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by Pirgozliev, V.R., Mansbridge, S.C., Whiting, I.M., Rose, S.P., Brearley, C.A. and Bedford, M.R.

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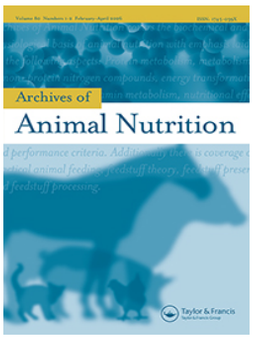

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Rethinking the maximum inclusion levels of exogenous phytase for enhanced turkey production performance

Vasil R. Pirgozliev[a], Stephen C. Mansbridge[a], Isobel M. Whiting[a], Stephen P. Rose[a], Charles A. Brearley[b] and Michael R. Bedford[c]

[a]National Institute of Poultry Husbandry, Harper Adams University, Newport, UK; [b]School of Biological Sciences, University of East Anglia, Norwich, UK; [c]AB Vista, Marlborough, UK

ABSTRACT

This study evaluated the effects of graded dietary phytase (PHY) inclusion on feed intake (FI), weight gain (WG), feed conversion ratio (FCR), nitrogen-corrected apparent metabolisable energy (AMEn), nutrient and mineral utilisation, and phytate degradation in growing turkeys. A positive control (PC) diet was formulated to meet or exceed breeder recommendations for nutrient content. A negative control (NC) diet was reformulated from the PC to contain the same calcium (Ca) to P ratio, but a reduced total P. The NC diet was subdivided and supplemented with PHY at 0, 100, 1000, 10,000, or 100,000 FTU/kg, resulting in six experimental diets. Seventy-two female BUT Premium turkey poults (mean body weight 1,885 g; SD ± 210 g) were randomly allocated to 36 raised-floor pens (two birds per pen; 0.36 m^2 per pen). Each diet was fed for 9 days, from 68 to 77 days of age to six replicate pens following randomisation. Birds fed the NC diet exhibited significantly reduced FI, WG, dry matter retention (DMR), and AMEn compared with birds fed the PC diet, confirming the adverse effects of marginal phosphorus supply. Supplementation with PHY at inclusion levels ≥1000 FTU/kg largely alleviated these deficiencies. Increasing dietary PHY concentration resulted in significant positive dose-dependent responses in WG, FCR, DMR, nitrogen retention, and AMEn. Marked reductions ($p < 0.001$) in excreta inositol phosphates (IP) were observed at PHY doses ≥1000 FTU/kg. Concentrations of IP and free inositol (IN) followed expected responses to increasing PHY dose, involving linear and quadratic components. Mineral retention coefficients were generally lower in turkeys fed the NC diet than in those fed the PC diet, except for phosphorus retention, which did not differ between control diets. Increasing PHY dose produced significant positive linear responses ($p < 0.001$) in the retention of calcium, magnesium, iron, potassium, manganese, zinc, and phosphorus, particularly at ≥1000 FTU/kg, Overall, the results confirm that turkeys respond to high PHY inclusion in a manner broadly consistent with broilers. Very high PHY levels (super- and mega-doses) provided continued additional benefits for growth performance beyond 1000 FTU/kg, along with continued improvements in nutrient retention, and the data suggest the optimum dose was beyond that investigated in this study.

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CONTACT Vasil R. Pirgozliev vpirgozliev@harper-adams.ac.uk

