Effects of added phytase on growth performance, carcass traits, and tibia ash of broiler chickens fed diets with reduced amino acid, crude protein, and phosphorus concentration

P. Hofmann,[*,1] J. Krieg,[*,1] M. Francesch,[†] D. Feuerstein,[‡] and M. Rodehutscord[*,2]

[*]*Institute of Animal Science, University of Hohenheim, Stuttgart 70599, Germany;* [†]*IRTA, Institute of Agrifood Research and Technology, Animal Nutrition, Constantí 43120, Spain; and* [‡]*BASF SE, Lampertheim 68623, Germany*

Primary Audience: Nutritionists

SUMMARY

This experiment was conducted to investigate the effect of phytase supplementation in diets reduced in amino acids (**AA**), crude protein (**CP**), and phosphorus (**P**) on growth performance, carcass traits, and tibia ash of broiler chickens. A total of 2,240 unsexed Ross 308 broiler chickens were used in 56 floor pens with 40 birds each and fed one of eight dietary treatments in seven replicates until 35 d of age. A positive control (**PC**) diet and diets with dietary AA/CP level reduced by 2, 4, and 6% were used with and without supplementation with 1,500 FTU phytase/kg. Starter, grower, and finisher diets were fed from d 1 to 10, d 10 to 25, and d 25 to 35, respectively. For the total period, no significant interaction effects between AA/CP level and phytase supplementation were detected for any measured traits. The ADG, ADFI, carcass weight, breast weight, and tibia ash weight were lower and FCR was higher compared to the PC diets when the AA/CP level was reduced by more than 2%. Phytase supplementation increased ADG, ADFI, final BW, and tibia ash weight. Tibia ash measurements showed that birds were adequately supplied with digestible phosphorus in all treatments, although dietary phosphorus and calcium were reduced in the phytase-supplemented diets. This enabled the feeding of broiler chickens without mineral phosphate supplements in grower and finisher diets. The results showed that supplementation with 1,500 FTU phytase/kg diminished the growth-decreasing effect of lower dietary AA/CP at all reduction steps.

Key words: amino acid reduction, phosphorus reduction, phytase, growth, bone mineralization, carcass, broiler chicken

2022 J. Appl. Poult. Res. 31:100258
https://doi.org/10.1016/j.japr.2022.100258

DESCRIPTION OF PROBLEM

In animal feeding, precise adjustment of nutrient concentrations of the feed to meet the demand of the animal is desirable in order to reduce feed costs and the excretion of nutrients. Concentrations of amino acids (**AA**) and CP in the feed are specifically relevant because they determine the excretion of nitrogen (**N**) that has negative effects on the environment.

[1] These authors contributed equally to this work.
[2]Corresponding author: inst450@uni-hohenheim.de

Lowering the AA and CP concentration in diets for broiler chickens is an effective strategy to lower the N-excretion of animals (Bregendahl et al., 2002; Hernández et al., 2012; Lemme et al., 2019). However, reduced dietary AA and CP can be accompanied by decreased growth when AA become growth-limiting (Hofmann et al., 2019; Hilliar et al., 2020; Hofmann et al., 2020). Increasing the prececal AA digestibility (**pcAAD**) is one possible tool to overcome this negative effect, as increasing proportions of digestible AA reduce the probability of any AA limiting growth.

The primary objective when adding phytase to the feed is to increase phosphorus (**P**) utilization by the animal by the hydrolysis of phytate (**$InsP_6$**). However, phytase could also increase the pcAAD between 1 and 6 percentage points in broiler chickens (Sommerfeld et al., 2018a; Siegert et al., 2019b; Krieg et al., 2020). Different possible modes of action for a phytase-induced increase in pcAAD have been discussed; these include binary and ternary phytate−AA complexes (Selle et al., 2000), dietary ingredients (Krieg et al., 2020), endogenous AA losses (Selle et al., 2012), microbial activity (Siegert et al., 2021), and phosphate being a kosmotropic agent that can reduce protein solubility (Selle et al., 2012). The predominant mechanism that cause the phytase-induced effect on pcAAD is not known (Selle et al., 2006, 2012; Selle and Ravindran, 2007). Nevertheless, an increase in pcAAD upon phytase supplementation, regardless of the cause, may contribute to enable further reduction of dietary AA and CP in practical feed formulations

