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The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna

Differences in phosphorus digestibility and metabolizable energy concentrations of rye- or wheat-based compound feeds in pigs

K. Schmitz, S. Kehraus and K.-H. Südekum*

University of Bonn, Institute of Animal Science, Endenicher Allee 15, D-53115 Bonn, Germany

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* Corresponding author:
e-mail: ksue@itw.uni-bonn.de

ABSTRACT. In this study, two trials were conducted to determine phosphorus (P) digestibility and metabolizable energy (ME) concentrations of compound feeds. The feeds were formulated with either wheat or hybrid rye supplemented with soybean meal (SBM) or rapeseed meal (RSM). The compound feeds were fed with (+) (trial 1) or without (−) (trial 2) phytase supplementation to estimate the effect of intrinsic phytase activity in wheat and rye. In addition, nitrogen (N) balance of the test rations was evaluated. The P content in each test ration, consisting of a basal ration (deficient in P) and a compound feed, was adjusted to keep digestible P below 2.0 g/kg dry matter. All compound feeds were tested in a duplicate 3 × 3 Latin Square design. Pigs were kept in metabolism crates for a 7-day adaptation period and a 5-day collection period during which faeces and urine were quantitatively collected. Phytase supplementation ($P < 0.05$) and the source of protein supplementation ($P < 0.05$) exerted an influence on P digestibility. Phytase supplementation levelled P digestibility, resulting in values of 70.2% and 69.5% for SBM-compound feed and RSM-compound feed, respectively. The type of cereal grain had no effect on P digestibility of compound feeds, indicating that intrinsic phytase did not show differential efficacy. The ME concentration of all compound feeds was high (≥ 14.2 MJ/kg dry matter) and appropriate for growing pigs. Phytase supplementation had no effect on ME concentration of compound feeds. Rye and RSM, containing higher fibre concentration than wheat and SBM, shifted N excretion from urine to faeces, which may help to reduce ammonia release from slurry.

Introduction

Rye and rapeseed meal (RSM) have emerged in recent years as attractive regional alternatives to conventional wheat and soybean meal (SBM)-based rations for pigs. Hybrid rye, known for its adaptability to challenging climatic conditions, has demonstrated comparable yields to wheat (Geiger and Miedaner, 2009). Ergot contamination in hybrid rye has been effectively reduced to the levels found in other cereal grain types using molecular breeding techniques (Miedaner and Geiger, 2015). Rye is rich in dietary fibre, offering potential health benefits through components such as arabinoxylans, fructans or β-glucans, as well as bioactive components (alkylresorcinols, lignans, etc.) found in close proximity to or bound to fibre (Jonsson et al., 2018).

Since excessive excretion of nitrogen (N) and phosphorus (P) in faeces and urine is a pollutant to the environment, and mineral P is a non-renewable resource, both N and P must be used efficiently and sustainably in animal nutrition. Factors affecting P digestibility, including total P concentration, phytate-P, and phytase activity exhibit considerable variation between and within cereal grain types (Schemmer et al., 2020) and oilseeds, with

particularly elevated P concentrations found in co-products of oilseed processing. Despite this, there have been limited efforts to study P digestibility and assess metabolizable energy (ME) values of rye- and RSM-based pig rations. Therefore, it appears reasonable to evaluate P digestibility, ME values and N balance of rye, especially hybrid rye, and RSM – considered regional feedstuffs in Central Europe – to comprehensively evaluate their environmental impact and production methods. The aim of this experiment was to compare the effects of compound feeds with wheat (W) or hybrid rye (R) combined with either SBM or RSM, and further supplemented with phytase (+) or non-supplemented (−), on P digestibility and ME concentrations. The hypothesis posited that hybrid rye and RSM could serve as viable alternatives to wheat and SBM, exhibiting comparable energy values. Furthermore, due to high endogenous phytase content in rye, it was anticipated that P digestibility might be even higher without phytase supplementation.

Material and methods

Rations

The compound feeds used in this study consisted of W, R, SBM and RSM. Each feed formulation comprised 70% cereal grain (CER) and 30% protein meal (PM), with (+) or without (−) phytase supplementation. These compound feeds were mixed proportionally with basal ration (BR) to obtain the test rations (TR), which were eventually fed to the animals. Throughout the formulation process, samples of the compound feed ingredients were systematically collected, both before and during the creation of the BRs and TRs. Before mixing the TRs, W and R were ground in a hammer mill using a 3.0 mm screen, and SBM and RSM were used as supplied. To determine P digestibility in compound feeds, it is crucial to maintain a suboptimal P supply in the fed rations, thereby minimising the regulatory excretion of P via faeces. Consequently, a BR was formulated (Table 1) low in P and supplemented with all other minerals and vitamins meeting the requirements. The concentration of digestible P (dP) in the TRs was adjusted to a maximum of 2.0 g/kg DM, following the recommendations of the Committee for Requirement Standards of the Society of Nutrition Physiology in Germany (GfE, 1994). This adjustment was based on the declared P content of the BR and the analysed P content and digestibility of ingredients, as outlined in DLG (2014).