1. Introduction

The application of dietary phytase (PHY) to enhance the utilisation of phosphorus (P), protein, and energy is a well-established strategy for improving the nutritional quality of poultry diets (Rodehutscord et al. 2022; Wolfrum et al. 2025). Phytase hydrolyses phytate, the primary storage form of P in plant ingredients, thereby releasing bound nutrients and reducing the need for inorganic P supplementation. In broiler nutrition, research has shown that "super doses" of PHY (i.e. >1500 U/kg) can markedly improve feed efficiency, particularly when birds are fed diets deficient in calcium (Ca) and available P (Walk et al. 2013). Subsequent studies demonstrated that such elevated PHY inclusion levels can lead to nearly complete phytate degradation, increased inositol (IN) concentrations in the gizzard, and improvements in growth performance, including feed efficiency (Walk et al. 2014). Interestingly, these benefits occurred without substantial changes in bone mineralisation: tibia ash in broilers fed super dosed PHY did not exceed values obtained with mineral-adequate or Ca- and P-supplemented diets. These findings suggest that the performance enhancements associated with PHY super dosing may stem from phytate destruction and subsequent IN release rather than solely from increased Ca and P supply. Despite the recognised benefits of PHY, responses vary depending on factors such as dosage, diet formulation, feeding duration, PHY source, and bird species (Sena et al. 2020; Cufadar et al. 2024; de Léo et al. 2025). Notably, Bedford and Rodehutscord (2024) highlighted that exogenous PHY can yield different outcomes in chickens versus turkeys. Additionally, it has been demonstrated that whilst chickens and turkeys can both have a similar intrinsic capacity for phytate degradation, prececal IP6 disappearance is more pronounced in broilers than in turkeys; however, turkeys appear more efficient than broilers at absorbing IN from the small intestine (Novotny et al. 2023a), suggesting that results observed in chickens may not be directly transferable to turkeys. Indeed, in studies using high PHY doses in turkeys, increased energy and nutrient availability and reduced phytate excretion were observed, yet growth performance remained unaffected (Pirgozliev et al. 2025). From a practical standpoint, determining the most profitable PHY dosing strategy requires understanding the capacity of a given PHY product to release P and its economic relationship with inorganic P sources (Wealleans et al. 2016). However, information on PHY super dosing in turkey diets remains limited. In particular, the effects of very high PHY doses on phytate degradation in reduced-P turkey diets are poorly understood. Therefore, the objective of the present experiment was to investigate the impact of very high levels of exogenous PHY on energy utilisation, nutrient availability, and phytate degradation in young turkeys fed reduced-P diets. Growth performance variables were also evaluated.

2. Materials and methods

2.1. Ethics statement

The experimental protocol received formal approval from the Harper Adams University Research Ethics Committee (project ID: 0202-201803-STAFF). Preparation of this manuscript followed the reporting standards set out in the ARRIVE 2.0 guidelines (Percie du Sert et al. 2020).

2.2. Experimental diets

A positive control (PC) diet was formulated to meet or exceed breeder recommendations (Aviagen Turkeys Ltd., Edinburgh, UK) for nutrient content (Table 1). A negative control (NC) diet was reformulated from the PC to contain the same calcium (Ca) to P ratio, but with a reduced total P level (Table 1). This NC batch was subsequently divided into five equal portions, to which graded levels of a commercial PHY (Quantum™ Blue, AB Vista, UK) were incorporated at 0, 100, 1000, 10,000 and 100,000 PHY units (FTU)/kg diet, respectively (Table 2).

Table 1. Ingredient composition (g/kg, as-fed) of the experimental Turkey diets formulated with either standard, positive control (pc), or lower, negative control (NC), phosphorus content.

Ingredients	PC [g/kg]	NC [g/kg]
Wheat	525.1	529.8
Maize gluten meal	25.0	25.0
Rye	20.0	20.0
Rape seed meal	50.0	50.0
Soybean meal (48 Crude Protein)	295.0	295.0
HCL Lysine	3.5	3.5
DL Methionine	3.5	3.5
L Threonine	0.9	0.9
Soya oil	30.0	30.0
Limestone flour tru.270	10.0	10.0
Dicalcium phosphate flour	30.0	26.3
Salt (NaCl)	3.0	3.0
Turkey premix[1]	4.0	4.0
Calculated provisions (as fed)		
Crude Fat g/[kg]	45.6	45.6
Crude Protein g/[kg]	241.2	241.2
Metabolisable Energy MJ/[kg]	12.16	12.16
Available Lysine g/[kg]	13.9	13.9
Methionine + Cysteine g/[kg]	10.8	10.8
Ca g/[kg]	12.8	11.2
Available P g/[kg]	6.8	5.3
Determined values (as fed)		
Dry matter g/[kg]	885	885
Crude Fat g/[kg]	42.4	38.1
Crude Protein g/[kg]	239.0	239.7
Gross Energy MJ/[kg]	16.58	16.22
Ca g/[kg]	19.1	12.8
Phytate P g/[kg]	3.1	3.0
Non-phytate P g/[kg]	7.9	4.9
Total P g/[kg]	11.0	7.9
Ash g/[kg]	84.4	71.1
IP3 nmol/g[3]	2589	2119
IP4 nmol/g[3]	6605	5746
IP5 nmol/g[3]	7973	9331
IP6 nmol/g[3]	2148	2890
Inositol nmol/g[3]	2670	2148

Note: [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.

