Rapeseed meal processing and dietary enzymes modulate excreta inositol phosphate profile, nutrient availability, and production performance of broiler chickens

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Rapeseed meal processing and dietary enzymes modulate excreta inositol phosphate profile, nutrient availability, and production performance of broiler chickens

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ABSTRACT This study aimed to assess the effect of rapeseed meal (**RSM**) processing method, where solvent extraction occurred under standard industry conditions (**ST**) or cold-pressed hexane extraction was employed (**MT**), and exogenous enzyme supplementation (phytase [**PHY**] and xylanase [**XYL**]) alone or in combination on key nutritional factors of broiler chickens. A randomized control experiment was performed using 144 male Ross 308 broilers in a 2 × 2 × 3 factorial arrangement. Three diets including a nutritionally complete wheat-based basal diet (**BD**), a diet containing 200 g/kg of RSM extracted under ST and another diet containing 200 g/kg of RSM extracted under MT were produced. Each diet was then split into 4 parts and was fed as is, or supplemented with PHY at 1,500 FTU/kg or XYL at 16,000 BXU/kg, alone or in combination, resulting in 12 diets in total. Response criteria: feed intake (**FI**), weight gain (**WG**), and feed conversion ratio (**FCR**), from 7 to 21 d age, AMEn, retention coefficients for dry matter (**DMR**), nitrogen (**NR**), fat (**FR**), and the profile of inositol phosphate esters (**IP2-6**) and myo-inositol (**MI**) in excreta. Diets containing MT had higher AMEn compared to ST diets ($P < 0.05$). There was RSM by PHY interaction for FI, as only birds fed MT diet responded to PHY supplementation with reduced FI and FCR ($P < 0.001$). Feeding XYL reduced overall FI and FCR ($P < 0.05$). Feeding PHY reduced IP6 and increased MI in excreta ($P < 0.001$). Feeding XYL and PHY in combination reduced MI in excreta compared to PHY only ($P = 0.05$). Compared to BD, birds fed RSM diets had an increased IP6 ($P < 0.05$) and MI concentration in excreta ($P < 0.01$). This may be due to IP ester differences in RSM and BD.

Key words: xylanase, phytase, phytate degradation, rapeseed meal, broiler chicken

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INTRODUCTION

With the sustained rise in the price of imported soybean meal (**SBM**) and its high environmental footprint, attention has been redirected toward the need to develop alternative protein sources for modern poultry production (Abdulla et al., 2017; Whiting et al., 2019; Karkelanov et al., 2021). Rapeseed is the most widely grown oilseed crop in the United Kingdom (**UK**) and Europe (Carré and Pouzet, 2014). Rapeseed meal (**RSM**), a co-product of the rapeseed oil recovery process, is an attractive alternative protein source for poultry (Kasprzak et al., 2016; Olukosi et al. 2017). Although the majority of currently available cultivars are registered as "double zero" (00) due to their low erucic acid and glucosinolate content, RSM is still high in non-starch polysaccharides (**NSP**) and phytate (Houdijk et al., 2017). Thus, formulating broiler diets using RSM remains challenging as its nutritive value is reportedly lower and more variable than SBM (Khajali and Slominski, 2012). In addition, Watts et al. (2020) demonstrated that the oil recovery methods, that is, traditional extraction or those that minimize the exposure of RSM to thermal treatments, can also impact on the feeding value of RSM for poultry.

Exogenous phytase (**PHY**) and xylanase (**XYL**) enzyme preparations are routinely used in poultry feed worldwide to improve phosphorus (**P**) nutrition and to mitigate the negative impact of phytate and of high

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dietary levels of NSP, especially in younger birds. Beyond these core reasons for including PHY and XYL, there is now significant interest in understanding the extra phosphoric effects of super-dosing PHY (Lee et al., 2017), giving a more complete destruction of the antinutritional factor phytate, the release of lower inositol phosphates, and production of myo-inositol (**MI**) in the digestive tract (Beeson et al., 2017; Sommerfeld et al., 2018; Pirgozliev et al., 2019a). More research is needed to study the interaction between exogenous XYL and super-dosed PHY on bird performance, dietary energy, and nutrients availability.

