG when feeding graded levels of PHY to turkeys at a similar age, although the FCR response was inconsistent (Pirgozliev et al., 2007; Ingelmann et al., 2018, 2019; Bassi et al., 2021; Novotny et al., 2023a, 2023b). The non-phytate P content in the diets in our study ranges between 4 and 8 g/kg, thus this may also explain the lack of a pronounced response in turkey growth performance.

Previous studies with young turkeys also found a linear increase in ME and nutrient retention coefficients with increased PHY concentrations (Pirgozliev et al., 2007; Bassi et al., 2021). The positive impact of exogenous phytase on P availability was found in other turkey experiments, although most of them used doses no higher than 1000 FTU/kg (Applegate et al., 2003; Kozlowski et al., 2010; Wealleans et al., 2016). An inclusion of 500 FTU/ kg feed may liberate 0.1 % of the phytate P (Choct, 2006), and in the current study, the inclusion of 12500 FTU/kg increased PR by approximately 10 %, which means that more phytate P was released and absorbed by turkeys' gastrointestinal tract. Bassi et al. (2021) found 14.6 % linear increase of PR when feeding 4000 FTU/kg in maize-soybean diet to 28d old turkeys. Thus, further confirming the importance of using PHY for environmental protection, especially in areas with high concentration of poultry farms (Toor et al., 2005). The difference of response to PHY observed by Bassi et al. (2021) and the recent study may be due to difference in dietary formulation, i.e. high RSM inclusion on the current study. Compared to SBM, RSM contains more fibre (Khajali, 2012), thus understandably, PHY is not as efficient as it would be in SBM diets. Involving xylanase enzymes in a combination with PHY in turkey studies warrant further investigation.

Pirgozliev et al. (2007) found a negative linear relationship between increase in AMEn and a decrease in mucin secretion/ endogenous losses in excreta. It has been speculated that PHY hydrolysed IP6 in diets, thus reducing their irritating impact on the GIT of turkeys and improving energy and nutrient utilisation. This is a part of so called extra-phosphoric effect of higher PHY inclusion that reduces the endogenous losses and increases mineral, amino acids and ME availability (Adeola and Cowieson, 2011). In the reported study, the

Table 4
Concentrations in excreta (nmol/mL) of inositol phosphate esters and inositol, and mineral retention coefficients of broiler chickens fed experimental diets.

Treatment		IP3[1]	IP4[1]	IP5[1]	IP6[1]	MYO[2]	CaR[3]	PR[4]
QB (FTU/kg)[5]								
0		3068[b]	2924[b]	5806[c]	33789[c]	4615	0.471[a]	0.482[a]
500		3503[c]	4246[c]	3010[b]	14491[b]	5459	0.480[ab]	0.483[a]
2500		2871[b]	4112[c]	657[a]	4105[a]	5243	0.480[ab]	0.492[a]
12500		1344[a]	942[a]	93[a]	1035[a]	5808	0.520[b]	0.530[b]
SEM[6]		163.7	314.5	252.9	1297.8	619.6	0.0097	0.0094
RSM[7]								
-		2662	2963	2144	12344	4851	0.480	0.516
+		2731	3149	2636	14365	5711	0.496	0.478
SEM		115.7	222.4	178.8	917.7	438.4	0.0068	0.0066
QB (FTU/kg)	RSM							
0 (PC)[8]	no	3152	2781	5145	31258	4442	0.577	0.546
0 (NC)[9]	no	2813	2586	4996	30983	4875	0.464	0.508
500	no	3633	4445	2861	13701	5738	0.469	0.504
2500	no	2824	3919	618	3619	4698	0.477	0.511
12500	no	1379	900	100	1075	4095	0.508	0.540
0	yes	3323	3262	6617	36594	4355	0.477	0.456
500	yes	3373	4047	3159	15282	5181	0.490	0.462
2500	yes	2917	4305	696	4590	5789	0.483	0.473
12500	yes	1310	983	86	995	7521	0.533	0.520
SEM		231.5	444.7	357.6	1835.4	876.7	0.0137	0.0133
Probabilities								
QB (FTU/kg)		<0.001	<0.001	<0.001	<0.001	0.586	0.004	0.002
L[10]		<0.001	<0.001	<0.001	<0.001	0.249	<0.001	<0.001
Q[11]		0.001	<0.001	<0.001	<0.001	0.740	0.332	0.290
Deviations		0.058	0.557	0.938	0.077	0.495	0.538	0.909
RSM		0.676	0.557	0.058	0.128	0.174	0.103	<0.001
QB x RSM		0.403	0.664	0.098	0.441	0.096	0.908	0.675
PC vs NC		0.382	0.643	0.775	0.822	0.709	<0.001	<0.001

[a,b,c] Means within the same column with different superscript letters differ statistically.
[1] IP3-6, inositol phosphate esters.
[2] MYO, myo-inositol.
[3] CaR, coefficient of Calcium retention.
[4] PR, coefficient of Phosphorus retention.
[5] QB, exogenous phytase enzyme.
[6] SEM, standard error of the mean.
[7] RSM, rapeseed meal.
[8] PC, positive control.
[9] NC, negative control.
[10] L, linear effects.
[11] Q, quadratic effects.

retention coefficients of DM, N, Ca and P responded in a linear dose dependent manner to PHY supplementation and were increased by 3.9, 6.0, 10.4 and 10.0 %, respectively.