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

	Expected	Analysed
Treatments[1]	Phytase PHY, [FTU/kg]	Phytase[2] PHY, [FTU/kg]
1	0	<50
2	0	<50
3	100	<50
4	1000	1130
5	10,000	14,100
6	100,000	132,000

Note: [1]Diets consisted in 6 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 P (3.2 g/kg) without PHY supplementation was fed as a negative control (NC); (3) Diet 2 supplemented with 100 FTU/kg; (4) Diet 2 supplemented with 1000 FTU/kg; (5) Diet 2 supplemented with 10,000 FTU/kg; (6) Diet 2 supplemented with 100,000 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.

All six diets were manufactured by Target Feeds Ltd (Whitchurch, SY13 2DX) and offered in a meal form to the turkeys. Titanium dioxide was added on the top of each diet at 5 g/kg as an indigestible marker.

2.3. Metabolisable energy and nutrient retention turkey study

A nutrient utilisation study was carried out with turkey poults during the period from 68 to 77 days of age. Female BUT Premium turkeys obtained as day-olds from a commercial supplier (Faccenda Foods Ltd., Dalton, UK) were brooded collectively in a single floor pen and provided a conventional wheat-soybean meal feeding regimen until 67 days of age. For the first 28 days, the starter ration delivered, per kilogram of feed, 11.85 MJ of AME, 265 g crude protein, 15.6 g digestible lysine, 12.2 g methionine plus cysteine, 14 g Ca, and 7.8 g available P. From 28 days onward, the birds were transitioned to the formulated PC diet. At 68 days of age, 72 poults (mean body mass 1885 g; SDEV ±210 g) selected at random were randomly allocated to 36 raised-floor pens (two birds per pen; floor space 0.36 m^2). Each pen contained a nipple drinker and a trough feeder, with unrestricted access to feed and water. Treatments were assigned to pens according to a randomised block design (spatial), providing six replicates per dietary group. The experimental facility was managed under commercial environmental standards, including negative-pressure ventilation and temperature and lighting schedules consistent with industry recommendations (Aviagen Turkeys Ltd., Edinburgh, UK). Following a six-day acclimation to the test diets, excreta were collected twice daily between 73 and 77 days of age. Samples were stored at 4°C, subsequently oven-dried at 60°C, and ground using a 0.75 mm screen. Feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) were recorded simultaneously during the entire study period.

2.4. Laboratory analysis

Titanium concentrations in diets and excreta were quantified following the procedure of Short et al. (1996). Dry matter was measured by oven-drying samples at 105°C to constant mass (AOAC 2006). Nitrogen content, used to calculate crude protein, was determined by combustion (AOAC 2006) using a LECO FP-528 analyser. Lipid content was assessed by diethyl ether extraction with a Soxtec apparatus (Foss Ltd.). Gross energy was analysed using an isoperibolic bomb calorimeter (Parr 6200), as outlined by Watts et al. (2020). Mineral analyses were obtained via inductively coupled plasma emission spectrometry as described elsewhere (Oduguwa et al. 2007; Whiting et al. 2022). Inositol and inositol phosphate fractions (IP6-3) were quantified following the method of Madsen et al. (2019). Phytase activity, specific to Quantum Blue, was measured using an ELISA-based assay (ESC SAM099), analogous to the method described by Engelen et al. (2001), in which one unit corresponds to the release of 1 µmol of inorganic phosphate per minute under assay conditions.

2.5. 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 metabolisable 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.

2.6. Statistical analysis

Statistical analyses were carried out on cage means (experimental unit) using GenStat (23rd edition) statistical software (IACR Rothamsted, Hertfordshire, UK). The data were analysed using the general ANOVA procedure, with blocks (random effect), and incorporating orthogonal polynomial contrasts to evaluate linear and quadratic trends associated with increasing PHY levels (fixed effect). Additionally, the PC and NC were compared with a single contrast comparison test. Prior to ANOVA, data were examined for homogeneity of variances and normality of residuals. Differences were considered statistically significant at $p < 0.05$. All data are presented as means together with their pooled standard errors of the means (SEMs).