Table 1. Ingredients (g/kg) and chemical composition of the basal ration, g/kg dry matter (DM)

Item	Ingredients	
Wheat starch, pregelatinised	624	
Beet pulp, dried	144	
Potato protein	82	
Blood plasma (poultry)	63	
Cellulose	21	
Soybean oil	16	
Vitamin and mineral premix[1]	50	
Analysed chemical composition	Phytase[2]	
	supplemented	unsupplemented
DM, g/kg	914	928
Ash	67.6	78.1
Crude protein	191	199
Ether extract	34.0	36.5
Crude fibre	42.2	34.4
aNDFom	219	190
ADFom	54.4	47.0
ADL	6.30	12.0
Starch[3]	533	528
Sugar	55.4	45.3
Ca	10.0	12.7
P	1.35	1.50
Digestible P[4]	0.76	0.69
Phytate-P	0.48	0.48
Phytase activity, U/kg DM	3673	381
Gross energy, MJ/kg DM	17.6	17.7
Metabolizable energy[4], MJ/kg DM	15.0	15.2

aNDFom – neutral detergent fibre assayed with heat stable amylase and expressed exclusive of residual ash, ADFom – acid detergent fibre expressed exclusive of residual ash, ADL – acid detergent lignin,[1] premix provided the following per kg diet: IU: vit. A 5 000, vit. D 500; µg: vit. B_{12} 25: mg: vit. E 28, vit B_1 4.3, vit. B_2 6.25, pantothenic acid 25, cholinchlorid 870, nicotinic acid 38, vit. B_6 7.5, vit. K 2.5, biotin 0.03, Zn 127.2, Mn 56.8, Fe 183.9, Cu 10.3, I 0.38, Se 0.50; g: lysin-HCl 3.8, tryptophan 1.3, Na 3.8, Mg 0.7; [2] Ronozyme HiPhos (37 500 FTU/g; 6-Phytase (EC 3.1.3.26) DSM, Heerlen, Netherlands); [3] polarimetric measurement; [4] calculated following GfE (2008)

The TRs were formulated by blending each compound feed into the BR at rates ranging from 390 g/kg to 600 g/kg DM. The BR, supplied in two parts by AGRAVIS Raiffeisen AG (Münster, Germany) as premix and other ingredients already mixed as a meal, underwent a final blending process at our institute. All rations (BR, TR) were prepared in one batch for each trial and stored in dry barrels at barn temperature until fed. For the first trial, a commercial phytase (6-Phytase (EC 3.1.3.26); Ronozyme HiPhos, 37500 FTU/kg; DSM, Heerlen, Netherlands) was provided in the premix on limestone as a carrier. In the second trial, an equivalent amount of limestone without phytase was added to the premix. Each BR and TR was offered as a meal to avoid heat effects of pelletisation-induced heat on endogenous phytase activity.

Animals and experimental procedures

The experiments conducted in this study received approval from the State Office for Nature, Environment and Consumer Protection (LANUV), North Rhine-Westphalia, Recklinghausen, Germany, under the file No. 81–02.04.2020.A055. The experiment was split in two trials with phytase supplementation (+) and the other without supplementation (−) due to the limited availability of metabolism crates. A total of 24 healthy male castrated crossbred pigs (German Landrace × Piétrain) were purchased from Campus Frankenforst, University of Bonn (Königswinter, Germany), with 12 pigs designated for each trial. The pigs in trial 1 had an initial mean (± standard deviation) body weight (BW) of 28.2 kg (± 6.0 kg) and age of 63 days (± 2 day), and 34.2 kg (± 5.8 kg) and 72 days (± 2 day) in trial 2. The health status of each pig was assessed daily.

For each trial, a new batch of the BR was mixed. Groups of six pigs were allotted to duplicate 3 × 3 Latin Squares, and three different rations were tested within each Latin Square. To ensure complete sets of Latin Squares, a BR, either (+) or (−), was assigned to each square. This design aimed to minimise the effects of age or BW of the pigs within each square. Each period consisted of a 7-day adaptation period and a 5-day collection period in metabolism crates. During the adaptation period, the pigs were housed pairwise in an indoor pen of 1.1 m × 1.7 m on sawdust bedding. Individual feeding was provided. Following this period, the pigs were transferred to metabolism crates (height = 55 cm; length = 95 cm; width = 52 cm) equipped with slatted floors, stainless steel troughs and separate collection trays for faeces and urine. Crates were oriented to allow visual contact between pigs. Room temperature was maintained at 22 ± 2 °C and a 10-h lighting programme was utilised. Throughout the whole experiment, the pigs were fed twice daily at 07:30 and 15:30. Meals were mixed with water immediately before feeding. Feed refusals were completely collected, weighed and dried to allow accurate determination of dry matter (DM) intake. After feeding, the pigs had free access to drinking water for at least 30 min. The rations were allocated based on the BW measurements of the pigs, taken at both the initiation and conclusion of each collection period. The feeding amounts corresponded to 2.0 to 2.5 times the maintenance requirement for ME (GfE, 2008). Feed samples for DM determination and calculation of DM intake were taken during preparation of meals, which were weighed at the beginning of each period and stored in polyethylene bags until feeding. Throughout a given period, the daily feed quantity offered in two meals was adjusted to the BW during the previous adaptation period, maintaining a constant during the subsequent collection period.