The aim of this experiment was to study the response of broiler chickens receiving diets formulated with 2 RSM samples, one obtained via conventional solvent extraction (**ST**) and the other produced under cold-pressed hexane extraction (**MT**), supplemented with PHY and XYL individually or in combination. The aim of the study was to measure the effect of the dietary treatments on AMEn, dry matter (**DMR**), nitrogen (**NR**) and fat (**FR**) retention coefficients, and the hydrolysis of inositol phosphate esters (**IP**) from phytate to lower IPs and MI. Feed intake (**FI**), weigh gain (**WG**), and feed conversion ratio (**FCR**) were also measured.

MATERIALS AND METHODS

The study procedures were approved by Harper Adams University Research Ethics Committee and reported here in accordance with the ARRIVE 2.0 guidelines (Percie du Sert et al., 2020).

Birds and Housing

Male Ross 308 broilers were obtained from a commercial hatchery at day old and were placed in a single floor pen and fed on a proprietary wheat-soya broiler ration until 7 d of age. The starter diet contained 12.38 MJ/kg AME and 216 g/kg CP and the main ingredients were wheat (603 g/kg), SBM (210 g/kg) and full fat soya (142 g/kg). On the first day of the experiment (7 d of age), the chicks were individually weighed and the heaviest and lightest birds discarded (in accordance with pre-determined inclusion and exclusion criteria that birds should be average commercial weight and good health), leaving 144 birds which were placed in 72 pens (2 birds per pen), following randomization. Standard temperature and lighting programs for Ross 308 broilers were used (Aviagen Ltd., Edinburgh, UK). Sample size determination was based on a priori information from previous similar studies. Animal well-being was checked daily.

Experimental Diets

Two RSM samples produced under different processing conditions were used in this study. A sample of conventionally solvent extracted RSM (ST) and cold-pressed hexane extracted RSM (MT) was obtained as previously described (Watts et al., 2020, 2021). In brief, conventionally solvent extraction includes two steps of cooking, first at 80 to 90°C to increase oil extraction efficiency, and second at 95 to 115°C for about an hour, when the residual hexane is flashed from the meal under pressure in a desolventising/toasting unit. The cold-pressed method employs a milder solvent extraction procedure by excluding the cooking step and cold-pressing the seed. The hexane temperature is maintained at approximately 50°C and the residual hexane is flashed out by injecting the meals with live steam.

A basal diet (**BD**) was designed and mixed to meet the nutritional requirements of the Ross 308 breed (Aviagen Ltd.; Table 1). The BD was then split in 3 parts, where in 2 parts, the RSM samples (Table 2) were incorporated at 200 g/kg (800 g of the BD + 200 g of each RSM sample), resulting in 3 diets. The three diets were then split in 4 parts each, with one part fed as is, and the other 3 parts supplemented either with PHY (1,500 FTU/kg; Quantum Blue 5G; AB Vista, Marlborough, UK; 5,000 FTU/g), XYL (16,000 BXU/

Table 1. Ingredient composition (g/kg, as-fed basis) and nutrient content of the experimental broiler chicken basal diet formulation.