3. Results

The analysed PHY activities were generally consistent with the intended inclusion rates of 0, 100, 1000, 10,000, and 100,000 FTU/kg (Table 2). No mortality occurred during the study. Treatment effects on growth performance, AMEn, and nutrient retention are summarised in Table 3. Relative to the PC diet, birds receiving the NC diet exhibited reduced FI ($p = 0.003$), WG ($p = 0.008$), AMEn ($p = 0.032$), AMEn:GE ratio ($p = 0.026$), dry matter retention (DMR; $p = 0.026$) and FR ($p = 0.019$). Supplementation with ≥1000 FTU/kg largely eliminated these deficits. Increasing dietary PHY concentration produced significant positive linear responses in WG ($p < 0.001$), reduced FCR ($p = 0.043$), AMEn ($p < 0.001$), AMEn:GE ($p < 0.001$), DMR ($p < 0.001$) and NR ($p < 0.001$). The effects of dietary treatments on IP and IN concentration in excreta are shown in Table 4 and there were no significant differences

Table 3. The effect of dietary treatments on daily FI, WG, FCR, AMEn, AMEn:GE and nutrient retention coefficients when fed to turkey poults from 68 to 77 days of age (excreta were collected from 73 to 77 days of age).

	FTU [kg]	FI [g/b/d]	WG [g/b/d]	FCR	AMEn [MJ/kg DM]	AMEn:GE	DMR	FR	NR
PC	0	192	113	1.706	13.46	0.755	0.691	0.865	0.512
NC	0	176	96	1.861	13.00	0.721	0.651	0.843	0.483
NC	100	171	98	1.772	13.09	0.725	0.653	0.856	0.506
NC	1000	181	104	1.751	13.52	0.749	0.689	0.866	0.554
NC	10,000	188	109	1.727	13.65	0.756	0.699	0.864	0.562
NC	100,000	181	118	1.527	13.73	0.761	0.707	0.862	0.589
SEM		4.0	3.7	0.0710	0.122	0.0068	0.0090	0.0076	0.0127
Probabilities									
PC vs NC		0.003	0.008	0.165	0.032	0.011	0.026	0.019	0.160
PHY		0.090	0.002	0.043	<0.001	<0.001	<0.001	0.238	<0.001
L		0.058	<0.001	0.005	<0.001	<0.001	<0.001	0.072	<0.001
Q		0.642	0.355	0.409	0.519	0.519	0.709	0.144	0.524
D		0.103	0.965	0.556	0.409	0.409	0.277	0.953	0.523

Note: FTU = phytase concentration in kg diet; FI = daily feed intake per bird; WG = daily weight gain per bird; FCR = feed conversion ratio; AMEn = nitrogen corrected apparent metabolisable energy; AMEn:GE = ratio between AMEn and gross energy (GE); DMR = coefficient of dry matter retention; FR = coefficient of fat retention; NR = coefficient of nitrogen retention; PC = positive control; NC = negative control; PHY = phytase; SEM = standard error of the mean; L = orthogonal polynomial contrast for linear response; Q = orthogonal polynomial contrast for quadratic response; D (deviation) = orthogonal polynomial contrast for deviation from linearity; There were 6 replications per treatment.

Table 4. The effect of dietary treatments on concentration of IP3, IP4, IP5, IP6 and in excreta of 77-day-old turkeys (diets fed from 68 to 77 days of age).

	FTU [kg]	IP3 [nmol/g]	IP4 [nmol/g]	IP5 [nmol/g]	IP6 [nmol/g]	IN [nmol/g]
PC	0	2458	4990	7077	35,337	94,990
NC	0	1884	3630	6018	31,423	69,838
NC	100	2347	5487	7397	33,776	56,533
NC	1000	4632	11,762	4481	15,534	68,132
NC	10,000	1792	2223	634	5446	80,160
NC	100,000	550	408	212	1943	77,481
SEM		188.8	542.6	395.7	1577.3	4935.2
Probabilities						
PC vs NC		0.097	0.084	0.257	0.299	0.056
PHY		<0.001	<0.001	<0.001	<0.001	0.025
L		<0.001	<0.001	<0.001	<0.001	0.022
Q		<0.001	<0.001	0.006	0.553	0.254
D		<0.001	<0.001	<0.001	<0.001	0.060

Note: FTU = phytase concentration in kg diet; IP3-6 = inositol phosphate esters; IN = inositol; PC = positive control; NC = negative control; PHY = phytase; SEM = standard error of the mean; L = orthogonal polynomial contrast for linear response; Q = orthogonal polynomial contrast for quadratic response; D (deviation) = orthogonal polynomial contrast for deviation from linearity; There were 6 replications per treatment.

