Phytase supplementation effects on amino acid digestibility depend on the protein source in the diet but are not related to $InsP_6$ degradation in broiler chickens

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ABSTRACT The objective was to determine phytase effects on prececal amino acid (**AA**) digestibility and phytate ($InsP_6$) breakdown when different oilseed meals were used in broiler chicken diets. The study included 14 diets: a corn-soybean meal (**SBM**) basal diet and 6 diets that contained SBM, rapeseed meal (**RSM**), and sunflower meal (**SFM**) with 2 inclusion levels at the expense of corn starch (150 and 300 g/kg SBM or SFM, or 100 and 200 g/kg RSM). Each diet was mixed with or without a phytase supplement of 1,500 FTU/kg. Diets were provided to broilers for 5 D. Digesta from the posterior half of the ileum were collected on day 21. The average essential AA digestibility, calculated by a regression approach, without and with phytase was 84 and 85% (SBM), 74 and 77% (SFM), and 66 and 73% (RSM), respectively. In the diets, phytase effects on AA digestibility were lower owing to other protein sources also present in the diet, but significant. Prececal $InsP_6$ disappearance was significantly affected by interactions between oilseed meal, inclusion level, and phytase supplementation. Overall, prececal $InsP_6$ disappearance was higher in SBM diets (52%) than in SFM diets (38%) and intermediate in RSM diets (43%). Across diets, phytase supplementation effects on prececal $InsP_6$ degradation linearly increased with the $InsP_6$ concentration of the diet up to 12 g/kg DM. The only exception from linearity was the diet with the high inclusion of SFM, which contained 15.9 g $InsP_6$/kg DM. In the ileal content, the concentration of *myo*-inositol was significantly increased by phytase supplementation, and this effect was highest in the diets that contained SBM as the only oilseed meal. Concentrations of lower inositol phosphates were increased by phytase supplementation, and this effect was most remarkable for Ins(1,2,3,4)P_4 and inositol tetrakisphosphates. The study showed that phytase effects on AA digestibility varied among the 3 tested oilseed meals, but these differences were not detectable in the diets containing these meals. Although phytase effects on ileal content of $InsP_6$ and its degradation products were substantial, they were not related to the effects on AA digestibility.

Key words: phytate, energy, protein feeds, regression

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INTRODUCTION

Phytases are widely used feed additives in nonruminant nutrition. The main function of phytase is the cleavage of *myo*-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate) ($InsP_6$) and its salts (phytate) to increase phosphorus (**P**) utilization by the animal. Phytase supplementation also affected amino acid (**AA**) digestibility of broiler chickens in some, but not all, studies (Ravindran et al., 1999; Rutherfurd et al., 2002; Rodehutscord et al., 2004; Kong and Adeola, 2014; Sommerfeld et al., 2018a,b). Inconsistent effects may partly be related to different protein sources used in the diet and by differences in $InsP_6$ storage in seeds (Erdman, 1979; Yiu et al., 1983; Adeola and Sands, 2003). In rapeseed, $InsP_6$ is located in globoid crystals within protein storage vacuoles (Gillespie et al., 2005) and is tightly associated with proteins (Yiu et al., 1983). In soybeans, $InsP_6$ is evenly distributed throughout the seed (Han and Wilfred, 1988). Its storage location is also different in sunflower seeds, in which $InsP_6$ is found in crystalloids or globoids within the kernel (Erdman, 1979; Allen and Arnott, 1981; Miller et al., 1986). These differences in $InsP_6$

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location and association with proteins among types of seeds might contribute to differences in AA digestibility that have been reported for different oilseed meals (e.g., Ravindran et al., 1999; Senkoylu and Dale 1999).

Values of AA digestibility are affected by endogenous protein secretion into the digestive tract and the different approaches used to consider basal endogenous AA losses. Often, basal endogenous AA loss is considered using estimates taken from literature but is also estimated independently using N-free diets. This presumes that basal endogenous protein loss is identical for the test feed and the N-free diet (Rutherfurd et al., 2002). Alternatively, AA digestibility is studied using the regression approach. The regression approach implies that basal endogenous losses are excluded in the way AA digestibility is calculated; thus, this is the approach with the highest accuracy (Ravindran et al., 2017). In this context, investigations of phytase effects are of specific interest because pure $InsP_6$ or phytate administered to chickens was found to increase mucin secretion (Onyango et al., 2009). This implies that basal endogenous AA losses may be different depending on the type of diet and whether $InsP_6$ is degraded in the digestive tract or not.

In the present study, we investigated whether the effects of phytase supplementation on AA digestibility differ among oilseed meals and diets containing different oilseed meals. We used rapeseed meal (**RSM**), soybean meal (**SBM**), and sunflower meal (**SFM**) and combined the measures of AA digestibility with $InsP_6$ degradation measures. The hypotheses were that differences in phytase effects on AA digestibility exist among oilseed meals and that these differences are reflected in differences in $InsP_6$ disappearance and formation of lower inositol phosphate isomers ($InsP_x$) and *myo*-inositol.

MATERIALS AND METHODS

Animals and Management

The experiment was conducted at the Agriculture Experiment Station of the University of Hohenheim in accordance with German Animal Welfare Legislation following approval of the Regierungspräsidium Tübingen, Germany (approval no. HOH49-17TE). Unsexed Ross 308 broilers were obtained from a commercial hatchery (Brüterei Süd ZN der Bwe-Brüterei Weser-Ems GmbH & Co. KG, Regenstauf, Germany). The hatchlings were allocated into groups of 15 and placed in 74 floor pens (115 × 230 cm ground area, 260 cm height). Seven pens each were used for the basal diet with and without phytase, and 5 pens for each of the other diets. The temperature in the animal house was continuously reduced from 34°C at the beginning to 26°C on day 21 of the experiment. The light regimen was 24L:0D during the first 3 D and 18L:6D thereafter. For the first 15 D, the birds were kept on wood shavings. On day 16, the litter was removed from the floor and birds were then kept on perforated floors until the end of the experiment. Birds were reallocated among pens on this day to achieve a similar animal weight (8,117 g ± 267 g) in each pen. The pens were randomly allocated to treatment diets in a completely randomized block design to achieve equal distribution of treatments within the building.

Diets

Feed and water were offered for ad libitum consumption throughout the experiment. A commercial starter was provided for the first 15 D and contained (per kg) 215 g CP, 11 g Ca, 5.5 g P, 12.5 MJ ME, 110 mg monensin sodium, 10 IU endo-1,4-β-xylanase, and 750 FTU of a 6-phytase (Deutsche Tiernahrung Cremer GmbH & Co. KG, Düsseldorf, Germany). A total of 14 experimental diets was mixed (Table 1). The basal diet consisted of mainly corn starch, corn, SBM, and corn gluten meal and was formulated to meet or exceed the recommendations of the Gesellschaft für Ernährungsphysiologie (GfE, 1999). Titanium dioxide (TiO_2, 5 g/kg) was used as an indigestible marker. In the other diets, corn starch was substituted for one of the oilseed meals at 2 different levels: 100 or 200 g RSM/kg (RSM1 and RSM2), 150 or 300 g SBM/kg (SBM1 and SBM2), and 150 or 300 g SFM/kg (SFM1 and SFM2). The inclusion of RSM was lower than that of the other meals because we wanted to avoid reduced feed intake of the birds at higher inclusion of RSM. The inclusion of oilseed meals at the expense of corn starch implied that differences in CP and AA content among diets originated only from the oilseed meals. One half of each diet was supplemented with 1,500 FTU phytase/kg (Natuphos E 5000 G, BASF SE, Germany) and labelled with "+", whereas the other half remained without a phytase supplement ("−"). All diets contained the same amount of monocalcium phosphate. The results of chemical analyses of all diets and the 3 oilseed meals are provided in Tables 2 and 3. Analyzed concentrations overall confirmed the calculated values (Table 2). Analyzed phytase activity was slightly higher than intended. The diets were produced by Research Diet Services (Research Diet Services BV, Hoge Maat 10, 3961NC, Wijk bij Duurstede, Netherlands) and provided to the broilers in pelleted form from day 16 to 21.

Measurements and Sampling Procedure

Animals were weighed on day 16 and day 21, and feed consumption within this period was determined on a pen basis. On day 21, all birds were weighed, stunned using a gas mixture (35% CO_2, 35% N_2, and 30% O_2), and then euthanized by CO_2 exposure. The posterior half of the section between Meckel's diverticulum and 2 cm anterior to the ileo-ceco-colonic junction was excised. The digesta was flushed out using ice-cold deionized water, pooled on a pen basis, and immediately frozen at −20°C until being freeze-dried.

Chemical Analyses

Samples of all diets were ground to pass through a 0.5-mm sieve (Ultra Centrifugal Mill ZM 200; Retsch

Table 1. Composition of the experimental diets (g/kg as fed, unless otherwise stated).

Diet	Basal	Basal+	RSM1	RSM2	RSM1+	RSM2+	SBM1	SBM2	SBM1+	SBM2+	SFM1	SFM2	SFM1+	SFM2+
Corn starch	300.00	300.00	200.00	100.00	200.00	100.00	150.00	0.00	150.00	0.00	150.00	0.00	150.00	0.00
SBM	0.00	0.00	0.00	0.00	0.00	0.00	150.00	300.00	150.00	300.00	0.00	0.00	0.00	0.00
RSM	0.00	0.00	100.00	200.00	100.00	200.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SFM	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	150.00	300.00	150.00	300.00
Corn	267.25	267.25	267.25	267.25	267.25	267.25	267.25	267.25	267.25	267.25	267.25	267.25	267.25	267.25
SBM	250.00	250.00	250.00	250.00	250.00	250.00	250.00	250.00	250.00	250.00	250.00	250.00	250.00	250.00
Corn gluten	75.00	75.00	75.00	75.00	75.00	75.00	75.00	75.00	75.00	75.00	75.00	75.00	75.00	75.00
Soybean oil	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00
L-Arginine·HCl	4.70	4.70	4.70	4.70	4.70	4.70	4.70	4.70	4.70	4.70	4.70	4.70	4.70	4.70
L-Valine	4.40	4.40	4.40	4.40	4.40	4.40	4.40	4.40	4.40	4.40	4.40	4.40	4.40	4.40
DL-Methionine	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
L-Lysine·HCl	2.70	2.70	2.70	2.70	2.70	2.70	2.70	2.70	2.70	2.70	2.70	2.70	2.70	2.70
Glycine	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
L-Threonine	2.30	2.30	2.30	2.30	2.30	2.30	2.30	2.30	2.30	2.30	2.30	2.30	2.30	2.30
L-Isoleucine	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80
L-Tryptophan	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
MCP	22.00	22.00	22.00	22.00	22.00	22.00	22.00	22.00	22.00	22.00	22.00	22.00	22.00	22.00
Limestone	8.60	8.60	8.60	8.60	8.60	8.60	8.60	8.60	8.60	8.60	8.60	8.60	8.60	8.60
Premix[1]	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Titanium dioxide	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
NaCl	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Phytase[2], FTU/kg feed	-	1,500	-	-	1,500	1,500	-	-	1,500	1,500	-	-	1,500	1,500

-, No exogenous phytase added; +, with phytase addition.
Numbers 1 and 2 indicate the inclusion level of the respective oilseed meal.
Abbreviations: MCP, monocalcium phosphate; RSM, rapeseed meal; SBM, soybean meal; SFM, sunflower meal.
[1]P-free vitamin/mineral premix.
[2]Phytase added on top of the diets.