Dietary ingredient	g/kg	g/kg	g/kg
RSM ST[1]	-	200.0	-
RSM MT[2]	-	-	200.0
Wheat	569.5	455.6	455.6
Maize gluten meal	10.0	8.0	8.0
Soybean meal	150.0	120.0	120.0
Full fat soybean meal	175.0	140.0	140.0
Monocalcium phosphate	20.0	16.0	16.0
Limestone	15.0	12.0	12.0
NaCl	3.8	3.0	3.0
Soya oil	40.0	32.0	32.0
Lysine HCL	4.1	3.3	3.3
Methionine	4.1	3.3	3.3
Threonine	2.0	1.6	1.6
Vitamin premix[3]	6.5	5.2	5.2
Total	1,000	1,000	1,000
Analyzed composition			
AME (MJ/kg)[4]	12.81	11.62	11.82
Dry matter (g/kg)	885	883	885
Gross energy (MJ/kg)	17.39	17.38	17.41
Oil (g/kg)	74	62	63
Crude protein (g/kg)	212	238	231
Ca (g/kg)	13.7	12.7	12.5
P (g/kg)	9.2	9.4	9.3
Phytate P (g/kg)	3.1	3.9	3.8
IP2 (nmol/g)[5]	2,928	2,392	2,366
IP3 (nmol/g)[5]	399	471	443
IP4 (nmol/g)[5]	2,956	2,547	2,497
IP5 (nmol/g)[5]	4,992	5,503	4,921
IP6 (nmol/g)[5]	2,128	9,721	10,799

[1]RSM ST, conventionally solvent extracted rape seed meal.
[2]RSM MT, cold-pressed hexane extracted rape seed meal.
[3]The premix contained vitamins and trace elements to meet breeder's recommendation (Aviagen Ltd., Edinburgh, UK). The premix provided (units/kg diet) retinol, 3,600 μg; cholecalciferol, 125 μg; μ-tocopherol, 34 mg; menadione, 3 mg; thiamin, 2 mg; riboflavin, 7 mg; pyridoxine, 5 mg; cobalamin, 15 μg; nicotinic acid, 50 mg; pantothenic acid, 15 mg; folic acid, 1 mg; biotin, 200 μg; iron, 80 mg; copper, 10 mg; manganese, 100 mg; cobalt, 0.5 mg; zinc, 80 mg; iodine, 1 mg; selenium, 0.2 mg; and molybdenum, 0.5 mg.
[4]The AME value was obtained via calculation.
[5]IP2-6, inositol phosphate esters.

Table 2. Chemical composition of the experimental conventionally solvent extracted (ST) and cold-pressed hexane extracted (MT) rapeseed meal samples (as-fed basis).

Determined values	ST	MT
Dry matter (g/kg)	877	884
AMEn (MJ/kg)[1]	6.88	7.86
Gross energy (MJ/kg)	17.36	17.47
Oil A (g/kg)	13.5	16.5
Crude protein (g/kg)	344	308
Ca (g/kg)	8.62	7.77
P (g/kg)	10.80	10.30
Soluble NSP[2] (g/kg)	55	80
Insoluble NSP[2] (g/kg)	174	156
Total NSP[2] (g/kg)	229	236
Total glucosinolates (μmol/g)	4.07	2.65
IP2 (nmol/g)[3]	275	121
IP3 (nmol/g)[3]	753	617
IP4 (nmol/g)[3]	936	657
IP5 (nmol/g)[3]	7,537	4,633
IP6 (nmol/g)[3]	39,839	45,427
Inositol (nmol/g)	2,432	3,723

[1]Determined in previous experiments (Watts et al., 2020, 2021).
[2]NSP, non-starch polysaccharides.
[3]IP2-6, inositol phosphate esters.

kg; Econase XT 25P; AB Vista; 160,000 BXU/g), or with the combination of 1,500 FTU/kg PHY + 16,000 BXU/kg XYL. Quantum Blue is an enhanced *E. coli* phytase, specifically designed to unlock nutrient potential from phytate. Econase XT 25P is a non-starch polysaccharide degrading enzyme based on endo-1,4-β-xylanase produced by a genetically modified strain of *Trichoderma reesei*. Twelve diets in total were fed during the study in mash form.

Experimental Procedures

Each experimental diet was fed to birds in 6 pens following randomization (pen was the experimental unit). Birds and feed were weighed on d 7 and d 21 to determine average daily FI, average daily WG, and FCR on a pen basis. Excreta were quantitatively collected each day for the last 4 d of the experiment (to avoid evaporation losses) and immediately dried at 60°C.