Table 5. The effect of dietary treatments on mineral retention coefficients of turkey poults (diets were fed from 68 to 77 days of age; excreta were collected from 73 to 77 days of age).

	FTU [kg]	CaR	MgR	FeR	KR	MnR	ZnR	PR	NaR	SR
PC	0	0.439	0.265	0.341	0.207	0.384	0.154	0.413	0.595	0.337
NC	0	0.256	0.095	0.176	0.061	0.116	0.010	0.368	0.354	0.408
NC	100	0.243	0.125	0.179	0.083	0.117	0.080	0.386	0.314	0.297
NC	1000	0.329	0.169	0.232	0.144	0.187	0.088	0.456	0.300	0.351
NC	10,000	0.360	0.247	0.262	0.190	0.238	0.164	0.460	0.464	0.365
NC	100,000	0.384	0.239	0.283	0.233	0.260	0.156	0.496	0.392	0.336
SEM		0.0326	0.0211	0.0236	0.0212	0.0250	0.0361	0.0202	0.0352	0.0416
Probabilities										
PC vs NC		<0.001	0.001	0.002	0.005	<0.001	0.006	0.156	<0.001	0.175
PHY		<0.001	<0.001	0.004	<0.001	<0.001	0.003	<0.001	0.025	0.463
L		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.056	0.571
Q		0.822	0.530	0.665	0.754	0.628	0.989	0.696	0.405	0.440
D		0.220	0.219	0.233	0.767	0.158	0.621	0.444	0.022	0.270

Note: FTU = phytase concentration in kg diet; CaR = coefficient of calcium retention; MgR = coefficient of magnesium retention; FeR = coefficient of iron retention; KR = coefficient of potassium retention; MnR = coefficient of manganese retention; ZnR = coefficient of zinc retention; PR = coefficient of phosphorus retention; NaR = coefficient of sodium retention; SR = coefficient of sulphur retention; PC = positive control; NC = negative control; PHY = phytase; SEM = standard error of the mean; L = orthogonal polynomial contrast for linear response; Q = orthogonal polynomial contrast for quadratic response; D (deviation) = orthogonal polynomial contrast for deviation from linearity; There were 6 replications per treatment.

between NC and PC ($p > 0.05$). Marked reductions in IP occurred at 1000 FTU/kg, and responses for both IP3-IP6 followed a curvilinear pattern ($p < 0.001$), involving linear and quadratic components. Excreta IP6 linearly decreased ($p < 0.001$) and IN concentration linearly increased ($p = 0.022$) with PHY supplementation. Mineral retention coefficients were generally lower in turkeys fed the NC diet compared with the PC, except for P retention, which did not differ ($p > 0.05$). Most mineral retention values increased at PHY doses ≥1000 FTU/kg. Increasing PHY dose resulted in significant positive linear responses for Ca, Mg, Fe, K, Mn, Zn, and P retention ($p < 0.001$) (See Table 5).

4. Discussion

In agreement with previous research (Cowieson et al. 2011; Lee et al. 2017), the present study demonstrates that PHY super dosing can substantially enhance nutrient utilisation and energy metabolism in growing turkeys. Birds fed the PC diet exhibited improved growth performance and increased energy and nutrient retention compared to NC, suggesting that turkeys may respond to higher than recommended available P levels, which may be provided due to the inclusion of the exogenous PHY (Pirgozliev et al. 2007; Bassi et al. 2021). These observations are consistent with established knowledge that inadequate levels of available P reduce feed intake, efficiency of nutrient use, and overall growth in poultry due to limitations in metabolic and skeletal function (Cowieson et al. 2011; Fernandes et al. 2019).

Supplementation with PHY at levels ≥1000 FTU/kg was sufficient to improve WG, FCR and nutrient utilisation to levels comparable to those achieved with the PC diet, indicating that PHY effectively compensated for the imposed P restriction. This is in agreement with other turkey studies feeding diets with less than 4 g/kg non-phytate P (Pirgozliev et al. 2007; Ingelmann et al. 2018, 2019; Bassi et al. 2021; Novotny et al. 2023a, 2023b), although greater dietary non-phytate P, e.g. over 4.0 g/kg, did not always produce a growth performance response (Applegate et al. 2003; Pirgozliev et al. 2025), though in the current study it did at 4.9 g/kg. It is therefore likely that some level of non-phytate P should be provided to ensure adequate P is available for systemic IP3+ synthesis following the generation of inositol from phytase super dosing.