Table 2. Analyzed concentrations of crude nutrients, amino acids, calcium, phosphorus, $InsP_6$, and phytase activity in the experimental diets (g/kg DM, unless otherwise stated).

Diet	Basal	Basal+	RSM1	RSM2	RSM1+	RSM2+	SBM1	SBM2	SBM1+	SBM2+	SFM1	SFM2	SFM1+	SFM2+
Dry matter	900.0	899.0	897.0	897.0	900.0	900.0	899.0	897.0	902.0	898.0	897.0	898.0	901.0	904.0
Organic matter	937.0	938.0	928.0	918.0	929.0	918.0	927.0	916.0	928.0	917.0	928.0	918.0	928.0	920.0
Crude protein	240.0	239.0	290.0	343.0	292.0	342.0	314.0	384.0	317.0	387.0	289.0	341.0	283.0	335.0
Ether extract	73.0	75.0	81.0	88.0	85.0	89.0	71.0	71.0	70.0	80.0	75.0	78.0	75.0	78.0
Crude fiber	19.0	18.0	40.0	58.0	40.0	59.0	24.0	31.0	24.0	27.0	47.0	75.0	43.0	76.0
GE, MJ/kg DM	19.5	19.3	19.0	20.0	19.6	20.0	19.7	19.9	19.7	19.9	19.6	20.0	19.6	20.0
Ala	13.3	12.9	15.5	17.8	15.7	18.0	16.6	19.8	16.4	20.1	15.5	17.9	15.1	17.5
Arg	17.9	17.2	20.9	24.1	21.2	24.4	23.7	28.6	23.4	29.1	22.1	26.8	22.0	26.3
Asx	22.5	22.1	26.3	30.1	26.5	30.4	31.5	40.1	31.3	40.9	27.3	32.1	26.7	31.6
Cys	3.5	3.5	4.7	5.8	4.8	5.9	4.5	5.6	4.6	5.7	4.4	5.1	4.3	4.9
Glx	43.3	42.3	51.3	60.6	52.3	61.2	56.7	70.0	56.5	71.2	53.5	63.4	52.1	62.2
Gly	12.9	12.6	15.5	18.3	15.5	18.7	16.2	19.1	16.1	19.4	16.2	19.7	15.9	19.2
His	6.7	7.0	8.3	9.9	8.3	9.7	8.6	10.9	8.4	10.8	7.9	9.2	7.5	9.0
Ile	10.6	9.4	12.5	14.6	12.7	15.3	14.8	16.8	14.5	18.1	12.8	15.4	13.1	15.0
Leu	23.1	21.9	26.3	30.0	26.6	30.4	29.0	34.0	28.6	35.1	26.2	29.7	25.7	29.0
Lys	13.3	12.9	16.1	19.0	16.3	19.4	18.2	22.5	18.2	23.0	15.2	17.3	15.0	16.9
Met	7.6	7.5	8.7	9.7	8.7	9.8	8.7	9.6	8.7	9.7	9.1	10.2	9.0	10.1
Phe	12.0	11.4	14.0	16.2	14.2	16.4	16.1	19.6	15.9	20.2	14.4	17.0	14.2	16.6
Pro	15.0	14.5	18.0	21.1	18.0	21.5	18.1	22.2	18.5	22.4	17.1	19.1	16.4	19.2
Ser	12.6	12.5	14.9	17.3	15.0	17.3	16.4	20.7	16.4	20.7	14.9	17.1	14.4	16.8
Thr	11.4	11.1	13.9	16.3	14.0	16.5	14.5	17.4	14.5	17.6	13.6	15.5	13.4	15.5
Trp	2.6	2.7	3.3	4.1	3.4	4.1	3.6	4.4	3.6	4.5	3.3	4.0	3.3	4.1
Tyr	8.5	8.1	10.0	11.6	10.1	11.8	11.2	13.6	11.1	14.0	9.9	11.3	9.6	11.1
Val	14.2	13.0	16.7	19.5	16.8	20.2	18.4	20.5	18.1	21.7	17.0	19.9	17.3	19.5
Phytase, FTU/kg	<60	1,760	<60	<60	1,930	2,040	<60	<60	1,770	1,700	<60	<60	1,930	1,920
Calcium	8.2	7.9	9.0	10.7	9.3	10.9	8.9	9.5	9.0	9.5	10.5	10.3	9.3	10.0
Phosphorus	8.7	8.4	9.8	11.9	10.3	12.1	9.9	11.0	10.0	11.1	11.3	12.9	10.6	12.3
$InsP_6$, µmol/g DM	11.7	11.5	15.6	19.4	15.9	19.5	14.8	17.8	14.6	17.9	17.5	24.3	18	23.9
$InsP_6$-P	2.2	2.1	2.9	3.6	3.0	3.6	2.8	3.3	2.7	3.3	3.3	4.5	3.3	4.4

Abbreviations: GE, gross energy; n.a., not analyzed; RSM1 and RSM2, rapeseed meal at 100 g/kg and 200 g/kg; SBM1 and SBM2, soybean meal at 150 g/kg and 300 g/kg; SFM1 and SFM2, sunflower meal at 150 g/kg and 300 g/kg.
+ = added phytase.

GmbH, Haan, Germany) or pulverized using a vibrating cup mill (PULVERISETTE 9; Fritsch GmbH, Idar-Oberstein, Germany). Digesta samples were pulverized using the same vibrating cup mill. Ground samples were analyzed for proximate nutrients and fiber fractions according to the methods of Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA), 2012. The concentrations of Ti, P, and Ca in pulverized feed and digesta samples were analyzed using inductively coupled plasma optical emission spectrometry after wet digestion (Zeller et al., 2015a). $InsP_3$ to InP_6 isomers were analyzed in pulverized feed and digesta samples, as described by Zeller et al. (2015a) with modifications noted by Sommerfeld et al. (2018b). Using this methodology, separation of enantiomers was not possible; therefore, we were unable to distinguish between the D- and L-forms. Some $InsP_3$ isomers could not be identified because standards were unavailable. Clear discrimination of the isomers Ins(1,2,6)P_3, Ins(1,4,5)P_3, and Ins(2,4,5)P_3 was not possible because they coeluted. *Myo*-inositol in feed and digesta samples was analyzed according to the study by Sommerfeld et al. (2018a) using gas chromatography coupled with mass spectroscopy after derivatization.

The concentrations of AA were determined according to a previously described method (Rodehutscord et al., 2004) with modifications (Siegert et al., 2017). In this assay, methionine and cysteine were determined as methionine sulphone and cysteic acid. The amide residue in the side group of asparagine (Asx) and glutamine (Glx) is lost during acid hydrolysis, and aspartic acid and glutamic acid are formed (Fontaine, 2003). Hence, aspartic acid was analyzed together with Asx and glutamic acid together with Glx. Determination of tyrosine and histidine might have been affected by the oxidation process (Mason et al., 1980). Tryptophan analysis followed previously described procedures (Fatufe et al., 2005). Phytase activity of the diets was analyzed by BASF SE (Ludwigshafen, Germany) according to method ISO EN 30024.

Calculations and Statistics

The ADG, ADFI, and gain:feed ratio (G:F) were calculated on a pen basis from day 16 to day 21 and corrected for mortality. Prececal $InsP_6$ degradation and pc digestibility of CP, AA, P, Ca, and gross energy (GE) (y) were calculated on a pen basis using the following equation:

$$y\left(\%\right) = 100 - 100 \times \left(\frac{Ti\ feed\ (g/kg\ DM)}{Ti\ digesta\ (g/kg\ DM)} \times \frac{Analyte\ digesta\ (g/kg\ DM)}{Analyte\ feed\ (g/kg\ DM)}\right) \quad [1]$$

Table 3. Chemical analyses of the used oilseed meals (g/kg DM, unless otherwise stated).

Oilseed meal	RSM	SBM	SFM
Organic matter	928.0	921.0	926.0
Crude protein	371.0	530.0	372.0
Ether extract	49.0	24.0	26.0
Crude fiber	154.0	46.0	213.0
GE (MJ/kg DM)	20.1	19.8	19.6
Ala	16.5	23.7	17.0
Arg	21.4	38.9	31.2
Asx	27.6	62.0	35.1
Cys	7.4	7.3	5.6
Glx	62.1	96.2	73.0
Gly	18.7	22.4	22.8
His	12.4	15.6	10.7
Ile	11.9	22.6	14.8
Leu	24.8	40.7	24.4
Lys	19.4	32.7	14.2
Met	7.0	7.4	9.1
Phe	14.4	27.2	17.4
Pro	22.1	27.9	16.3
Ser	17.3	28.8	17.4
Thr	16.7	21.4	15.0
Trp	4.9	6.9	5.2
Tyr	10.5	18.1	9.9
Val	15.7	23.2	17.8
Ca	7.8	3.4	5.5
P	10.7	7.8	13.5
Glucosinolates, g/kg	3.3	n.a.	n.a.
ADFom, g/kg	192	n.a.	180
aNDFom, g/kg	262	n.a.	192
Starch, g/kg	19	18	54
Total sugar, g/kg	99	105	74
TIA, g/kg	n.a.	3.06	n.a.
Urease activity, mg N/g min 30°C	n.a.	<0.02	n.a.
Reactive lysine, %	n.a.	95	n.a.
Protein digestibility index, %	n.a.	11.2	n.a.
Nitrogen solubility index, %	n.a.	15	n.a.