In turkeys, a beneficial effect of exogenous PHY supplementation on P availability (prececal digestibility (pcdP) or total tract retention) have been reported: Applegate et al. (2003) reported 9.0 % improved P total tract retention with 500 FTU/kg compared to control; Ingelmann et al. (2019) found 10.0 % increase in pcd P in with 500 FTU/kg; Kozlowski et al. (2010) achieved a 16 % increase in the pcd P digestibility with 1000 FTU in diets; Novotny et al. (2023a) found 37.4 % increase in prececal P digestibility in with 1500 FTU/kg; Bassi et al. (2021) reported 26 % increase in P total tract retention with 4000 FTU in diets. Interestingly, compared to control, Beeson et al. (2017), reported 13.9 % improved total tract P retention with 500 FTU/kg, but no difference was observed with 1500 FTU/kg diet. Adebiyi and Olukosi (2015) did not find a response to P digestibility in young turkeys when supplementing semi-synthetic diets with 1000 FTU of *E. coli* PHY. The lack of a PHY effect in that particular study was explained by the low phytate P content of the diets.

The improvement in Ca retention with dietary PHY supplementation agrees with those of previous studies in turkeys, in which the Ca digestibility significantly increased in *E. coli* PHY supplemented feed (Kozlowski et al., 2010; Ingelmann et al., 2018, 2019; Bassi et al. 2021; Novotny et al., 2023a). Adeola and Cowieson (2011) explained the positive effect of PHY on the Ca availability in non-ruminants with reduced formation of insoluble Ca-phytate complexes in the small intestine due to lower proportions of InsP3–6 entering this section. The up-regulated absorption of Ca in response to increased P availability may also be a reason for the observed improvement in Ca retention (Ingelmann et al., 2018, 2019). This also may explain the higher Ca retention coefficient in the PC compared to NC in the reported study. Additionally, the PC diet has higher P and Ca contents compared to the NC diet, thus further supporting the higher CaR and PR in the PC.

The increase in AMEn and nutrient retention was coupled with the reduction of the concentration of IP6 and IP5 isomers in this study. The results suggest that very high levels of PHY are capable of reducing the IP6 concentration in the excreta by 97 %. Similarly, IP5 concentration in the excreta is decreased by almost 99 % of the NC value. Both, IP6 and IP5, are highly potent chelaters of minerals and may interfere with digestion of protein. This may explain the positive linear improvement in AMEn with increasing level of supplementary PHY, in keeping with the theory that a high IP6 concentration can inhibit pepsin secretion and therefore protein digestion. As noted earlier, the lack of performance response to PHY in this study is most probably due to the short feeding period. In agreement with Bedford and Walk (2016), IP4 and IP3 initially increase by 45 % and 14 %, respectively with a standard 500 FTU/kg PHY, however, superdosing PHY at 12 500 FTU/kg resulted in a reduction of 32 % and 44 %, respectively, from the NC. In agreement with Ingelmann et al. (2018, 2019), IP5 represents the major isomer in excreta. Pirgozliev et al. (2022) reported an increase in MYO excretion with 1500 FTU in chickens fed 20 % RSM although no response on MYO concentration in excreta was found in the current study. This may be due

to a significant difference noted between chickens and turkeys, namely the turkey seems to be almost 7 times more effective at absorbing inositol from the small intestine than the broiler (Novotny et al., 2023b). Thus, proportionately more of the IP6 is hydrolysed to IP1 then to inositol by endogenous phosphatases and quantitatively absorbed in the turkey. It is also possible that some of the released MYO was fermented by the microflora in the gastrointestinal tract of the birds.

Conclusion

The reported results confirm that super dosing of phytase in rapeseed meal fed turkeys is an effective strategy for improving the nutritional value of diets through the reduction of the anti-nutritional factors IP6 and IP5. Results indicate improvements in metabolizable energy and nutrient retention coefficients. The positive responses observed in this study suggest that dietary phytate was the main obstacle in utilising the nutrients from the diets.

Disclosures

The authors report no potential conflict of interest.

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