The linear improvements observed in AMEn, nutrient and mineral retention with increasing PHY dose agree with previous reports (Pirgozliev et al. 2007, 2025; Bassi et al. 2021), further suggest that the enzyme facilitated progressive liberation of phytate-bound nutrients, leading to more efficient digestion and metabolism. The lack of a quadratic component to any of the parameters determined suggests that the optimum dose could still be higher than tested here and highlights the problems in past research where incremental doses have been linearly rather than logarithmically spaced.

In turkeys, exogenous PHY supplementation generally enhances P availability, as evidenced by increased ileal P digestibility or P retention. Improvements of 9–10% have been reported with 500 FTU/kg (Applegate et al. 2003; Ingelmann et al. 2019), while higher inclusions yielded greater responses, including 16% with 1000 FTU/kg (Kozlowski et al. 2010), 37.4% with 1500 FTU/kg (Novotny et al. 2023a), and 26% with 4000 FTU/kg (Bassi et al. 2021). Pirgozliev et al. (2025) observed a gradual increase in P retention by 10% from 0 to 12,500 FTU/kg in turkeys fed rapeseed meal-based diets. In contrast, Beeson et al. (2017) observed improved total tract P retention at 500 FTU/kg but not at 1500 FTU/kg, suggesting some the responses are not unequivocal. Furthermore, Adebiyi and Olukosi (2015) reported no effect of 1000 FTU/kg *E. coli* PHY in young turkeys, likely due to the low dietary phytate P concentration. Variability in responses to dietary PHY across studies is largely attributable to differences in dietary formulation, including ingredient composition, phytate content, minerals and available P levels. Nevertheless, these findings consistently underscore the importance of PHY supplementation as a strategy to improve P utilisation and reduce environmental P excretion but also question whether doses employed have gone high enough to determine where the true optimum lies.

The remaining mineral retention data also highlight the efficacy of PHY super dosing in turkeys. Retention coefficients for Ca, Mg, Fe, K, Mn and Zn all increased linearly with escalating PHY dose. This again suggests the optimum dose lies beyond the range tested in this work, even though it is higher than ever tested before in turkeys. The absence of differences in P retention between NC and PC diets likely reflects the reduced available P in the NC diet, making retention values somewhat insensitive to intake.

The observed improvement in Ca retention with dietary PHY supplementation is consistent with previous turkey studies reporting increased Ca digestibility following *E. coli* PHY inclusion (Kozlowski et al. 2010; Ingelmann et al. 2018, 2019; Bassi et al. 2021; Novotny et al. 2023a; Pirgozliev et al. 2025). The enhanced Ca availability is attributed to reduced formation of insoluble Ca – phytate complexes in the small intestine, resulting from lower concentrations of IP3-IP6 (Adeola and Cowieson 2011). Additionally, increased P availability may upregulate Ca absorption, further contributing to improved Ca retention (Ingelmann et al. 2018, 2019). This mechanism likely explains the higher Ca retention coefficient observed in the PC compared with the NC, as the PC diet contained higher Ca and P levels, supporting greater Ca and P retention.

Nonetheless, the clear linear enhancement of mineral retention with increasing PHY may suggest improved solubilisation and intestinal absorption as phytate may have been progressively degraded. The results in this study suggest that very high dosage of PHY can reduce the IP6 excreta concentration by 95%. Similarly, IP5 concentration in the excreta was decreased by almost 96.5% of the NC value. Both, IP6 and IP5, are highly potent chelators of minerals and may interfere with digestion of nutrients (Cowieson et al. 2011; Bedford and Walk 2016). This may explain the positive linear improvement in WG and FCR with increasing level of supplementary PHY, in keeping with the theory that a high IP6 concentration can inhibit gastrointestinal enzymes secretion and therefore nutrient digestion.

In accordance with Bedford and Walk (2016) IP3 and IP4 initially increased by 25% and 51%, respectively with 100 FTU/kg phytase, but super dosing PHY at 100 000 FTU/kg resulted in a reduction of 71% and 89%, respectively, compared with the NC. Whilst individual IP esters are still highly capable of binding Mg and first-order transition metals such as Zn, Cu and Fe (Persson et al. 1998), it is the overall pattern of IP esters that is important since they are co-dependent. These elements are critical for the maintenance of the immune system and for activation of digestive proteases amongst their many functions.