Glucosinolates (ISO 9167-1:1992), total sugar (determined as glucose, Luff-Schoorl titrimetry), starch (heat stable α-amylase assay), TIA (NEN-EN-ISO 14902), urease activity, (NEN 3557:1995nl), reactive lysine (ANAL-10334), PDI (water soluble nitrogen after extraction using an Ultra-Turrax and centrifugation), and NSI (NEN-3517) were analyzed by NutriControl analytical solutions (Veghel, Netherlands).

Abbreviations: GE, gross energy; n.a., not analyzed; NSI, nitrogen solubility index; PDI, protein dispersibility index; RSM, rapeseed meal; SBM, soybean meal; SFM, sunflower meal; TIA, trypsin inhibitor activity.

Daily nutrient intake was calculated as the product of feed intake (g DM/D) and analyzed nutrient concentrations in the diets (g/kg DM). The amount of nutrient digested per day was calculated by multiplying daily intake and determined digestibility. Statistical evaluation of all traits determined for the diets was performed according to the following model:

$$y_{ijkl} = \mu + meal_i + phytase_j + level_k + meal_i \times level_k + phytase_j \times level_k + meal_i \times phytase_j + meal_i \times phytase_j \times level_k + block_l + e_{ijkl} \quad [2]$$

where y_{ijkl} is the mean value of each treatment, μ is the mean of all treatments, $phytase_j$ is the fixed effect of phytase supplementation j (0 or 1,500 FTU/kg), $level_k$ is the fixed effect of the inclusion level k (1 or 2) of the oilseed meal ($meal_i$, RSM, SBM, or SFM), $block_l$ is the random block effect, and e_{ijk} is the residual error.

Effects of phytase in the basal diet were evaluated for all traits according to the following model:

$$y_{ij} = \mu + phytase_i + block_j + e_{ij} \quad [3]$$

For the single oilseed meals (not diets), pc CP and AA digestibility were calculated as the slope of linear regressions between the ingested and digested amounts. Because the differences in intake of CP and AA originated only from the respective oilseed meal, the slope of the regression reflected the pc CP and AA digestibility of this oilseed meal. The following model was applied to determine the effect of phytase supplementation on pc CP and AA digestibility of the oilseed meals (Siegert et al., 2019a):

$$dig_{kl} = \alpha_{kl} + ing_{kl} \times \beta_{kl} + e_{kl} \quad [4]$$

where dig_{kl} is the daily amount of CP or AA pc digested in combinations of oilseed meal k and phytase supplementation l, α_{kl} is the intercept of combinations of oilseed meal k and phytase supplementation l, ing_{kl} is the daily amount of ingested CP or AA in combinations of oilseed meal k and phytase supplementation l, β_{kl} is the digestibility of CP or AA in combinations of oilseed meal k and phytase supplementation l, and e_{kl} is the residual error. The assumption of linearity between intake and digested amounts of CP and AA has been confirmed in previous studies (Rodehutscord et al., 2004; Rezvani et al., 2008; Kluth and Rodehutscord, 2009).

Regressions to calculate pc AA digestibility, comparisons among the slopes, and analysis of variance for all other traits were performed using PROC MIXED (version 9.4 of the SAS system for Windows, 2016; SAS Institute Inc., Cary, NC). Significant diet effects were determined using t tests if $P \leq 0.05$. Normal distribution and homogeneity of variance were tested before statistical analysis.

RESULTS

Performance Traits

Mortality was low (1.4%) and not related to any treatment. Phytase supplementation had no effect on performance traits when the basal diet was fed (Table 4). In the diets with different inclusion levels of the oilseed meals, ADG ranged between 66 g/D (RSM2+ and RSM2−) and 77 g/D (SBM1+) (Table 5). For ADG, the interaction between meal and level was significant ($P = 0.031$), indicating that increasing the inclusion rate of SBM but not for the other meals led to a decrease in ADG. The ADFI ranged between 75 g/D (SBM2+) and 84 g/D (SFM1+) and was significantly influenced

Table 4. Performance traits and prececal disappearance/digestibility of nutrients in the basal diet with (+) or without (−) phytase supplementation (least square means, pooled SEM; n = 7 pens per diet).

Trait	Phytase −	Phytase +	SEM	*P* value
Performance				
ADG, g/D	68	67	2.5	0.662
ADFI, g/D	87	84	2.7	0.202
G:F, g/g	0.79	0.80	0.02	0.679
Prececal disappearance/digestibility, %				
Essential amino acids				
Arg	93	93	0.2	0.106
His	87[b]	88[a]	0.5	0.030
Ile	89	88	0.7	0.450
Leu	89	89	0.6	0.990
Lys	91	91	0.4	0.245
Met	95	95	0.4	0.940
Phe	88[a]	88[a]	0.6	0.721
Thr	85[a,b]	86[a]	0.6	0.441
Trp	84	85	0.9	0.211
Val	90	90	0.6	0.571
Nonessential amino acids				
Ala	88	88	0.7	0.918
Asx	84	85	0.6	0.130
Cys	77	78	0.8	0.654
Glx	90	91	0.4	0.230
Gly	88	88	0.5	0.375
Pro	88	88	0.5	0.720
Ser	85	86	0.6	0.122
Tyr	88	88	0.7	0.702
$InsP_6$	26[b]	83[a]	2.1	<0.001
P	68[b]	72[a]	1.7	0.040
Ca	55	51	0.6	0.191
CP	88	89	0.5	0.459
GE	85	85	0.5	0.793

Values in the same column not sharing the same superscript letter are significantly different ($P \leq 0.05$).

Abbreviations: ADG, average daily weight gain; ADFI, average daily feed intake; Ca, calcium; CP, crude protein; GE, gross energy; G:F, gain:feed ratio; InsP6, phytate; P, phosphorus.

Table 5. ADG, ADFI, and G:F of broiler chickens in the experimental period of 5 D (least square means, pooled SEM; n = 5 pens per diet).

Meal	Level	Phy	ADG, g/D	ADFI, g/D	G:F, g/g
RSM	1	−	69	80	0.87
	2	−	66	77	0.86
	1	+	70	81	0.87
	2	+	66	76	0.88
SBM	1	−	75	81	0.92
	2	−	69	77	0.90
	1	+	77	83	0.92
	2	+	67	75	0.90
SFM	1	−	69	81	0.86
	2	−	70	81	0.87
	1	+	72	84	0.86
	2	+	70	80	0.88
		SEM	2.8	2.1	0.015
2-way interactions					
Meal × level					
RSM	1		70[b,c]	81	0.87[c]
	2		66[b]	76	0.87[c]
SBM	1		76[a]	82	0.92[a]
	2		68[b,c]	76	0.90[b]
SFM	1		71[b]	83	0.86[c]
	2		70[b]	80	0.88[c]
		SEM	2.4	1.8	0.013
Main effects					
RSM			68	78[b]	0.87
SBM			72	79[b]	0.91
SFM			71	81[a]	0.87
		SEM	2.2	1.6	0.011
	1		72	82[a]	0.88
	2		68	78[b]	0.88
		SEM	2.2	1.5	0.011
		−	70	79	0.88
		+	71	80	0.88
		SEM	2.2	1.5	0.011
P value					
Meal			0.014	0.028	<0.001
Phy			0.463	0.610	0.378
Level			<0.001	<0.001	0.876
Meal × level			0.031	0.188	0.028
Phy × level			0.237	0.056	0.499
Meal × Phy			0.897	0.872	0.919
Meal × Phy × level			0.905	0.909	0.780

Values in the same column and within the same subheading not sharing the same superscript letter are significantly different ($P \leq 0.05$).

Level 1 = 150 g/kg SBM, 100 g/kg RSM, 150 g/kg SFM; level 2 = 300 g/kg SBM, 200 g/kg RSM, 300 g/kg SFM.

− = without added phytase; + = added phytase.

Abbreviations: Phy, phytase; RSM, rapeseed meal; SBM, soybean meal; SFM, sunflower meal.

by the oilseed meal and inclusion level. ADFI was highest when SFM was used and declined at the higher level of inclusion. The gain:feed ratio ranged between 0.86 (RSM2− and SFM1+) and 0.92 (SBM1+ and SBM1−) and decreased with an increase in the inclusion level of SBM, but not for the other oilseed meals, which lead to a meal × level interaction.

Prececal AA Digestibility

Phytase supplementation did not significantly affect AA digestibility of the basal diet except for His (Table 4). The AA digestibility of the other experimental diets was significantly increased by phytase supplementation by 1 or 2 percentage points (pp), independent of the oilseed meal and the level at which it was included (Table 6). The interaction meal × level was significant, except for Ile, Leu, Met, Val, and Ala. When this interaction was significant, an increase in the inclusion level of SBM either increased or did not affect pc AA digestibility. For RSM and SFM diets, an increase in the inclusion level led to a decrease or did not affect pc AA digestibility. The pc digestibility of Ile, Leu, Met, Val, and Ala was highest in SBM diets and lowest in RSM diets (Ile and Val), or it was not different between RSM and SFM diets (Leu and Ala).

The digestibility of the single oilseed meals was determined from regression analysis (Table 7). Based on the average of all AA, pc digestibility was higher by 7 pp in RSM, 3 pp in SFM, and 1 pp in SBM upon phytase supplementation. In RSM, pc digestibility was significantly increased by phytase supplementation for Arg (6 pp), Ile (14 pp), Lys (8 pp), Pro (11 pp), and Val (11 pp) ($P \leq 0.04$). In SBM and SFM, pc digestibility of Cys was increased by 8 pp ($P = 0.039$) and 10 pp ($P = 0.037$), respectively. Changes in pc digestibility of other essential AA ranged from −2 pp to +9 pp in RSM, −4 to +3 pp in SBM, and −1 to +4 pp in SFM. Numerical changes in digestibility of nonessential AA

Table 6. Prececal amino acid digestibility (%) of the diets with (+) and without phytase supplementation (least square means, pooled SEM; n = 5 pens per diet).