Urine and faeces were systematically collected in a quantitative manner. Urine was collected in a refrigerated plastic container containing 10% (v/v) sulphuric acid to ensure acidification to a pH ≤ 3.0. Each morning after feeding, urine was weighed and subsamples collected. The plastic containers were subsequently emptied and prepared for the next collection cycle. Faeces were collected twice daily following feeding. Faeces and urine were promptly frozen at −18 °C for the 5-day collection period as a pooled sample and stored until analyses.

Chemical analyses

All feedstuffs, including ingredients for both the BR and TR, underwent grinding with a centrifugal mill (Type Z100, Retsch GmbH, Haan, Germany) utilising a 1 mm mesh screen for subsequent analyses. After thawing, urine and faeces were homogenised. Faecal samples were lyophilised (P18K-E-6, Piatkowski Forschungsgeräte, München, Germany) and ground following the same procedure as described for feedstuffs. All chemical analyses were conducted in duplicate according to the standards of VDLUFA (2012). The following parameters were determined in feedstuffs: DM (3.1), ash (8.1), crude protein (CP, N · 6.25; 4.1.1), ether extract after HCl digestion (EE; 5.1.1b), crude fibre (CF, 6.1), neutral detergent fibre treated with amylase and expressed exclusive of residual ash (aNDFom; 6.5.1), acid detergent fibre expressed exclusive of residual ash (ADFom; 6.5.2), acid detergent lignin (ADL; 6.5.3), minerals phosphorus (10.6) and calcium (Ca; 10.3), and reducing sugars (7.1.1). Ingredients were also analysed for ND insoluble CP (NDICP) and AD insoluble CP (ADICP), and TRs were analysed for NDICP, following the method described by Licitra et al. (1996). Starch (7.2.1) and phytase activity (27.1) were determined at LUFA Nord-West (Oldenburg, Germany) in samples that were refrigerated until shipment to preserve phytase activity. Phytate was analysed at the Institute of Animal Science, University of Hohenheim, Stuttgart, Germany, following Zeller et al. (2015) and using high-performance ion exchange chromatography (Dionex ICS-3000, using Dionex CarboPac® PA

200 column, Idstein, Germany). An adiabatic bomb calorimeter (C 200, Ika-Werke GmbH & Co. KG, Staufen, Germany) was used to analyse the heat of combustion of feedstuffs, faeces and urine (in triplicate after lyophilisation).

Ash, N, P and Ca contents were analysed in thawed urine samples as described above. Additionally, urea and ammonia were analysed using an urea/ammonia assay (R-Biopharm AG, Arc. No. 10542946035; Darmstadt, Germany). DM, ash, N, P and Ca contents in fresh faeces samples were analysed as described above. Moreover, the previously specified methods were also applied to lyophilised samples: CF, aNDFom, ADFom, ADL, NDICP and ADICP.

Calculations and statistical analyses

P digestibility in the TRs was calculated according to GfE (1994) as follows:

$$\text{digestibility of } P_{TR} = \frac{P_{intake} - P_{output}}{P_{intake}},$$

where: P_{intake} represents total P intake (g) and P_{output} total faecal P output (g) during the 5-day collection period. P digestibility of the compound feed was determined by difference GfE (1994):

$$\text{digestibility of } P_{compound\ feed} =$$

$$= \frac{\text{digestibility of } P_{TR} - [\text{digestibility of } P_{BR} \times (1 - a)]}{a}$$

with a = analysed $P_{compound\ feed}$ (g/kg DM) × inclusion level of compound feed in TR) / analysed P $content_{TR}$ (g/kg DM)

The ME of the corresponding compound feed was calculated by proportionally subtracting the ME of the corresponding BR from the ME value of the TR. Following Mason and Frederiksen (1979), NDIN (= NDICP/6.25) in faeces was considered as indigestible dietary N and subtracted from total faecal N, leaving metabolic faecal N (mfN).

Data analysis was conducted using the MIXED procedure of SAS (version 9.4; SAS Institute, Inc., Cary, NC, USA). The normal distribution of the results was checked using the Kolmogorov-Smirnov and Shapiro-Wilk tests. If necessary, outliers were identified using a boxplot and eliminated before statistical analysis to ensure normal distribution. In this model, the treatment was divided into its factors, with CER (n = 2), phytase supplementation (n = 2), PM (n = 2) and period (n = 3) included as fixed effects and analysed separately. The animal was considered as a random effect. The level of significance was set at $P < 0.05$. The results of the treatments are presented as least squares means.