Chemical Analyses

Dietary and excreta samples were milled through a 0.5-mm sieve before analysis. Diets and excreta samples were subsequently analyzed for dry matter content (**DM**), gross energy (**GE**), nitrogen (**N**), fat, IP2-6, and MI. Minerals (calcium, Ca; phosphorus, P) in diet and RSM were analyzed as previously described (Tanner et al., 2002). The activity of PHY and XYL was 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). Dry matter, gross energy, nitrogen, and fat in dietary and excreta samples were determined as described elsewhere (Abdulla et al., 2021).

The NSP content of the BD and RSM samples were determined following the method of Englyst (1994). Minerals in the BD and RSM samples were measured as described by Tanner et al. (2002). Total glucosinolate content was determined using high performance liquid chromatography (ISO 9167, 1992). Analysis for phytate (IP6), IP2-5, and MI was performed according to methods described previously (Madsen et al., 2019; Pirgozliev et al., 2019b). The AMEn of diets were calculated following the method of Hill and Anderson (1958). The coefficients of nutrient retention were determined as the difference between intake and voiding of the nutrient, divided by their respective intake.

Statistical Analysis

Statistical comparisons were performed using the general ANOVA procedure of Genstat 19th edition (VSN International Ltd, IACR Rothamsted, Hertfordshire, UK) in a 2 × 2 × 3 factorial arrangement, with main effects of phytase, xylanase, and diet type, for growth performance measures: FI, WG, FCR, AMEn, nutrient retention, and ileal phytate degradation. All data were checked for normality and homogeneity of residuals prior to ANOVA.

RESULTS

The BD (Table 1) met the diet specification for this strain of broiler chicken (Aviagen Ltd.). The chemical composition of the RSM samples is summarized in Table 2. The AMEn values of the RSM samples used in MT and ST diets were determined previously (Watts et al., 2020, 2021) and were 11.90 and 11.70 MJ/kg, respectively. The crude protein content in ST sample was higher when compared to MT sample, 344 vs. 308 g/kg. The calculated AMEn of MT and ST diets were reduced by approximately 0.9 and 1.1 MJ/kg, and dietary CP was 232 and 239 g/kg, respectively. There was variation within IP in the RSM samples; most noticeable for IP2, IP5 and IP6. The MT sample had 3723 nmol/g MI content compared to 2432 nmol/g in ST sample. The analyzed PHY and XYL activity in the diets was slightly variable but close to the expected 1,500 FTU/kg or 16,000 BXU/kg, respectively (Table 3). Dietary phytate P was lower in the BD, 0.303 (g/100 g) and slightly higher in ST and MT diets, 0.392 vs. 0.385 (g/100 g), respectively.

There were no bird mortalities during the experiment. The effects of experimental treatments on broiler growth performance are shown in Table 4. There was an RSM by PHY interaction in FI, as only birds fed MT diet responded to PHY supplementation with reduced FI ($P < 0.05$) and there was no response in birds fed BD and ST diets. Feeding XYL significantly reduced overall FI ($P < 0.05$). Birds fed BD diet had greater WG compared to the rest ($P < 0.001$). Feeding XYL improved feed efficiency, that is, reduced FCR ($P < 0.001$). There was RSM by PHY interaction for FCR as only bird fed

Table 3. Analyzed enzyme activities in experimental diet samples.

	Expected		Determined		
Treatments[1]	Phytase, FTU/kg	Xylanase, BXU/kg	Phytase[2], FTU/kg	Xylanase[3], BXU/kg	Phytate P[4] (g/100 g)
1	0	0	< 50	< 2,000	0.299
2	1,500	0	1790	< 2,000	0.311
3	0	16,000	< 50	20,700	0.305
4	1,500	16,000	1,720	18,500	0.295
5	0	0	< 50	< 2,000	0.379
6	1,500	0	1,350	< 2,000	0.403
7	0	16,000	< 50	18,800	0.389
8	1,500	16,000	1730	19,300	0.397
9	0	0	< 50	< 2,000	0.375
10	1,500	0	1,530	< 2,000	0.393
11	0	16,000	< 50	19,400	0.381
12	1,500	16,000	1,560	19,700	0.389