			Essential amino acids										Nonessential amino acids							
Meal	Level	Phy	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Ala	Asx	Cys	Glx	Gly	Pro	Ser	Tyr
RSM	1	−	90	83	85	86	86	94	84	81	81	86	85	80	76	87	84	83	81	83
	2	−	88	82	84	84	84	93	83	79	80	85	84	78	75	86	82	80	79	82
	1	+	91	85	87	88	88	94	86	82	82	88	86	82	77	89	85	84	82	85
	2	+	89	82	86	86	86	93	85	80	81	87	85	80	76	88	83	81	80	84
SBM	1	−	91	85	88	88	90	94	87	84	83	89	86	83	74	89	86	85	83	87
	2	−	91	86	88	88	90	94	87	85	85	89	87	84	76	89	86	86	85	88
	1	+	92	86	89	88	90	94	87	85	84	89	87	84	78	90	86	87	85	88
	2	+	92	86	89	88	91	94	88	85	85	90	87	85	79	90	86	87	86	89
SFM	1	−	90	82	85	85	86	94	84	81	81	87	84	79	74	88	81	84	80	84
	2	−	91	82	86	85	86	94	85	82	82	87	85	80	74	88	79	83	79	84
	1	+	92	83	87	87	88	94	86	83	82	89	86	82	77	89	83	85	82	86
	2	+	92	83	87	87	87	94	87	83	83	88	86	82	76	90	81	85	81	86
		SEM	0.3	0.5	0.7	0.7	0.4	0.4	0.6	0.6	0.7	0.6	0.7	0.5	0.7	0.4	0.4	0.5	0.5	0.6
2-way interactions																				
Meal × level																				
RSM	1		90^{c}	84^{b}	86	87	87^{b}	94	85^{b}	82^{b}	81^{d}	87	86	81^{c}	$77^{a,b}$	88^{c}	84^{b}	83^{c}	82^{c}	84^{c}
	2		89^{d}	82^{c}	85	85	85^{c}	93	84^{c}	80^{c}	81^{d}	86	84	79^{d}	75^{c}	87^{d}	83^{c}	81^{d}	79^{e}	83^{d}
SBM	1		92^{a}	85^{a}	88	88	90^{a}	94	87^{a}	84^{a}	84^{b}	89	87	83^{b}	$76^{b,c}$	$89^{a,b}$	86^{a}	86^{a}	84^{b}	87^{b}
	2		92^{a}	86^{a}	89	88	90^{a}	94	88^{a}	85^{a}	85^{a}	89	87	84^{a}	78^{a}	$90^{a,b}$	86^{a}	87^{a}	85^{a}	88^{a}
SFM	1		91^{b}	83^{c}	86	86	87^{b}	94	85^{b}	82^{b}	81^{d}	88	85	81^{c}	$76^{b,c}$	89^{c}	82^{d}	85^{b}	$81^{d,c}$	85^{c}
	2		91^{b}	82^{c}	87	86	87^{b}	94	86^{b}	82^{b}	83^{c}	88	85	81^{c}	$75^{b,c}$	$89^{b,c}$	80^{e}	84^{b}	80^{d}	85^{c}
		SEM	0.2	0.4	0.6	0.6	0.4	0.3	0.5	0.5	0.6	0.5	0.6	0.4	0.5	0.3	0.3	0.4	0.4	0.5
Main effects																				
RSM			89	83	85^{c}	86^{b}	86	93^{b}	85	81	81	86^{c}	85^{b}	80	76	88	84	82	80	84
SBM			92	86	89^{a}	88^{a}	90	94^{a}	87	84	84	89^{a}	87^{a}	84	77	90	86	86	85	88
SFM			91	82	86^{b}	86^{b}	87	94^{a}	86	82	82	88^{b}	85^{b}	81	76	89	81	84	81	85
		SEM	0.2	0.3	0.5	0.5	0.4	0.3	0.4	0.4	0.5	0.5	0.6	0.4	0.3	0.3	0.3	0.4	0.3	0.5
	1		91	84	87	87	88	94	86	83	82	88	86	82	76	89	84	85	82	85
	2		91	83	87	86	87	94	86	82	83	88	85	81	76	89	83	84	82	85
		SEM	0.2	0.3	0.5	0.5	0.3	0.3	0.4	0.4	0.5	0.5	0.6	0.4	0.3	0.3	0.3	0.3	0.3	0.5
		−	90^{b}	83^{b}	86^{b}	86^{b}	87^{b}	94^{b}	85^{b}	82^{b}	82^{b}	87^{b}	85^{b}	81^{b}	75^{b}	88^{b}	83^{b}	83^{b}	81^{b}	85^{b}
		+	91^{a}	84^{a}	87^{a}	87^{a}	88^{a}	94^{a}	87^{a}	83^{a}	83^{a}	88^{a}	86^{a}	82^{a}	77^{a}	89^{a}	84^{a}	85^{a}	83^{a}	86^{a}
		SEM	0.2	0.3	0.5	0.5	0.3	0.3	0.4	0.4	0.5	0.5	0.6	0.3	0.3	0.3	0.3	0.3	0.3	0.5
P values																				
Meal			<0.001	<0.001	<0.001	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.0306	<0.001	<0.001	<0.001	<0.001	<0.001
Level			0.013	0.036	0.644	0.087	<0.001	0.053	0.673	0.048	0.030	0.083	0.135	0.325	0.790	0.338	<0.001	0.008	0.015	0.844
Phy			<0.001	0.001	<0.001	<0.001	<0.001	0.011	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Meal × level			<0.001	<0.001	0.079	0.083	<0.001	0.156	<0.001	<0.001	0.010	0.101	0.065	<0.001	0.005	0.006	0.001	<0.001	<0.001	0.004
Meal × Phy			0.383	0.256	0.108	0.187	0.201	0.435	0.185	0.160	0.430	0.088	0.234	0.220	0.136	0.343	0.131	0.589	0.602	0.519
Level × Phy			0.782	0.384	0.871	0.644	0.751	0.503	0.673	0.580	0.370	0.882	0.433	0.735	0.653	0.933	0.641	0.979	0.384	0.659
Meal × level × Phy			0.577	0.559	0.194	0.879	0.508	0.882	0.874	0.713	0.694	0.239	0.958	0.865	0.964	0.928	0.612	0.585	0.907	0.954

Level 1 = 150 g/kg SBM, 100 g/kg RSM, 150 g/kg SFM; level 2 = 300 g/kg SBM, 200 g/kg RSM, 300 g/kg SFM. Values in the same column within a subheading not sharing the same superscript letter are significantly different ($P \leq 0.05$).

Abbreviations: Phy, phytase; RSM, rapeseed meal; SBM, soybean meal; SFM, sunflower meal.

Table 7. Effect of a phytase supplementation on prececal CP and amino acid digestibility (%) of RSM, SBM, and SFM in broiler chickens calculated by regression analysis.

		RSM			SBM			SFM		
Phytase		−	+	P value	−	+	P value	−	+	P value
CP		63	67	0.324	83	84	0.951	72	74	0.577
	SE	3.1	2.8		2.1	2.0		2.8	2.6	
Essential amino acids										
Arg		73	79	0.040	88	90	0.337	86	88	0.352
	SE	2.5	1.9		1.3	1.2		1.4	1.3	
His		68	66	0.705	84	81	0.363	66	65	0.729
	SE	3.0	3.4		2.2	2.6		3.4	3.8	
Ile		66	80	0.004	86	89	0.227	80	84	0.311
	SE	0.4	2.4		2.3	1.6		2.9	2.2	
Leu		66	75	0.158	82	84	0.507	73	75	0.798
	SE	4.8	3.5		2.9	2.4		4.2	3.6	
Lys		65	73	0.030	88	90	0.612	73	74	0.955
	SE	2.7	2.2		1.5	1.4		3.3	3.0	
Met		84	86	0.518	89	85	0.298	88	88	0.855
	SE	2.7	2.3		2.6	2.7		1.9	1.7	
Phe		67	76	0.065	84	87	0.373	79	81	0.496
	SE	3.9	2.9		2.0	1.7		2.8	2.5	
Thr		60	67	0.100	81	81	0.949	70	74	0.422
	SE	3.1	2.6		2.4	2.2		3.2	2.7	
Trp		71	70	0.756	84	84	0.825	77	77	0.817
	SE	2.9	2.2		2.2	2.3		2.7	2.5	
Val		67	78	0.013	84	87	0.382	80	83	0.351
	SE	3.5	2.3		2.7	1.9		2.8	2.2	
Nonessential amino acids										
Ala		65	73	0.160	81	81	0.945	73	74	0.905
	SE	4.4	3.5		2.9	2.7		3.7	3.4	
Asx		57	65	0.174	82	84	0.588	69	73	0.288
	SE	4.3	3.6		1.7	1.6		2.9	2.7	
Cys		70	74	0.154	73^{b}	81^{a}	0.039	66^{b}	76^{a}	0.037
	SE	2.5	2.2		2.7	2.6		3.2	3.3	
Glx		74	80	0.061	86	88	0.422	82	86	0.161
	SE	2.5	2.1		1.5	1.4		1.9	1.7	
Gly		66	72	0.143	81	82	0.848	62	66	0.177
	SE	3.1	2.5		2.5	2.3		2.1	2.0	
Pro		56	66	0.034	82	84	0.700	70	76	0.328
	SE	3.6	2.9		2.9	2.7		4.6	3.7	
Ser		58	63	0.363	83	84	0.925	63	66	0.510
	SE	4.2	3.8		2.3	2.3		3.8	3.7	
Tyr		64	72	0.104	86	88	0.460	75	77	0.634
	SE	4.1	3.1		2.3	2.0		3.9	3.3	

Values in the same row and oilseed meal not sharing a common superscript letter are significantly different ($P \leq 0.05$).

Abbreviations: CP, crude protein; RSM, rapeseed meal; SBM, soybean meal; SE, standard error of the estimated slope; SFM, sunflower meal.

ranged between 4 pp and 8 pp in RSM, 0 pp and 2 pp in SBM, and 1 pp and 6 pp in SFM.