Results

Animals

All pigs were healthy throughout the experiment. However, some animals, particularly those fed the BR, refused up to 20% of their daily ration during a single collection period.

Chemical composition

The planned P and dP contents of the BRs were 0.6 g P/kg and 0.3 g dP/kg, respectively; however, the analysed P and dP concentrations were higher (Table 1). Consequently, the analysed P and dP contents in the TRs were also higher than calculated (Table 2). Both BRs were prepared using the same formulation except for the phytase supplementation, yet CF, aNDFom, and sugar contents, as well as phytase activity of the (+)BR were higher compared to the (−) BR. Differences between the CER and PM were due to their belonging to different species (Table 3); therefore, only the respective types were compared among themselves. The TRs were calculated for a concentration of 2.0 g dP/kg DM, leading to different inclusion levels of the compound feed in individual TRs. The CP was lower in the (+)TRs compared to the (−)TRs (Table 2). All (+)TRs or R-TRs had a phytase activity greater than 1000 FTU/kg DM, while the (−)W-TR remained below 500 FTU/kg DM (Table 2). The GE content in all TRs was similar.

Phosphorus and metabolisable energy

Phosphorus digestibility values and ME concentrations of compound feeds are shown in Table 4. P digestibilities of the (+) compound feeds were 11.8 percentage units higher ($P < 0.05$) compared to the (−) compound feeds, and 3.0% higher ($P < 0.05$) in the compound feeds with SBM compared to those with RSM. Phosphorus digestibility of the (−) compound feed containing SBM or RSM was 60.7% and 55.4%, respectively, whereas the (+) compound feed showed similar values of 70.2% and 69.5%, respectively. The ME concentration was higher ($P < 0.05$) in wheat-based compound feeds and those with SBM compared to rye-based and RSM-containing compound feeds, respectively. Phytase supplementation had no effect on ME concentration.

Nitrogen balance

There was no effect found of CER on N intake (Table 5). However, urinary N excretion was higher (1.96 g/day; $P < 0.05$) in pigs fed the W-TR compared to the R-TR. In contrast, faecal N excretion in the W-TR-fed pigs was lower (1.47 g/day; $P < 0.05$) compared to pigs fed the R-TR (Table 5);

Table 2. Chemical composition of test rations in, g/kg dry matter (DM)

Item	Basal ration							
	Cereal grain							
	wheat				rye			
	Phytase							
	supplemented		unsupplemented		supplemented		unsupplemented	
	Protein meal							
	SBM	RSM	SBM	RSM	SBM	RSM	SBM	RSM
DM, g/kg	892	898	904	911	903	906	915	915
Organic matter	951	943	950	942	951	943	950	949
Ash	48.8	57.3	49.5	57.6	48.6	56.6	50.1	51.0
Crude protein	216	196	223	208	208	195	216	193
Ether extract	29.4	32.4	30.3	32.9	27.2	30.1	28.0	30.1
Crude fibre	38.7	46.6	38.4	52.2	33.4	44.5	30.9	67.0
aNDFom	213	231	249	204	223	225	245	249
ADFom	66.1	73.7	72.8	70.1	51.9	72.4	57.5	72.0
ADL	15.1	19.0	11.0	23.0	23.6	22.6	11.0	27.0
NDICP	76.9	63.4	74.9	70.0	80.0	81.8	68.2	64.5
Starch[1]	511	510	518	512	502	493	498	495
Sugar	56.8	56.9	50.4	51.7	67.3	65.5	63.6	62.8
Ca	5.90	7.73	4.84	7.11	5.16	7.11	4.86	5.49
P	2.83	3.23	3.07	3.63	2.88	3.34	3.09	4.07
Digestible P[2]	2.05	2.26	1.85	1.92	1.93	2.26	1.75	2.23
Phytate	5.68	5.94	6.20	6.80	5.54	5.81	5.74	7.66
Phytate-P	1.60	1.67	1.75	1.91	1.56	1.64	1.62	2.16
Phytate-P of P, %	56	52	57	53	54	49	52	53
Phytase activity, U/kg DM	2169	1875	422	458	2664	2555	1466	1454
Gross energy, MJ/kg DM	18.1	17.9	18.1	18.0	18.0	17.9	18.0	18.0

aNDFom – neutral detergent fibre assayed with heat stable amylase and expressed exclusive of residual ash, ADFom – acid detergent fibre expressed exclusive of residual ash, ADL – acid detergent lignin, NDICP – neutral detergent insoluble crude protein, SBM – soybean meal, RSM – rapeseed meal; [1] polarimetric measurement; [2] calculated following GfE (1994)

Table 3. Chemical composition of compound feed ingredients, g/kg dry matter (DM) unless stated otherwise