[1]Diets consisted in 12 experimental treatments: (1) diet formulated without rape seed meal (RSM) without phytase or xylanase; (2) diet formulated without RSM with phytase without xylanase; (3) diet formulated without RSM without phytase with xylanase; (4) diet formulated without RSM with phytase and with xylanase; (5) diet containing RSM produced at standard temperature (ST) without phytase or xylanase; (6) diet containing ST with phytase without xylanase; (7) diet containing ST without phytase with xylanase; (8) diet containing ST with phytase and with xylanase; (9) diet containing RSM produced at mild temperature (MT) without phytase or xylanase; (10) diet containing MT with phytase without xylanase; (11) diet containing MT without phytase with xylanase; (12) diet containing MT with phytase and with xylanase.

[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.

[3]One 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.

[4]Phytate phosphorus was determined via NIR.

MT diet responded to PHY supplementation with reduced FCR ($P = 0.001$) and there was no response in birds fed BD and ST diets.

The BD had the highest AMEn followed by MT and ST diets, respectively ($P < 0.05$). The DMR and NR coefficients were higher for BD ($P < 0.05$), but did not differ between MT and ST diets ($P > 0.05$). Both, ST and MT diets had higher FR coefficients than BD ($P < 0.001$).

The profile of the IP and MI concentrations in excreta in relation to the experimental treatments is detailed in Table 5. Feeding PHY reduced IP6 ($P < 0.001$) and increased IP3 ($P < 0.001$) phosphates, although dietary XYL increased IP3 ($P<0.05$), IP4 ($P < 0.001$) and IP5 ($P<0.05$) phosphates in excreta. Feeding PHY alone increased MI in excreta although XYL did not change MI when fed alone and even reduced it when in combination with PHY ($P = 0.05$). Birds fed BD had less IP6 ($P < 0.05$) and MI ($P < 0.001$) phosphates in excreta compared to ST and MT fed birds. There was a PHY × RSM interaction for IP5, as the reduction in InsP5 differed between treatments and was lower ($P < 0.05$) for MT compared to BD and ST, 47% vs. 83%, respectively. Dietary PHY also interacted with RSM for IP4 in excreta as the concentration was increased by a greater magnitude in BD and ST diets in comparison to MT ($P < 0.05$). There was an RSM × Enzyme supplementation interaction ($P < 0.001$) for IP2, as BD diet responded to enzyme supplementation via reducing IP2 concentration in excreta, although it was not the case for ST and MT diets and indeed the IP2 levels in these diets were lower than in the BD.

DISCUSSION

The overall BW of birds fed BD was 807 g, or approximately 20% below the Ross 308 broiler target body weight for commercial flocks. This was expected due to the feeding of mash diets (rather than pelleted diets fed commercially), thus the reduced performance compared to large commercial flocks was anticipated (Pirgozliev et al., 2016; Yang et al., 2020), but was not considered detrimental to the study aims. The further reduction in WG of birds fed ST and MT diets was also expected and may be attributed to the low AMEn and high NSP contents of dietary RSM compared to the BD diet. While amino acid digestibility was not measured, this could be another explanation for the low performance.

The positive response of MT diets to PHY supplementation on FI and FCR agrees with previous research. Watts et al. (2020) found that oil recovery method that minimises the exposure of RSM to thermal treatments and by adding a suitable enzyme there is scope to increase the nutritional value of RSM for broilers and increase its utilization in modern poultry production. Collectively, the higher AMEn and trends observed in overall higher DMR and NR coefficients of MT compared to ST diet further reflect on the fact that less heat damage was incurred to the RSM during cold press hexane extraction. This is further supported by Olukosi et al. (2017), who found that reducing the exposure of the RSM to preliminary thermal treatments prior to solvent extraction and desolventising/toasting contributed to 1.3 MJ/kg greater ME in the final meal. Confirming previous findings, dietary XYL reduced FI (Pirgozliev et al., 2015) and FCR (Olukosi et al., 2020; Pirgozliev et al., 2021). The marginal improvement in growth performance variables in birds fed both enzymes agrees with the view that feeding a combination of enzymes can have a positive additive effect on growth performance of poultry (Olukosi et al., 2010; Abdulla et al., 2017). However, the diets fed were sufficient in P and other nutrients, thus the magnitude of the responses to the enzymes can be expected to be low (Cabahug et al., 1999).