Prececal Digestibility of CP, $InsP_6$, P, Ca, and GE

Phytase supplementation significantly increased pc $InsP_6$ disappearance and pc P digestibility of the basal diet, but not pc CP and pc GE digestibility (Table 4). Phytase supplementation also significantly increased pc P digestibility of oilseed meal–containing diets by an average of 7 pp (Table 8). Phytase supplementation increased pc $InsP_6$ disappearance to the same extent in both SBM diets; however, $InsP_6$ disappearance was lower at high inclusion levels in RSM and SFM diets (RSM: 10 pp, $P = 0.005$; SFM: 12 pp, $P = 0.011$) and without (RSM: 16 pp, $P < 0.001$; SFM: 10 pp, $P = 0.006$) phytase supplementation. In the RSM diets, pc CP and P digestibility were decreased by increasing the inclusion level (2 pp each; $P < 0.001$), but no significant changes for SBM and SFM diets were found. An increase in the inclusion level led to a decrease in pc Ca digestibility in SFM diets (4 pp, $P = 0.022$), but not in SBM and RSM diets, leading to a meal × level and phytase × level interaction. Supplementation of phytase led to a decrease in Ca digestibility by 5 pp at the low inclusion level and reached the same level as in the diets with the high inclusion level. For pc GE digestibility, meal × level, phytase × level, and meal × phytase interactions were significant. Increases in oilseed meal inclusion decreased GE digestibility by 4 pp in SBM diets and by 9 and 8 pp in RSM and SFM diets, respectively. Supplementation of phytase led to an increase in pc GE digestibility only at the low inclusion level (2 pp). Supplementation of phytase increased pc GE digestibility only in SFM diets.

Table 8. Prececal $InsP_6$ disappearance and digestibility (%) of CP, P, Ca, and GE of the experimental diets (least square means, pooled SEM, n = 5 pens per diet).

			Disappearance or digestibility, %				
Meal	Level	Phy	CP	$InsP_6$	P	Ca	GE
RSM	1	−	85	19^{e,f}	61	51	76
	2	−	83	2^{g}	58	46	67
	1	+	86	80^{a,b}	68	47	76
	2	+	85	70^{c,d}	66	46	68
SBM	1	−	87	20^{e,f}	65	55	78
	2	−	88	22^{e}	66	57	75
	1	+	88	86^{a}	74	52	79
	2	+	88	81^{a,b}	77	57	74
SFM	1	−	85	2^{g}	58	52	72
	2	−	85	13^{f}	57	45	65
	1	+	87	75^{b,c}	63	45	76
	2	+	86	63^{d}	63	43	66
		SEM	0.5	3.5	1.5	2.3	0.8
2-way interactions							
Meal × level							
RSM	1		86^{b}	49	64^{b}	49^{b}	76^{b}
	2		84^{c}	36	62^{c}	46^{b,c}	67^{e}
SBM	1		87^{a}	53	69^{a}	54^{a}	79^{a}
	2		88^{a}	52	71^{a}	57^{a}	75^{c}
SFM	1		86^{b}	38	61^{b}	48^{b}	74^{d}
	2		86^{b}	38	60^{b}	44^{c}	66^{f}
		SEM	0.4	3.0	1.3	1.9	0.7
Phy × level							
	1	−	86	13	62	53^{a}	75^{b}
	2	−	85	12	60	49^{b}	69^{c}
	1	+	87	80	68	48^{b}	77^{a}
	2	+	86	72	69	49^{b}	69^{c}
		SEM	0.4	2.8	1.2	1.7	0.7
Meal × Phy							
RSM		−	84	11	60	49	72^{b}
		+	86	75	67	47	72^{b}
SBM		−	87	21	66	56	77^{a}
		+	88	84	76	55	77^{a}
SFM		−	85	8	58	49	69^{c}
		+	86	69	63	44	71^{b}
		SEM	0.4	2.7	1.3	1.9	0.7
Main effects							
RSM			85	43	63	48	72
SBM			88	52	70	55	77
SFM			86	38	60	46	70
			0.3	2.6	1.1	1.7	0.7
	1		86	47	65	50	76
	2		86	42	64	49	69
		SEM	0.3	2.6	1.1	1.6	0.7
		−	85	13	61^{b}	51	72
		+	87	76	68^{a}	48	73
		SEM	0.3	2.6	1.1	1.6	0.7
Meal			<0.001	<0.001	<0.001	<0.001	<0.001
Phy			0.058	<0.001	<0.001	0.008	0.009
Level			<0.001	0.003	0.450	0.202	<0.001
Meal × level			0.002	0.002	0.019	0.016	<0.001
Phy × level			0.296	0.016	0.139	0.033	0.024
Meal × Phy			0.749	0.736	0.084	0.583	0.007
Meal × Phy × level			0.919	0.002	0.981	0.813	0.102

Level 1 = 150 g/kg SBM, 100 g/kg RSM, 150 g/kg SFM; level 2 = 300 g/kg SBM, 200 g/kg RSM, 300 g/kg SFM. − = without added phytase; + = added phytase. Values in the same column and within the same subheading not sharing the same superscript letter are significantly different ($P \leq 0.05$).

Abbreviations: Ca, calcium; CP, crude protein; GE, gross energy; P, phosphorus; Phy, phytase; RSM, rapeseed meal; SBM, soybean meal; SFM, sunflower meal.

Concentrations of $InsP_x$ and Myo-Inositol in Ileal Digesta

In ileal digesta, the concentrations of $InsP_6$ and Ins(1,2,4,5,6)P_5 were decreased by phytase supplementation in all diets ($P < 0.001$; Table 9), whereas concentrations of *myo*-inositol and $InsP_4$ isomers were increased by phytase supplementation ($P < 0.05$). The concentration of Ins(1,2,3,4,5)P_5 was significantly increased upon phytase supplementation in RSM and SFM diets, but not in SBM diets. Significant effects of oilseed meal and inclusion level were found for $InsP_6$ and Ins(1,2,4,5,6)P_5, with concentrations decreasing from SFM to RSM and SBM and higher concentrations with high meal inclusion. The proportion of InsP isomers in the sum of all detected InsP isomers is illustrated in

Table 9. Concentration of phytate ($InsP_6$, InsP isomers, and *myo*-inositol [μmol/g DM]) in the digesta of the small intestine of broiler chickens fed the experimental diets (least square means, pooled SEM; n = 5 pens per diet).

					Ins(…)P_4		Ins(…)P_5			
Meal	Level	Phy	*Myo*-inositol	$InsP_3$[1]	(1,2,3,4)	(1,2,5,6)	(1,2,3,4,5)	(1,2,4,5,6)	(1,2,3,4,6)	$InsP_6$
RSM	1	−	3.3	0.3	0.4	0.6	2.5	2.6	1.0	46.9
	2	−	2.9	<LOQ	0.3	0.7	2.8	3.0	1.1	50.6
	1	+	10.6	10.0	7.6	4.7	4.4	1.5	<LOQ	11.7
	2	+	10.6	7.2	8.7	5.2	6.5	2.1	0.2	15.8
SBM	1	−	4.0	<LOQ	0.3	0.6	2.3	2.5	0.8	46.2
	2	−	6.2	<LOQ	0.4	0.6	2.3	2.3	0.8	47.4
	1	+	17.0	9.6	6.6	4.0	3.1	1.0	nd	8.1
	2	+	19.4	5.3	6.3	3.4	3.8	1.2	nd	11.3
SFM	1	−	3.2	0.3	0.7	0.9	4.1	3.2	1.0	53.8
	2	−	4.4	0.3	0.8	1.0	4.6	3.3	1.1	53.5
	1	+	12.8	10.3	9.5	5.9	6.8	2.1	0.2	16.2
	2	+	11.7	6.1	8.9	5.0	8.6	2.6	0.3	22.9
		SEM	1.34	0.70	0.71	0.42	0.78	0.26	0.05	2.40
2-way interactions										
Meal × Phy										
RSM		−	3.1^{c}	-	0.4^{c}	0.6^{c}	2.6^{d}	2.8	1.0	48.7
		+	10.6^{b}	8.6	8.2^{a}	5.0^{a}	5.5^{b}	1.8	-	13.8
SBM		−	5.1^{c}	-	0.4^{c}	0.6^{c}	2.3^{d}	2.4	0.8	46.8
		+	18.2^{a}	7.4	6.4^{b}	3.7^{b}	$3.5^{c,d}$	1.1	-	9.7
SFM		−	3.8^{c}	0.3	0.8^{c}	0.9^{c}	$4.4^{b,c}$	3.2	1.0	53.7
		+	12.2^{b}	8.2	9.2^{a}	5.4^{a}	7.7^{a}	2.3	0.2	19.5
		SEM	1.07	0.49	0.56	0.32	0.62	0.23	0.04	2.02
Main effects										
RSM			6.8	-	4.3	2.8	4.0	2.3^{b}	-	31.2^{b}
SBM			11.7	-	3.4	2.2	2.9	1.8^{c}	-	28.3^{c}
SFM			8.0	4.2	5.0	3.2	6.0	2.8^{a}	0.6	36.6^{a}
		SEM	0.90	0.35	0.47	0.26	0.53	0.21	0.04	2.02
	1		8.5	-	4.2	2.8	3.9^{b}	2.2^{b}	-	30.5^{b}
	2		9.2	-	4.2	2.6	4.8^{a}	2.4^{a}	-	33.6^{c}
		SEM	0.84	-	0.43	0.24	0.50	0.21	-	1.97
		−	4.0	-	0.5	0.7	3.1	2.8^{a}	1.0	49.8^{a}
		+	13.7	8.1	7.9	4.7	5.5	1.8^{b}	-	14.3^{b}
		SEM	0.84	0.35	0.43	0.24	0.62	0.21	0.04	1.97
P value										
Meal			<0.001	0.235	0.003	0.001	<0.001	<0.001	<0.001	<0.001
Phy			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Level			0.283	<0.001	0.874	0.518	0.018	0.009	0.002	<0.001
Meal × level			0.237	0.528	0.656	0.354	0.580	0.097	0.378	0.716
Phy × level			0.676	0.006	0.978	0.390	0.100	0.078	0.428	0.075
Meal × Phy			0.002	0.892	0.025	0.023	0.049	0.288	0.365	0.365
Meal × Phy × level			0.641	-[2]	0.491	0.416	0.818	0.884	-[2]	0.283

Level 1 = 150 g/kg SBM, 100 g/kg RSM, 150 g/kg SFM; level 2 = 300 g/kg SBM, 200 g/kg RSM, 300 g/kg SFM. − = without added phytase; + = added phytase; values in the same column and within the same subheading not sharing the same superscript letter are significantly different ($P \leq 0.05$).

Abbreviations: <LOQ, not quantifiable in most samples; nd, not detectable in most samples; Phy, phytase; RSM, rapeseed meal; SBM, soybean meal; SFM, sunflower meal.

[1]At least one of the following isomers: Ins(1,2,6)P_3, Ins(1,4,5)P_3, Ins(2,4,5)P_3.