Item	Wheat	Rye	SBM	RSM
DM, g/kg	877	907	892	889
Ash	17.6	17.6	68.2	77.6
Crude protein	119	94.0	512	413
Ether extract	25.9	21.9	27.3	33.5
Crude fibre	30.7	21.4	43.0	128
aNDFom	129	130	212	340
ADFom	46.0	51.0	179	224
ADL	11.0	11.0	27.0	9.00
NDICP	19.5	17.4	152	107
ADICP	1.42	11.4	37.0	36.0
Starch[1]	698	657	66.0	61.0
Sugar	36.1	63.5	108	103
Ca	0.16	0.19	2.11	7.96
P	3.38	3.19	6.99	12.6
Phytate	8.32	7.26	12.0	28.3
Phytate-P	2.34	2.04	3.38	7.97
Phytate-P of P, %	69	64	48	63
Phytase activity, U/kg DM	505	3278	n.d.	n.d.
Gross energy, MJ/kg DM	18.0	17.6	19.3	19.5

aNDFom – neutral detergent fibre assayed with heat stable amylase and expressed exclusive of residual ash, ADFom – acid detergent fibre expressed exclusive of residual ash, ADL – acid detergent lignin, NDICP – neutral detergent insoluble crude protein, ADICP – acid detergent insoluble crude protein, SBM – soybean meal, RSM – rapeseed meal, n.d. – not determined; [1] polarimetric measurement

the same phenomenon was observed for mfN, which was lower (1.29 g/day; $P < 0.05$) in pigs fed W-TR (Table 6). An effect of PM on N intake was observed, resulting in higher (1.50 g/day; $P < 0.05$) intake recorded for pigs fed the SBM-TR compared to pigs fed the RSM-TR. This was reflected in higher (1.65 g/day; $P < 0.05$) urine N excretion of the SBM-TR-fed animals compared to the RSM-TR group. Conversely, faecal N excretion was lower (1.16 g/day; $P < 0.05$) in pigs fed the SBM-TR than in pigs fed the RSM-TR, which was also reflected in lower mfN (0.89 g/day; $P < 0.05$) and NDIN (0.15 g/day; $P < 0.05$) excretion in pigs fed RSM-TR. Nitrogen balance was higher (1.26 g/day) in pigs fed SBM-TR compared to RSM-TR. An effect of phytase supplementation on N intake was detected, resulting in lower (4.22 g/day; $P < 0.05$) intake of pigs fed the (+)TR compared to pigs fed the (−)TR, as reflected in lower (5.00 g/day; $P < 0.05$) urinary N excretion of the (+)TR-fed animals. However, no effect of phytase supplementation was observed on total faecal N excretion or mfN excretion (Table 6), which, combined with lower N intake, resulted in an 8.7% higher ($P < 0.05$) N utilisation efficiency of pigs fed the (+)TR compared to the (−)TR.

Table 4. Phosphorus digestibility (%) and metabolizable energy (MJ/kg dry matter (DM) in compound feed presented as least squares means

Item	Cereal grain								SEM	*P*-value			
	wheat				rye								
	Phytase												
	supplemented		unsupplemented		supplemented		unsupplemented						
	Protein meal												
	SBM	RSM	SBM	RSM	SBM	RSM	SBM	RSM		CER	Phyt	PM	R
Phosphorus digestibility	73.4	71.1	62.6	55.0	67.0	67.9	58.8	55.9	2.13	0.09	<0.05	<0.05	<0.05
Metabolizable energy	15.6	14.8	16.0	14.9	15.2	14.5	15.2	14.2	0.19	<0.05	0.86	<0.05	0.10

SBM – soybean meal, RSM – rapeseed meal, SEM – standard error of the means, CER – cereal grain, Phyt – phytase supplementation, PM – protein meal, R – round; $P < 0.05$ indicates that data are significantly different

Table 5. Nitrogen balance (g/day) and efficiency of N utilisation (%) of test rations presented as least squares means

Item	Basal ration								SEM	*P*-value			
	Cereal grain												
	wheat				rye								
	Phytase												
	supplemented		unsupplemented		supplemented		unsupplemented						
	Protein meal												
	SBM	RSM	SBM	RSM	SBM	RSM	SBM	RSM		CER	Phyt	PM	R
N intake	35.8	34.8	40.7	38.8	35.1	35.8	41.4	37.6	0.95–1.04	0.94	<0.05	<0.05	<0.05
Urinary N excretion	12.8	11.7	18.3	17.3	11.4	10.4	17.1	13.5	0.875	<0.05	<0.05	<0.05	<0.05
Faecal N excretion	3.97	4.85	3.75	5.15	4.98	5.88	5.63	7.08	0.335	<0.05	0.12	<0.05	0.49
N balance	19.2	18.3	19.4	16.3	18.8	19.5	18.7	16.9	1.02	0.84	0.20	<0.05	<0.05
Efficiency of N utilisation	53	52	47	42	54	55	45	45	2.2	0.63	<0.05	0.32	0.30

N – nitrogen, CER – cereal grain, Phyt – phytase supplementation, PM – protein meal, SBM – soybean meal, RSM – rapeseed meal, SEM – standard error of the means, R – round; SEM is stated as a range due to different n for test rations (n = 6), when a correction for outliers was made if the whole data set was not normally distributed; $P < 0.05$ indicates that data are significantly different