Table 4. Selected productivity variables of broiler chickens, dietary metabolizable energy and nutrient retention coefficients.

			FI[1] (g/b)	WG[2] (g/b)	FCR[3] (g/g)	AMEn[4] (MJ/kg)	DMR[5]	NR[6]	FR[7]
PHY[8]	PHY	XYL[9]							
−			816	627	1.311	13.07	0.686	0.637	0.690
+			773	621	1.250	13.10	0.689	0.648	0.694
SEM[10]			18.3	8.6	0.0271	0.093	0.0055	0.0066	0.0095
XYL									
−			831	614	1.357	13.06	0.687	0.641	0.687
+			759	634	1.205	13.11	0.688	0.644	0.696
SEM			18.3	8.6	0.0271	0.093	0.0055	0.0066	0.0095
RSM[11]									
BD[12]			807	690[a]	1.170	13.39[c]	0.704[b]	0.655[b]	0.636[b]
ST[13]			781	593[b]	1.319	12.85[a]	0.674[a]	0.626[a]	0.720[a]
MT[14]			797	589[b]	1.354	13.05[b]	0.686[ab]	0.647[ab]	0.719[a]
SEM			22.4	10.5	0.0332	0.114	0.0067	0.0081	0.0117
Interactions									
	−	−	858	617	1.403	13.05	0.685	0.639	0.688
	−	+	774	637	1.220	13.09	0.687	0.636	0.691
	+	−	804	612	1.312	13.08	0.689	0.643	0.686
	+	+	743	631	1.189	13.13	0.690	0.653	0.701
SEM			25.9	12.1	0.0383	0.131	0.0077	0.0093	0.0135
RSM									
BD	−		792[a]	692	1.145[a]	13.31	0.699	0.651	0.619
BD	+		821[ab]	689	1.194[a]	13.48	0.709	0.658	0.653
ST	−		759[ac]	585	1.299[b]	12.72	0.664	0.608	0.721
ST	+		803[a]	601	1.339[b]	12.98	0.683	0.645	0.719
MT	−		897[b]	604	1.490[c]	13.18	0.695	0.654	0.729
MT	+		697[c]	574	1.218[ab]	12.86	0.676	0.641	0.709
SEM			31.7	14.8	0.0469	0.161	0.0094	0.0114	0.0165
RSM									
BD	−	−	829	687	1.205	13.16	0.689	0.648	0.590
BD	+	−	898	685	1.312	13.56	0.717	0.658	0.648
BD	−	+	769	696	1.108	13.45	0.710	0.655	0.648
BD	+	+	762	693	1.101	13.39	0.701	0.658	0.658
ST	−	−	806	571	1.410	12.85	0.671	0.613	0.746
ST	+	−	829	599	1.387	12.95	0.681	0.639	0.720
ST	−	+	713	600	1.188	12.59	0.657	0.602	0.697
ST	+	+	777	602	1.291	13.01	0.685	0.651	0.718
MT	−	−	939	592	1.592	13.13	0.695	0.657	0.728
MT	+	−	685	552	1.237	12.72	0.670	0.634	0.689
MT	−	+	854	616	1.388	13.23	0.696	0.650	0.729
MT	+	+	708	597	1.199	13.00	0.683	0.649	0.728
SEM			44.9	21.0	0.0663	0.227	0.0134	0.0161	0.0233
P-values									
PHY			0.107	0.650	0.117	0.786	0.679	0.257	0.773
XYL			0.007	0.113	<0.001	0.701	0.876	0.743	0.492
RSM			0.718	<0.001	<0.001	0.005	0.008	0.042	<0.001
PHY × XYL			0.658	0.943	0.437	0.962	0.895	0.502	0.641
RSM × PHY			<0.001	0.316	0.001	0.162	0.122	0.099	0.257
RSM × XYL			0.436	0.660	0.803	0.660	0.807	0.988	0.183
RSM × PHY × XYL			0.325	0.725	0.276	0.424	0.303	0.773	0.291