[2]No *P* values given due to values under the limit of quantification or detection.

Figure 1. The proportion of $InsP_6$ was reduced from 84% in SFM and 88% in SBM diets to 31% in SBM and 37% in SFM diets. In contrast, the increase was particularly pronounced for $InsP_4$ and $InsP_3$ isomers.

DISCUSSION

One hypothesis of the present study was that phytase effects on pc AA digestibility differed among oilseed meals used in the diets. This hypothesis was confirmed by calculations made for the single oilseed meals: The average increase in AA digestibility was 7 pp in RSM, whereas it was 3 pp in SFM and 1 pp in SBM. Because AA digestibility in the absence of phytase was lower in RSM and SFM than in SBM, phytase supplementation reduced the differences in AA digestibility levels among oilseed meals although they did not cease it (Figure 2). In the oilseed meal–containing diets, this difference in a phytase effect among the meals was no longer visible. While overall the phytase effect on AA digestibility of the diets was significant, an interaction of phytase with the type and level of oilseed meal was not found (Table 5). The differences found in single meals were not detectable in diets likely because the effect of type of oilseed meal was diluted by the other feed ingredients. At the high level of inclusion in the diet, the proportion of CP that originated from RSM was 22% and from SFM 33%. The remainder originated from the basal diet, and

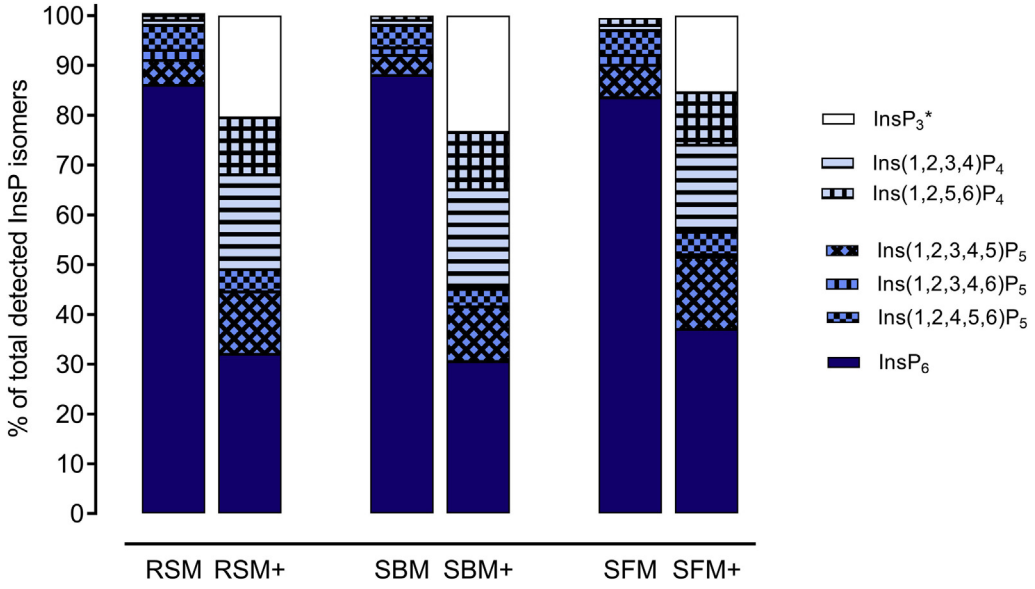

Figure 1. Changes in the proportion of InsP isomers in the sum of all detected InsP isomers in the ileal digesta of 21-day-old boiler chickens by phytase supplementation (+). Birds were provided diets that contained either rapeseed meal (RSM), soybean meal (SBM), or sunflower meal (SFM) (data pooled across 2 inclusion levels). *At least one of the following isomers: Ins(1,2,6)P_3, Ins(1,4,5)P_3, Ins(2,4,5)P_3.

phytase did not significantly affect AA digestibility of the basal diet, except that of His (Table 4). This might be taken as an example of how important it is to distinguish between a raw material and a diet when enzyme effects on AA digestibility are studied.

One approach to explain phytase effects on AA digestibility is the cleavage of phytate-protein complexes. Such an effect should be reflected in phytase effects being specifically pronounced for the AA that are highly abundant in protein fractions associated with $InsP_6$. This was not observed in the present study. For soybeans and soy products, an association of $InsP_6$ with β-conglycinin and glycinin has been described (Prattley and Stanley, 1982; Nishinari et al., 2018), which are the main protein fractions of soybeans (Wagner and Sorgentini, 1996). In these protein fractions, the concentration of Cys is low, whereas those of Arg, Asx, and Glx are relatively high (Riblett et al., 2001). Cruciferin and napin are the main storage proteins in rapeseed (Aider and Barbana, 2011), and they are rich in Arg, Glx, and Leu. In sunflower seeds, the main AA are Asx, Arg, and Glx (Conde et al., 2005). In the present study, the effect of phytase supplementation on digestibility of the highly abundant AA in the respective proteins was not systematically higher than that for other AA, although $InsP_6$ degradation was remarkably increased. This lack of congruence, together with the overall low phytase effects on AA digestibility in SBM, indicated that enzyme accessibility of protein-phytate complexes may not be the limiting factor for AA digestibility in SBM. Although effects of phytase supplementation on pc AA digestibility were stronger in RSM and SFM than those in SBM, the results imply that cleavage of protein-phytate complexes was not the main factor causing the increase in pc AA digestibility in these oilseed meals.

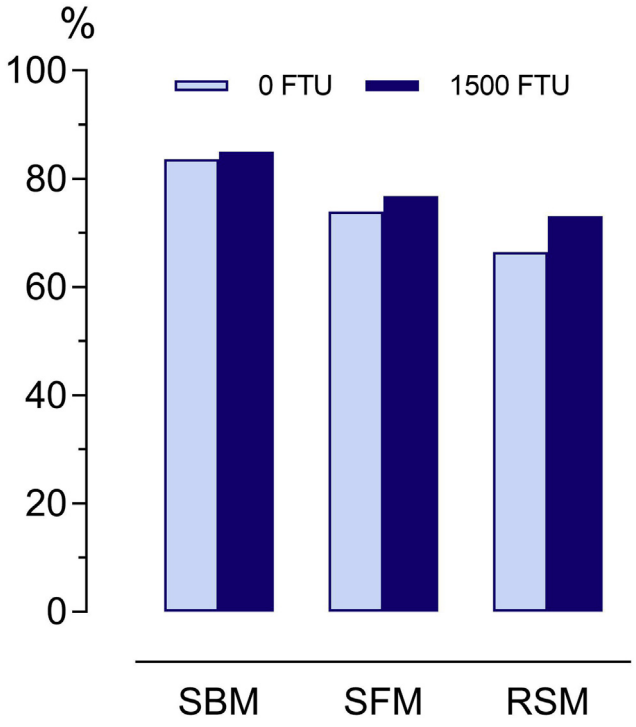

Figure 2. Effect of supplemented phytase on the mean AA digestibility of soybean meal (SBM), sunflower meal (SFM), and rapeseed meal (RSM) determined using the regression approach. The *P* values for individual amino acids of each oilseed meal are provided in Table 7.

Another possible reason for a phytase-induced increase in pc AA digestibility is the reduction of endogenous AA loss. However, the present data obtained for the diets provided no indication that the increase in AA digestibility upon phytase supplementation was caused by lower endogenous AA losses. Endogenous proteins of broilers contain high proportions of Asx, Glx,

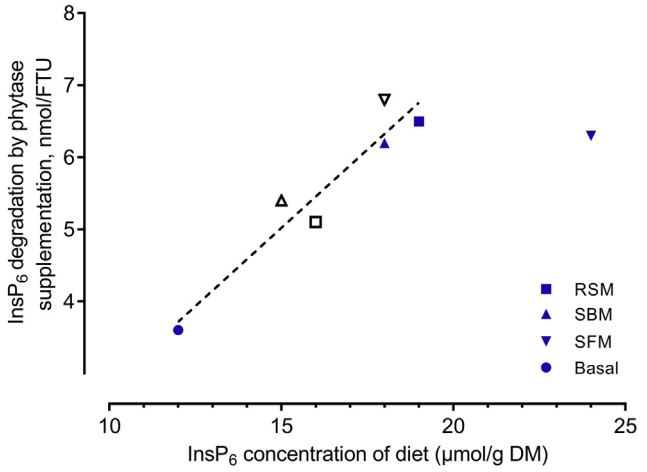

Figure 3. Prececal $InsP_6$ degradation caused by phytase supplementation (1,500 FTU/kg) to the basal diet and diets with inclusion of rapeseed meal (RSM), soybean meal (SBM), and sunflower meal (SFM) at 2 levels each (open symbols = 150 g/kg for SBM and SFM, 100 g/kg for RSM; filled symbols = 300 g/kg for SBM and SFM, 200 g/kg for RSM), on the expense of corn starch (300 g/kg, Basal). Linear regression (without SFM2): $y = -1.51 + 0.44\times$ ($R^2 = 0.91$, RSME = 0.38).

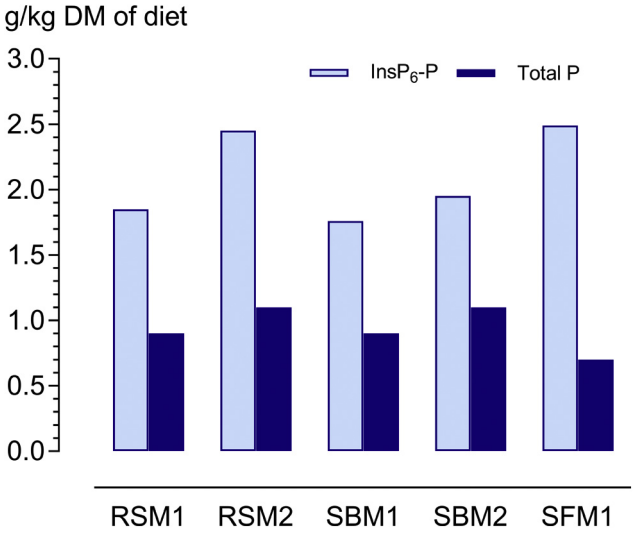

Figure 4. Increments in the degraded amount of $InsP_6$-P and total P due to phytase supplementation (1,500 FTU/kg) in soybean meal-corn–based diets (SBM) or diets with rapeseed meal (RSM) and sunflower meal (SFM). Diet SFM2 is not included because probably the $InsP_6$ content was too high as discussed in the text.

and Thr (Kluth and Rodehutscord, 2009). The overall phytase effect on digestibility of these AA in the diets was +1.4 pp, which is similar to the average effect on all AA of +1.3 pp (Table 6). Using the data from regression analysis, digestibility of Asx, Glx, and Thr also was not higher than the average for all AA, perhaps except for that of SFM, where the increase in digestibility of these 3 AA was 1 pp higher than the average (Table 6). This led us conclude that the effects on endogenous protein secretion likewise were not the reason for AA digestibility effects of phytase. This is consistent with conclusions drawn in previous studies (Borda-Molina et al., 2019; Siegert et al., 2019b). If endogenous secretion is affected in the anterior digestive tract, reabsorption in the more distal sections might be responsible for the effects not measured in the terminal ileum.