Table 6. NDIN and ADIN intake and faecal excretion and metabolic faecal nitrogen (g/day) by the group fed the test ration presented as least squares means

Item	Basal ration								SEM	*P*-value			
	Cereal grain												
	wheat				rye								
	Phytase												
	supplemented		unsupplemented		supplemented		usupplemented						
	Protein meal												
	SBM	RSM	SBM	RSM	SBM	RSM	SBM	RSM		CER	Phyt	PM	R
Intake													
NDIN	15.0	11.3	13.9	13.0	13.5	15.0	13.1	12.5	0.125	0.52	0.10	<0.05	<0.05
ADIN	0.502	1.26	0.400	1.17	0.541	1.36	0.800	1.23	0.025	<0.05	0.51	<0.05	<0.05
Faeces													
NDIN	0.393	0.517	0.351	0.620	0.492	0.642	0.457	0.503	0.0397	0.12	0.40	<0.05	0.52
ADIN	0.558	0.512	0.525	0.682	0.480	0.575	0.542	0.704	0.0424	0.85	<0.05	<0.05	<0.05
mfN	3.58	4.33	3.40	4.53	4.49	5.24	5.18	6.10	0.306–0.319	<0.05	0.19	<0.05	<0.05

NDIN – neutral detergent insoluble nitrogen, ADIN – acid detergent insoluble nitrogen, mfN – metabolic faecal nitrogen, CER – cereal grain, Phyt – phytase supplementation, PM – protein meal, SBM – soybean meal, RSM – rapeseed meal, SEM – standard error of the mean, R – round; SEM is given as a range due to different n for test rations (n = 6) when correction was made for outliers if the entire data set was not normally distributed; $P < 0.05$ indicates that data are significantly different

Discussion

Animals and experimental procedure

Deviating from the recommendations of GfE (1994), limestone, as a phytase carrier, was mixed in both BRs, so that the only difference between the two BRs was phytase activity. The BR, as suggested by GfE (1994), was formulated to provide no more than 6 g Ca/kg DM and approx. 1 g P/kg DM. In the present experiment, the Ca:P ratio in the (+)BR was

7.4:1, and in the (−)BR, it was 8.5:1. Recommendations for P supply for growing pigs in Germany are based on dP, and the Ca:dP ratio in pig rations should be between 2:1 and 3:1 (DLG, 2010). In our study, the Ca:dP ratio of the TRs ranged from 2.5 to 3.7. The (−)W-RSM-TR and (+)R-RSM-TR rations had Ca:dP ratios of 3.7 and 3.1, respectively, which was slightly higher than the recommended level. An unbalanced ratio of Ca:dP may negatively affect P digestibility, as it may cause formation of mineral complexes with phytate (Dersjant-Li and Dusel, 2019). Klein et al. (2022) demonstrated that adding limestone corresponding to 8.5 g Ca/kg DM instead of 5.4 g Ca/kg DM (Ca:dP: 3:1 vs. 1.9:1) reduced phytate degradation in the hindgut, but without affecting P digestibility. As the actual Ca:dP ratio did not affect the efficiency of P utilisation, the addition of limestone evidently had no negative effect on P digestibility.

The dP content in three TRs was above the targeted 2 g/kg DM, with a maximum value of 2.26 g/kg DM. Since there was no regulatory excretion via urine (data not shown), these concentrations still ensured a suboptimal supply. Using a BR as the control group and calculating P digestibility of the compound feed, it was assumed that the results were corrected for endogenous losses. Thus further corrections, as described by She et al. (2018), by estimating the standardised total tract digestibility (STTD) values, were not considered beneficial.

Chemical composition

Ration

The differences in chemical composition between the two BRs could be due to the feed manufacturing processes, given that the BRs were produced in two different batches. The variation and composition between the two BRs were consistent with those of Schemmer et al. (2020), except for the Ca content, and consequently, ash, which were higher in the present experiment due to the inclusion of limestone.

Urine

The influence of P intake with drinking water on P digestibility values can be neglected because P concentration in drinking water was below 0.01 mg/l (Stadtwerke Bonn, personal communication, 2021). Daily urinary P excretion was low (<0.04 g/day; data not shown) and consistent with values recommended by GfE (1994) and Schemmer et al. (2020), indicating adequate dP concentrations in the diets, which allowed almost complete utilisation of absorbed P by the pigs and consequently did not affect the measured P digestibility values.