[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]PHY, exogenous phytase enzyme.
[9]XYL, exogenous xylanase enzyme.
[10]SEM, standard error of the mean.
[11]RSM, rapeseed meal.
[12]BD, basal diet.
[13]ST − diet containing conventionally solvent extracted RSM.
[14]MT, diet containing cold-pressed hexane extracted RSM.
[a,b,c]Means within the same column with different superscript letters differ statistically.

As metabolizable energy is a measurement of the available energy in carbohydrates, fats, and proteins it was expected that supplementing PHY and XYL would not greatly influence AMEn in a nutritionally sufficient diet. The lack in AMEn response coupled with the lack of response to enzyme supplementation of dietary DMR, NR, and FR coefficients.

The theory of enzymatic breakdown of phytate compounds distinguishes between liberation of phytate molecules from complexes with other matter components and enzymatic cleavage of phosphate residues on the myo-inositol ring (Zyla et al., 2004). The stepwise manner of dephosphorylation of IP6 (Greiner et al., 2000) will lead to a release of different InsP

Table 5. Concentrations of inositol phosphate esters and inositol in excreta (nmol/mL) of broiler chickens fed experimental diets.

			IP2[1]	IP3[1]	IP4[1]	IP5[1]	IP6[1]	Inositol
PHY[2]	PHY	XYL[3]						
−			1,855	810	2,261	5,072	39,301	3,535
+			1,777	1,772	4,988	2,987	11,455	9,243
SEM[4]			63.9	46.1	138.3	98.8	596.4	246.5
XYL								
−			1,882	1,211	3,277	3,883	24,651	6,619
+			1,750	1,370	3,972	4,175	26,105	6,159
SEM			63.9	46.1	138.3	98.8	596.4	246.5
RSM[5]								
BD[6]			2,652	1,298	3,910	3,500	23,800[b]	4,415[b]
ST[7]			1,515	1,359	3,811	4,587	26,075[a]	6,985[a]
MT[8]			1,282	1,215	3,153	4,001	26,259[a]	7,768[a]
SEM			78.3	56.5	169.4	121.1	730.4	301.9
Interactions								
	−	−	2,080	764	2,093	5,056	38,661	3,416[a]
	−	+	1,630	855	2,429	5,087	39,942	3,654[a]
	+	−	1,684	1,658	4,460	2,710	10,641	9,822[c]
	+	+	1,870	1,885	5,515	3,263	12,268	8,665[b]
SEM			90.4	65.2	195.6	139.8	843.4	348.6
RSM								
BD	−		2,891	748	2,254[a]	4,526[b]	38,235	1,931
BD	+		2,412	1,847	5,566[c]	2,475[d]	9,365	6,899
ST	−		1,471	861	2,403[a]	5,932[a]	40,256	3,974
ST	+		1,558	1,858	5,219[c]	3,241[c]	11,895	9,996
MT	−		1,203	819	2,127[a]	4,757[b]	39,414	4,700
MT	+		1,361	1,610	4,178[b]	3,244[c]	13,105	10,836
SEM			110.7	79.9	239.6	171.2	1,032.9	426.9
RSM								
BD	−	−	3,620[d]	636	2,108	4,708	38,557	1,317
BD	+	−	2,292[c]	1,832	4,945	2,195	7,947	6,965
BD	−	+	2,162[c]	861	2,401	4,343	37,913	2,545
BD	+	+	2,533[c]	1,862	6,188	2,755	10,782	6,832
ST	−	−	1,484[ab]	819	2,190	5,825	38,762	3,907
ST	+	−	1,464[ab]	1,646	4,542	2,882	11,283	11,332
ST	−	+	1,459[ab]	903	2,615	6,040	41,749	4,042
ST	+	+	1,653[b]	2,070	5,896	3,600	12,506	8,659
MT	−	−	1,137[a]	837	1,982	4,635	38,663	5,026
MT	+	−	1,297[ab]	1,496	3,895	3,054	12,693	11,168
MT	−	+	1,269[ab]	802	2,272	4,878	40,184	4,374
MT	+	+	1425[ab]	1,724	4,461	3,435	13,517	10,504
SEM			156.5	113.0	338.8	242.1	1,460.8	603.7
P values								
PHY			0.393	<0.001	<0.001	<0.001	<0.001	<0.001
XYL			0.149	0.018	<0.001	0.041	0.090	0.193
RSM			<0.001	0.201	0.005	<0.001	0.036	<0.001
PHY × XYL			<0.001	0.302	0.072	0.067	0.838	0.050
RSM × PHY			0.010	0.154	0.037	0.005	0.428	0.328
RSM × XYL			0.002	0.583	0.610	0.560	0.862	0.105
RSM × PHY × XYL			<0.001	0.203	0.727	0.520	0.414	0.270