Comparisons of AA digestibility values across studies should be conducted with caution. Assay details have large effects on the determined digestibility values. Moreover, processing details vary among cracking plants, and pc AA digestibility of oilseed meals in nonruminants is strongly influenced by processing details, such as heat treatment (Goodarzi Boroojeni et al., 2014; Bryan et al., 2017). Despite limitations in comparability among studies, the results of Senkoylu and Dale (1999) are in accordance with the results of the present study where digestibility of most AA was lower for RSM than that of SBM and intermediate for SFM. Cruciferin had lower solubility than glycinin, and cruciferin formed more insoluble aggregates than glycinin when heated (Ramlan et al., 2002). The protein solubility of oilseed meals can be used as an indicator for reduced protein and AA digestibility in broilers caused by overprocessing (Parsons, 1996). We did not measure protein solubility of the oilseed meals; however, the combination of cruciferin content and heat treatment could be a reason for the AA digestibility to be lower in RSM than in SBM in the present study. Proteins from SFM and sunflower cake have a high solubility, similar to SBM (Schingoethe and Ahrar, 1979; Salgado et al., 2011). The inclusion of high levels of SFM in broiler diets was reported to reduce DM digestibility (Rama Rao et al., 2006). The high crude fiber level of SFM and its association with reduced DM and nutrient digestibility (Rama Rao et al., 2006; de Vries, 2015) and energy digestibility in the present study perhaps were the reasons for lower AA digestibility values than those of SBM, although protein solubility might not have been reduced. Neutral detergent insoluble nitrogen (**NDiN**) might also have contributed to the variability of pc AA digestibility among the oilseed meals. The NDiN concentration of RSM was negatively correlated with Lys digestibility in laying hens (Rezvani et al., 2012), and crude fiber and NDiN concentrations were positively correlated (Mustafa et al., 1996). Hence, the different fiber and NDiN levels could also be causative for the differences in AA digestibility among the oilseed meals. This might explain the lower level of pc AA digestibility for RSM and SFM, despite phytase supplementation (Figure 2).

In the present study, pc $InsP_6$ degradation was low overall when phytase was not supplemented. This can be explained by MCP and limestone contained in the diets (Rodehutscord, 2017a). However, $InsP_6$ degradation was remarkably increased by phytase supplementation although to a different extent among the diets. When calculated across all diets, the amount of $InsP_6$ that disappeared upon phytase supplementation showed a linear response to increasing $InsP_6$ concentration in the diet, except for that of SFM2 (Figure 3). The calculated equation showed that the efficiency of the supplemented

phytase increased by 0.44 nmol/FTU per each incremental μmol of $InsP_6$ contained in the diet up to 19 μmol $InsP_6$/kg DM (12.5 g $InsP_6$/kg DM). The type of oilseed meal apparently was not a relevant determinant in this relationship, except for the high level of SFM inclusion. The reasons for deviating results of SFM2 compared to that of the other treatments are unclear. One possible reason is an upper limit of $InsP_6$ degradation capacity. An upper level of pc $InsP_6$ degradation was indicated in the P digestibility ring test (Rodehutscord et al., 2017b). However, the ring test was conducted using diets without phytase supplementation. Because differences in $InsP_6$ concentration in the present study were achieved by oilseed meal inclusion, changes in the $InsP_6$ concentration cannot be distinguished from other intrinsic factors of the oilseed meals. Other factors, such as high content of fiber fractions in SFM (Rama Rao et al., 2006; de Vries 2015), might have been involved, as discussed before.

The phytase supplementation caused an increase in P digestibility that was lower than the increase in $InsP_6$-P disappearance in all diets (except SFM2) (Figure 4). This is a consequence of incomplete dephosphorylation of $InsP_6$. Although some part of the $InsP_6$ contained in the diet was completely dephosphorylated by the end of the ileum as indicated by remarkably increased *myo*-inositol concentrations, a greater part remained in the form of $InsP_4$, $InsP_3$, and likely, $InsP_2$ and $InsP_1$ isomers.

The present study does not provide evidence that the effects of phytase supplementation on pc CP and AA digestibility can be predicted from effects on pc $InsP_6$ degradation and vice versa. Significant correlations with pc AA digestibility were determined for digesta concentrations of $InsP_6$, Ins(1,2,5,6)P_4, Ins(1,2,3,4)P_4, and $InsP_3$ (data not shown). These significant correlations were mainly caused by the separation of the data points into 2 clusters of observations with and without phytase supplementation. Within the 2 clusters, the data points were randomly scattered and no connection between pc AA digestibility and the concentration of InsP isomers was observed.

Concentrations of $InsP_6$, Ins(1,2,4,5,6)P_5, and Ins(1,2,3,4,6)P_5 isomers in the digesta were significantly reduced by phytase supplementation, whereas concentrations of Ins(1,2,3,4,5)P_5, $InsP_4$ isomers, $InsP_3$ isomers, and *myo*-inositol were significantly or numerically increased in all diets. The shift from higher to lower InsP isomers and *myo*-inositol was similar for all diets (Figure 1). The used phytase is a hybrid designed from genes of *Hafnia* sp., *Yersinia mollaretii*, and *Buttiauxella gaviniae* and was classified as a 6-phytase (Rychen et al., 2017). The present results confirmed this classification. The isomer Ins(1,2,3,4,5)P_5 had the highest concentration among all $InsP_5$ isomers in the digesta after phytase supplementation. Based on the description of the degradation pathway of 6-phytase by Pontoppidan et al. (2012) and the concentrations of InsP isomers in the present study, the used phytase seems to have a degradation pathway involving Ins(1,2,3,4,5)P_5 and Ins(1,2,3,4)P_4 as main degradation products. Ins(1,2,5,6)P_4 was also increased in the ileum, which indicates a highly active alternative degradation pathway of the phytase product. This can probably be explained by the combination of genes of 3 different microorganisms. In studies that used a modified *Escherichia coli* 6-phytase, the Ins(1,2,5,6)P_4 was the $InsP_4$ isomer that was found in the ileum in specifically high concentrations (Zeller et al., 2015b; Sommerfeld et al., 2018b).

Prececal GE digestibility of the basal diets was significantly higher than that in all other diets, which can be explained by the high concentration of corn starch in the basal diets. Consistent with this, pc GE digestibility was lower for the diets with the higher inclusion rates of each oilseed meal, independent of phytase supplementation. In the basal diet and in the diets containing RSM and SBM, phytase did not change pc GE digestibility. This is consistent with the results of Zaefarian et al. (2013) who found no influence of 500 FTU phytase on pc GE digestibility of corn-SBM-based diets in 21-day-old broilers. In the present study, the GE digestibility was higher (within each inclusion rate) for the SBM than RSM diets and lowest for SFM diets. These findings further support the theory that the fiber fractions in the diets, especially the relatively high fiber content in SFM, considerably influenced the nutritive value of the diets and the oilseed meals.

In conclusion, the results of the present study demonstrated that the effects of phytase supplementation on pc AA digestibility and on pc $InsP_6$ degradation can differ among oilseed meals. This suggests that phytase dosage might be optimized based on the composition of the diet. Increased pc AA digestibility seemed to be independent from basal endogenous AA losses and not related to disappearance of $InsP_x$.

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Conflict of Interest Statement: Dieter Feuerstein is an employee of BASF SE. The remaining authors declare that they have no conflicts of interest.

REFERENCES

Adeola, O., and J. S. Sands. 2003. Does supplemental dietary microbial phytase improve amino acid utilization? A perspective that it does not. J. Anim. Sci. 81:E78–E85.

Aider, M., and C. Barbana. 2011. Canola proteins: composition, extraction, functional properties, bioactivity, applications as a food ingredient and allergenicity – a practical and critical review. Trends Food Sci. Technol. 22:21–39.

Allen, R. D., and H. J. Arnott. 1981. Effects of exogenous enzymes on oilseed protein bodies [Yucca rupicola, Helianthus annuus]. Scan. Elec. Microsc. (USA) 3:561–570.

Borda-Molina, D., T. Zuber, W. Siegert, A. Camarinha-Silva, D. Feuerstein, and M. Rodehutscord. 2019. Effects of protease and phytase supplements on small intestinal microbiota and amino acid digestibility in broiler chickens. Poult. Sci. 98:2906–2918.