Phosphorus digestibility

Phytate-P must be enzymatically cleaved to be available to animals. While the activity of phytase plays a pivotal role in determining the extent of this effect, factors related to animals, diet and measurements may exert additional influences. The intrinsic phytase activity of CER varied significantly. Interestingly, no discernible effect of CER on P digestibility was observed. The differences in P digestibility could be attributed to the phytate-P content and its proportion in total P, especially in PM. This aligns with the findings of Rodehutscord et al. (1996), who tested P digestibility of wheat, SBM and their combination without phytase supplementation. The latter authors demonstrated the additivity of P digestibility of individual components and suggested no effect of internal wheat phytase on SBM P digestibility. Similar studies on other cereal grains also found no correlation between intrinsic phytase activity and P digestibility values (Hovenjürgen et al., 2003; Schemmer et al., 2020; Klein et al., 2021). Nevertheless, the findings of Archs Toledo et al. (2020) showed a positive effect of endogenous phytase of hybrid rye on P digestibility of maize grain-SBM rations. A possible explanation for this discrepancy could be attributed to the location of intrinsic phytase and phytate-P. Phytase tends to accumulate near its substrate until it is hydrolysed during germination. Notably, phytate is primarily stored in other tissues, such as the aleurone layer of wheat and rye, soybean cotyledon or maize germ (Madsen and Brinch-Pedersen, 2020). Klein et al. (2021) postulated that endogenous phytases might not be able to hydrolyse phytate from other sources in feed. However, grinding may increase the accessibility to softer parts such as the germ or endosperm. The observation by Archs Toledo et al. (2020) could potentially be linked to the accessibility of rye internal phytase to phytate-P in the germ of maize, compared to phytate-P in more resistant cotyledons of granulated SBM or RSM.

Studies have shown that the effect of microbial phytase supplementation increases almost linearly up to 1000 FTU/kg, reaching an asymptote at approx. 1800–2000 FTU/kg (Dersjant-Li and Dusel, 2019; Rosenfelder-Kuon et al., 2020). In our study, phytase activity in the (+)TR was >1875 FTU/kg DM, implying the attainment of the maximum phytase effect. The difference in P digestibility

of the (+)TR and (−)TR suggests that the endogenous phytase, compared to its commercial microbial counterpart, may not have been sufficiently resistant to pH or proteases active in the stomach or other factors affecting its activity (Dersjant-Li et al., 2015; Dersjant-Li and Dusel, 2019). For instance, dietary fibre components can exert a confining effect on phytate-P (Pettersson and Pontoppi, 2013) or lead to a higher viscosity of digesta, hindering phytases from reaching their substrate and impeding their efficiency.

The phosphorus digestibility of wheat and rye in the (−) compound feed was 59% and 57%, respectively, while in the (+) compound feed, it was 72% and 67%, respectively. These values were consistent with Düngelhoef et al. (1994), who tested wheat as a single component and found P digestibility of 62% (± 3%) in rations without phytase supplementation, and 74% (± 3%) in rations with phytase addition (750 FTU/kg). Schemmer et al. (2020) reported a mean P digestibility of 59% in wheat without phytase supplementation, but a significantly lower value for rye of only 45%. McGhee and Stein (2019) tested three different hybrid rye and wheat grains, both supplemented with phytase (1000 FTU/kg) and unsupplemented. They obtained STTD values of P for unsupplemented hybrid rye ranging from 49% to 56%, 37% for wheat, and 62–71% and 58% for supplemented grains, respectively. The values for rye were similar to the P digestibility of the R-compound feed analysed in this study. Generally, a substantial variability exists in phytase activity, phytate-P content and P digestibility between and within different cereal grains. This variability is partly attributed to disparities in the methods employed for digestibility determination and the genotype of cereal grains within a species (Schemmer et al., 2020). Rodehutscord et al. (1997) investigated P digestibility of SBM and RSM, finding 37% for SBM in the ration without phytase supplementation, and 76% in the phytase-supplemented (750 FTU/kg) ration; the corresponding values for RSM amounted to 24% and 73%, respectively. Consistent with these observations, our study also demonstrated that phytase supplementation exerted a stronger effect on RSM-compound feed, particularly at a higher phytate-P concentration. Consequently, both supplemented compound feeds achieved a P digestibility of approximately 70%. Nevertheless, the digestibility of the (−) compound feed containing PM (61% in SBM, and 55% in RSM) was relatively high compared to most values reported in the literature (Rodehutscord et al., 1997; DLG, 2014; Mejicanos et al., 2016). This suggests that the inclusion of the grain ingredient enhanced the overall P digestibility of the compound feed. The overall outcomes of the experiment were consistent with the findings of She et al. (2017; 2018), who analysed STTD of P in SBM (57% and 66%, respectively) and RSM (39% and 45%, respectively) without supplements. The latter study also investigated different levels of microbial phytase (500, 1000 and 1500 FTU) supplementation, resulting in even higher STTDs (SBM: 82%, 90% and 90%; RSM: 70%, 72% and 77%, respectively). Similar to CER, the variability in PM values could be due to different concentrations of phytate-P and their proportion relative to total P, enzyme-substrate relationships or the selected method of P digestibility determination. Nevertheless, PM in the (+) compound feed reached a P digestibility of 70%, a remarkably high value for a mixed dry ration.