This may explain the positive and linear improvement of WG and FCR with increasing PHY dose, as it is not until the highest dose fed in this study is utilised that performance is maximised, which correlates with minimum levels of all IP esters and as such compromise mineral status of the bird and efficacy of digestive proteases which rely on these metals for activation.

However, a previous turkey study (Pirgozliev et al. 2025) showed that super dosing PHY at 12,500 FTU/kg reduced IP3 and IP4 by only 32% and 44%, which is half that compared to the recent study. The response patterns for IP6, IP5, IP4 and IP3 clearly show that once more than 100 FTU/kg PHY is fed the levels of IP6 and IP5 fall and result in an accumulation of IP4 and IP3. It is only when 10,000 FTU/kg or higher are fed that IP4 and IP3 levels begin to fall, and both fall considerably with the highest dose employed. Indeed at 100,000 FTU, the total content of IP esters (IP6-3) falls by 65%

which may explain the incremental improvements in digestibility and performance implicated by the linear responses noted, since increased IN by itself has not consistently been shown to improve performance in broilers (Pirgozliev et al. 2019; Arthur 2024). This suggests that even trivial remnants of IP6 and lower esters should be avoided if performance is to be optimised. This is in contrast with previous reports in broilers showing near-complete phytate disappearance at high PHY concentrations (Walk et al. 2014). Although the only difference between NC and PC in excreta was IN concentration, the strong dose-dependent reduction in IP supports a mechanistic link between phytate breakdown and the improvements in energy and nutrient retention.

An interesting finding of this work is that the use of 100,000 FTU improved WG, FCR and PR compared with 1000 FTU, or less. This highlights that the response is clearly log-linear and not quadratic, as is often modelled in most PHY papers. The excreta IN levels increased with increased PHY as is usually found in broilers (Walk et al. 2014; Kriseldi et al. 2021; Pirgozliev et al. 2022). However, turkeys may be more efficient at the absorption of IN compared with broilers (Novotny et al., 2023a). As a result, it would be expected that blood plasma levels of IN in turkeys fed the highest dose of PHY would continue to increase until the threshold was reached where it starts to be catabolised and may have therefore been responsible for the FCR improvement noted, as is the case with broilers (Lee et al. 2017), though FCR responses to IN are known to be inconsistent (Arthur et al. 2019; Pirgozliev et al. 2019). This work confirms that the response to PHY does not reach an asymptote in turkeys and that broiler data is likely not applicable in super-dosed turkeys. These findings contribute valuable new evidence towards defining optimal PHY dosing strategies for turkey nutrition and support the potential for PHY to reduce reliance on inorganic P sources in commercial turkey production.

5. Conclusion

Overall, the findings confirm that turkeys respond to high PHY inclusion in a manner broadly consistent with broilers. The present data also indicate that very high super doses (>10000 FTU/kg) provide additional benefit beyond 1000 FTU/kg, particularly with respect to growth performance, and that mega dosing (>100000 FTU/kg) may even be warranted for turkeys, depending on economic cost of inclusion. These findings contribute valuable new evidence towards defining optimal PHY dosing strategies for turkey nutrition and support the potential for PHY to reduce reliance on inorganic P sources in commercial turkey production.

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Author contributions

CRediT: **Vasil R. Pirgozliev:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing; **Stephen C.**

Mansbridge: Investigation, Methodology, Software, Writing – review & editing; **Isobel M. Whiting:** Data curation, Investigation, Methodology, Project administration, Writing – review & editing; **Stephen P. Rose:** Software, Visualization, Writing – review & editing; **Charles A. Brearley:** Formal analysis, Investigation, Methodology; **Michael R. Bedford:** Funding acquisition, Investigation, Methodology, Writing – review & editing.

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ORCID

Vasil R. Pirgozliev http://orcid.org/0000-0002-4213-7609
Stephen C. Mansbridge http://orcid.org/0000-0003-4246-9782
Isobel M. Whiting http://orcid.org/0000-0003-2160-3583
Stephen P. Rose http://orcid.org/0000-0001-6459-597X
Charles A. Brearley http://orcid.org/0000-0001-6179-9109
Michael R. Bedford http://orcid.org/0000-0002-5308-4290

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