[1]IP2-6, inositol phosphate esters.
[2]PHY, exogenous phytase enzyme.
[3]XYL, exogenous xylanase enzyme.
[4]SEM, standard error of the mean.
[5]RSM, rapeseed meal.
[6]BD, basal diet.
[7]ST, diet containing conventionally solvent extracted RSM.
[8]MT, diet containing cold-pressed hexane extracted RSM.
[a,b,c,d]Means within the same column with different superscript letters differ statistically.

(and isomers). As expected (Pirgozliev et al., 2019a; Olukosi et al 2020; Kriseldi et al. 2021), feeding phytase reduced the excreta concentration of IP6-5. However, the degree of dephosphorylation for IP5 differed between treatments, as it was lower for MT compared to BD and ST, 46 vs. 83%, respectively. Despite the higher IP6 in MT sample compared to ST sample, the IP6 in excreta in birds fed those diets did not differ. Higher PHY doses can further boost the breakdown of phytate compared to 'regular' doses, thus the super PHY dose was possibly efficient enough to improve MI release, hence the lack of interaction between PHY and XYL.

The phytate in wheat and rapeseeds resides in the aleurone layer and in the cotyledons respectively and is strongly associated with fibers, thus is expected that exogenous xylanases should increase access of phytase to phytate resulting in increased IP hydrolysis and releasing more MI. However, no such interactive effect was observed in this study, which agrees with Zeller et al. (2015) and Olukosi et al. (2020). The increased MI excreta levels in PHY fed birds suggests that P net

absorption was primarily driven by PHY supplementation, with no further effects observed with XYL supplementation, which agrees with other studies (Olukosi and Adeola, 2008; Olukosi et al., 2008; Tiwari et al., 2010).

Feeding PHY and XYL together led to reduced excreta MI concentration by 11.8% when compared to feeding PHY only. The reason for XYL × PHY interaction on excreta MI is unclear. The difference in excreta MI between PHY and PHY x XYL fed birds in this study was 12%, or 1,157 nmol MI only. The MI has been determined on excreta which was oven dried for at least 48 h at 60°C, thus microbial proliferation cannot be excluded. The results suggest that although statistically, the difference in MI may not be biologically significant.

In conclusion, the current study indicates that the RSM samples shared several similarities in their responses in terms of nutrient retention and IP hydrolysis, but there are differences in their responses in terms of growth performance and AMEn in presence of exogenous PHY. Whether these are driven by differences between the oil recovery methods, that is, traditional extraction or those that minimize the exposure of RSM to thermal treatments, or influenced in addition by dietary requirements of broiler chickens at this age, need further investigation.

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DISCLOSURES

The authors report no potential conflicts of interest.

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