Energy concentration

Differences in ME concentrations were influenced by individual chemical composition of CER or PM. Wheat contained more starch and CP compared to rye, leading to a higher ME concentration (0.6 MJ/kg DM). Likewise, SBM contained more CP and less fibre than RSM, contributing to a higher ME concentration (0.9 MJ/kg DM). The lowest ME concentration among the compound feeds tested was obtained for the (−)R-RSM compound feed (14.2 MJ/kg DM), which was still a high value suitable for pig rations. McGhee and Stein (2020) observed no effect of hybrid rye rations on ME concentration, while Arredondo et al. (2019) found no effect of an increase in phytase activity from 0 to 2550 FTU/kg on ME of a ration based on maize grain and soybean meal. The results of both of these studies were consistent with the present findings. Wilke (2020) reported that feed intake and daily weight gain in weaned piglets were neither influenced by the substitution of wheat grain with rye grain nor by SBM and RSM, even when high proportions of rye grain (60%) and RSM (28%) were incorporated. Notably, the feed conversion ratio was predominantly influenced by the elevated proportion of rapeseed meal in the compound feed, a factor that can be further attributed to the lower ME content determined in this study.

Nitrogen balance

The TRs were not intended to be isonitrogenous or isoenergetic. The adjustment of the dP content to 2 g/kg DM in the TR, resulted in varying proportions

of the BR to compound feed. Generally, across all TRs, higher N intakes in the SBM-TRs and (−)TRs correlated with elevated urinary N excretion.

Although pigs fed the R-TR or W-TR had equal N intake, urinary N excretion was 1.93 g N/day higher in the latter; the difference can be entirely attributed to 2.00 g/day urea-N and interpreted as excess excretion. Conversely, in pigs fed the W-TR, faecal excretion was 1.46 g N/day lower, which was further reflected in a 1.29 g N/day lower mfN compared to theR-TR. The higher content of fermentable fibre (aNDFom) in the R-TR likely led to increased fermentation in the large intestine. Consequently blood urea-N was transferred to the large intestine and utilised to support microbial growth (Bindelle et al., 2008). The shift of N excretion from urine to faeces, bound in microbial biomass, was reflected by higher mfN excretion of animals fed the R-TR.

The elevated CP concentration in SBM and the greater proportion of SBM than RSM in the TRs (on average 18% SBM vs. 14% RSM) resulted in a 1.50 g N/day higher N intake by pigs fed the SBM-TR compared to pigs fed the RSM-TR. Fibre, i.e., cell-wall material, can encapsulate nutrients, and thus hinder their digestion (Agyekum and Nyachoti, 2017). In addition, RSM contains polyphenols, such as tannins, which can bind protein, thereby potentially reducing protein digestibility in RSM compared to SBM (Choi et al., 2015). The higher N intake of the SBM-TR and its better availability due to the lower fibre concentration and different fibre composition compared to RSM was reflected, on the one hand, in a 1.65 g N/day higher urinary N excretion, and a 1.88 g N/day higher urea-N excretion in pigs fed the SBM-TR compared to the RSM-TR. On the other hand, the 1.16 g N/day lower faecal N excretion of pigs fed the SBM-TR compared to RSM-TR, and especially the 0.15 g N/day lower ($P < 0.05$) faecal NDIN, reflected improved digestibility due to the lower amount of undigested dietary N. The higher NDIN digestibility (calculated from Table 6) of the SBM-TR, in addition to the factors mentioned above, was also related to the higher phytate-P content in RSM than SBM, with phytate-bound protein being recovered in the NDIN fraction and unavailable to the animal. The lower mfN excretion in pigs fed the SBM-TR compared to the RSM-TR group was primarily associated with the total intake of fermentable fibre. All these factors resulted in lower nutrient utilisation of the RSM-TR in the small intestine, with more N and carbohydrates entering the large intestine, where they could serve as substrates for microbial digestion and fermentation.

The reduced intake of 4.22 g N/day in animals fed the (+)TRs compared to the (−)TRs was reflected in a 4.98 g N/day lower urinary N excretion, of which 4.12 g N/day was urea-N. However, no effect of phytase supplementation was observed on total faecal N excretion or on mfN, which, in addition to lower N intake and even lower urinary N excretion, resulted in a higher N utilisation of pigs fed the (+)TRs compared to the (−)TRs. Once again, the evident oversupply of nitrogen resulted in direct excretion in the urine and was not conducive to optimal N metabolism. In addition to other fibre-related factors (e.g., pH reduction in the large intestine), feeding fermentable fibre in combination with a reduction in N intake, especially precaecally indigestible N fractions, can effectively reduce ammonia emissions in manure, as urinary urea N is more susceptible to rapid decomposition (Bindelle et al., 2008).

Conclusions

The combination of rye and rapeseed meal proved to be a valuable alternative with regard to P digestibility, metabolizable energy content and N excretion, to a wheat-soybean meal-based ration for growing pigs and can be recommended. Supplementation with phytase is essential from the perspective of good agricultural practice, demonstrating benefits both ecologically and economically.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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