Bryan, D. D. S. L., J. L. MacIsaac, B. Rathgeber, N. McLean, and D. M. Anderson. 2017. Meal residual oil level and heat treatment

after oil extraction affects the nutritive value of expeller pressed canola meal for broiler chickens. Can. J. Anim. Sci. 96:658–667.
Conde, J. M., M. d. M. Y. Escobar, J. J. Pedroche Jiménez, F. M. Rodríguez, and J. M. Rodríguez Patino. 2005. Effect of enzymatic treatment of extracted sunflower proteins on solubility, amino acid composition, and surface activity. J. Agric. Food Chem. 53:8038–8045.
de Vries, S. 2015. Fiber in poultry nutrition; bonus or burden? Proc. Eur. Symp. Poult. Nutr. 20:40–47.
Erdman, J. W. 1979. Oilseed phytates: nutritional implications. J. Am. Oil Chem. Soc. 56:736–741.
Fatufe, A. A., F. Hirche, and M. Rodehutscord. 2005. Estimates of individual factors of the tryptophan requirement based on protein and tryptophan accretion responses to increasing tryptophan supply in broiler chickens 8-21 days of age. Arch. Anim. Nutr. 59:181–190.
Fontaine, J. 2003. Amino acid analysis of feeds. Pages 15–40 in Amino Acids in Animal Nutrition. J. P. F. D'Mello, ed. CABI Publishing, Willingford, Oxon, UK, Cambridge, MA.
Gesellschaft für Ernährungsphysiologie (GfE) 1999. Empfehlungen zur Energie- und Nährstoffversorgung der Legehennen und Masthühner (Broiler). DLG-Verlag, Frankfurt am Main, Germany.
Gillespie, J., S. W. Rogers, M. Deery, P. Dupree, and J. C. Rogers. 2005. A unique family of proteins associated with internalized membranes in protein storage vacuoles of the Brassicaceae. Plant J. 41:429–441.
Goodarzi Boroojeni, F., A. Mader, F. Knorr, I. Ruhnke, I. Röhe, A. Hafeez, K. Männer, and J. Zentek. 2014. The effects of different thermal treatments and organic acid levels on nutrient digestibility in broilers. Poult. Sci. 93:1159–1171.
Han, Y. W., and A. G. Wilfred. 1988. Hydrolysis of phytate in soybean and cottonseed meals by Aspergillus ficuum phytase. J. Agric. Food Chem. 36:259–262.
Kluth, H., and M. Rodehutscord. 2009. Effect of inclusion of cellulose in the diet on the inevitable endogenous amino acid losses in the ileum of broiler chicken. Poult. Sci. 88:1199–1205.
Kong, C., and O. Adeola. 2014. Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. Asian-Australas. J. Anim. Sci. 27:917–925.
Mason, V. C., M. Rudemo, and S. Bech-Andersen. 1980. Hydrolysate preparation for amino acid determinations in feed constituents. 6. The influence of phenol and formic acid on the recovery of amino acids from oxidized feed proteins. Z. Tierphysiol. Tierernahr. Futtermittelkd. 43:35–48.
Miller, N., H. E. Pretorius, and L. J. Du Toit. 1986. Phytic acid in sunflower seeds, pressed cake and protein concentrate. Food Chem. 21:205–209.
Mustafa, A. F., D. A. Christensen, and J. J. McKinnon. 1996. Chemical characterization and nutrient availability of high and low fiber canola meal. Can. J. Anim. Sci. 76:579–586.
Nishinari, K., Y. Fang, T. Nagano, S. Guo, and R. Wang. 2018. Soy as a food ingredient. Pages 149–186 in Proteins in Food Processing. R. Y. Yada, ed. Woodhead Publishing is an imprint of Elsevier, Duxford, UK.
Onyango, E. M., E. K. Asem, and O. Adeola. 2009. Phytic acid increases mucin and endogenous amino acid losses from the gastrointestinal tract of chickens. Br. J. Nutr. 101:836–842.
Parsons, C. M. 1996. Digestible amino acids for poultry and swine. Anim. Feed Sci. Technol. 59:147–153.
Pontoppidan, K., V. Glitsoe, P. Guggenbuhl, A. P. Quintana, C. S. Nunes, D. Pettersson, and A.-S. Sandberg. 2012. In vitro and in vivo degradation of myo-inositol hexakisphosphate by a phytase from Citrobacter braakii. Arch. Anim. Nutr. 66:431–444.
Prattley, C. A., and D. W. Stanley. 1982. Protein-phytate interactions in soybeans. I. Localization of phytate in protein bodies and globoids. J. Food Biochem. 6:243–254.
Rama Rao, S. V., M. V. L. N. Raju, A. K. Panda, and M. R. Reddy. 2006. Sunflower seed meal as a substitute for soybean meal in commercial broiler chicken diets. Br. Poult. Sci. 47:592–598.
Ramlan, M., N. Maruyama, M. Adachi, N. Hontani, S. Saka, N. Kato, Y. Ohkawa, and S. Utsumi. 2002. Comparison of protein chemical and physicochemical properties of rapeseed cruciferin with those of soybean glycinin. J. Agric. Food Chem. 50:7380–7385.
Ravindran, V., S. Cabahug, G. Ravindran, and W. L. Bryden. 1999. Influence of microbial phytase on apparent ileal amino acid digestibility of feedstuffs for broilers. Poult. Sci. 78:699–706.
Ravindran, V., O. Adeola, M. Rodehutscord, H. Kluth, J. D. van der Klis, E. van Eerden, and A. Helmbrecht. 2017. Determination of ileal digestibility of amino acids in raw materials for broiler chickens – results of collaborative studies and assay recommendations. Anim. Feed Sci. Technol. 225:62–72.
Rezvani, M., H. Kluth, C. Elwert, and M. Rodehutscord. 2008. Effect of ileum segment and protein sources on net disappearance of crude protein and amino acids in laying hens. Br. Poult. Sci. 49:28–36.
Rezvani, M., H. Kluth, M. Bulang, and M. Rodehutscord. 2012. Variation in amino acid digestibility of rapeseed meal studied in caecectomised laying hens and relationship with chemical constituents. Br. Poult. Sci. 53:665–674.
Riblett, A. L., T. J. Herald, K. A. Schmidt, and K. A. Tilley. 2001. Characterization of β-conglycinin and glycinin soy protein fractions from four selected soybean genotypes. J. Agric. Food Chem. 49:4983–4989.
Rodehutscord, M. 2017a. Advances in understanding the role of phytate in phosphorus and calcium nutrition of poultry. Pages 165–180 in Achieving Sustainable Production of Poultry Meat. T. Applegate, ed. Burleigh Dodds Science Publishing, Cambridge, UK.
Rodehutscord, M., O. Adeola, R. Angel, P. Bikker, E. Delezie, W. A. Dozier III, M. Umar Faruk, M. Francesch, C. Kwakernaak, A. Narcy, C. M. Nyachoti, O. A. Olukosi, A. Preynat, B. Renouf, A. Saiz del Barrio, K. Schedle, W. Siegert, S. Steenfeldt, M. M. van Krimpen, S. M. Waititu, and M. Witzig. 2017b. Results of an international phosphorus digestibility ring test with broiler chickens. Poult. Sci. 96:1679–1687.
Rodehutscord, M., M. Kapocius, R. Timmler, and A. Dieckmann. 2004. Linear regression approach to study amino acid digestibility in broiler chickens. Br. Poult. Sci. 45:85–92.
Rutherfurd, S. M., T. K. Chung, and P. J. Moughan. 2002. The effect of microbial phytase on ileal phosphorus and amino acid digestibility in the broiler chicken. Br. Poult. Sci. 43:598–606.
Rychen, G., G. Aquilina, G. Azimonti, V. Bampidis, M. d. L. Bastos, G. Bories, A. Chesson, G. Flachowsky, J. Gropp, B. Kolar, M. Kouba, M. López-Alonso, S. López Puente, A. Mantovani, B. Mayo, F. Ramos, M. Saarela, R. E. Villa, R. J. Wallace, P. Wester, P. Brantom, N. A. Dierick, B. Glandorf, L. Herman, S. Kärenlampi, J. Aguilera, M. Anguita, and P. S. Cocconcelli. 2017. Safety and efficacy of Natuphos® E (6-phytase) as a feed additive for avian and porcine species. EFSA J. 15:5042.
Salgado, P. R., S. E. Molina Ortiz, S. Petruccelli, and A. N. Mauri. 2011. Sunflower protein concentrates and isolates prepared from oil cakes have high water solubility and antioxidant capacity. J. Am. Oil Chem. Soc. 88:351–360.
Schingoethe, D. J., and M. Ahrar. 1979. Protein solubility, amino acid composition, and biological value of regular and heat-treated soybean and sunflower meals. J. Dairy Sci. 62:925–931.
Senkoylu, N., and N. Dale. 1999. Sunflower meal in poultry diets: a review. Worlds Poult. Sci. J. 55:153–174.
Siegert, W., J. Boguhn, H. P. Maurer, J. Weiss, T. Zuber, J. Möhring, and M. Rodehutscord. 2017. Effect of nitrogen fertilisation on the amino acid digestibility of different triticale genotypes in caecectomised laying hens. J. Sci. Food Agric. 97:144–150.
Siegert, W., C. Ganzer, H. Kluth, and M. Rodehutscord. 2019a. Effect of amino acid deficiency on precaecal amino acid digestibility in broiler chickens. J. Anim. Physiol. Anim. Nutr. 103:723–737.
Siegert, W., T. Zuber, V. Sommerfeld, J. Krieg, D. Feuerstein, U. Kurrle, and M. Rodehutscord. 2019b. Prececal amino acid digestibility and phytate degradation in broiler chickens when using different oilseed meals, phytase and protease supplements in the feed. Poult. Sci. 98:5700–5713.
Sommerfeld, V., M. Schollenberger, I. Kühn, and M. Rodehutscord. 2018a. Interactive effects of phosphorus, calcium, and phytase supplements on products of phytate degradation in the digestive tract of broiler chickens. Poult. Sci. 97:1177–1188.
Sommerfeld, V., S. Künzel, M. Schollenberger, I. Kühn, and M. Rodehutscord. 2018b. Influence of phytase or myo-inositol supplements on performance and phytate degradation products in the crop, ileum, and blood of broiler chickens. Poult. Sci. 97:920–929.
Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA) 2012. Handbuch der

Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch), Bd. III. Die chemische Untersuchung von Futtermitteln. VDLUFA-Verlag, Darmstadt.

Wagner, J. R., and D. A. Sorgentini. 1996. Thermal and electrophoretic behavior, hydrophobicity, and some functional properties of acid-treated soy isolates. J. Agric. Food Chem. 44:1881–1889.

Yiu, S. H., I. Altossar, and R. G. Fulcher. 1983. The effects of commercial processing on the structure and microchemical organization of rapeseed. Food Struct. 2:165–173.

Zaefarian, F., L. F. Romero, and V. Ravindran. 2013. Influence of a microbial phytase on the performance and the utilisation of energy, crude protein and fatty acids of young broilers fed on phosphorus-adequate maize- and wheat-based diets. Br. Poult. Sci. 54:653–660.

Zeller, E., M. Schollenberger, I. Kühn, and M. Rodehutscord. 2015a. Hydrolysis of phytate and formation of inositol phosphate isomers without or with supplemented phytases in different segments of the digestive tract of broilers. J. Nutr. Sci. 4:e1.

Zeller, E., M. Schollenberger, M. Witzig, Y. Shastak, I. Kühn, L. E. Hoelzle, and M. Rodehutscord. 2015b. Interactions between supplemented mineral phosphorus and phytase on phytate hydrolysis and inositol phosphates in the small intestine of broilers. Poult. Sci. 94:1018–1029.