Supplementation of phytase has been shown to increase weight gain, carcass weight, and breast weight of broiler chickens receiving diets with reduced dietary Lys (Selle et al., 2007; Walk and Rao, 2019). In addition, it was found by Wang et al. (2021) that an increased FCR of broiler chickens fed diets with 15 g/kg lower CP concentration compared to a diet with adequate dietary CP can be compensated by phytase supplementation of 1,500 FTU/kg. However, it is not known up to which level of dietary AA and CP the growth-limiting effect of AA/CP reduction in broiler chickens can be compensated by phytase supplementation over the entire period of growth.

Therefore, the objective of the present study was to evaluate whether the reduced growth of broiler chickens caused by a reduction in dietary AA and CP of 2, 4, and 6% can be diminished or overcome by supplementation with 1,500 FTU phytase/kg over the entire production period. Carcass traits were measured to investigate the effect of phytase supplementation in AA and CP reduced diets on these traits. Tibia ash was measured to investigate the effect on bone mineralization of phytase supplementation and the associated reduction in mineral P supply of diets supplemented with phytase.

MATERIALS AND METHODS

Birds and Housing

The experiment was carried out at the Experimental Farm of the Institute of Agrifood Research and Technology, (**IRTA**), Spain, and followed the EU principles for care and use of animals in research (EU, 2010). A total of 2,240 unsexed Ross 308 broiler hatchlings were supplied by a commercial hatchery and allocated randomly to 56 floor pens (2 m × 1.97 m) of 40 birds each, so that a similar mean bird weight was achieved in every pen. Each of the 8 dietary treatments was assigned to 7 pens following a completely randomized block design. The feeding regime was comprised of a starter phase from placement until d 10, a grower phase from d 10 to 25, and a finisher phase from d 25 to 35, resulting in 24 different diets. Feed and water were provided ad libitum for the entire experiment. The temperature was set to 32 to 34˚C on the first 2 d and then gradually decreased by 2˚C per week to 19 to 21˚C at the end of the experiment. The following lighting program was applied: 24L:0D for the first 2 d, 18L:6D from d 3 to 7, and 16L:8D from d 8 until the end of the experiment. Pens were bedded with wood shavings that were renewed when necessary.

Experimental Diets

The diets were based mainly on corn and a mixture of soybean meal, rapeseed meal, and sunflower meal as protein sources (Table 1). Four dietary concentrations of Lys, Met+Cys, Thr and

Table 1. Ingredients and calculated composition of the experimental diets (g/kg).

	Starter phase[1]								Grower phase[2]								Finisher phase[3]							
AA/CP level	PC		−2%		−4%		−6%		PC		−2%		−4%		−6%		PC		−2%		−4%		−6%	
Phytase	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+
Corn	465.4		478.7		491.3		503.3		507.2		520.1		531.6		544.3		535.3		546.9		558.1		569.6	
SBM44	192.3		186.4		181.0		175.5		102.5		99.2		96.3		93.0		60.5		58.6		56.8		55.0	
SBM48	42.5		41.2		40.0		38.8		101.5		98.2		95.3		92.0		121.6		117.9		114.3		110.5	
RSM	10.1		9.8		9.5		9.2		102.5		99.2		96.3		93.0		101.6		98.5		95.5		92.4	
SFM	197.3		191.3		185.7		180.1		102.5		99.2		96.3		93.0		101.6		98.5		95.5		92.4	
Soybean oil	50.0		50.0		50.0		50.0		50.0		50.0		50.0		50.0		50.0		50.0		50.0		50.0	
L-Lysine•HCl	4.8		4.7		4.6		4.6		3.2		3.2		3.2		3.2		2.3		2.3		2.3		2.3	
DL-Methionine	3.1		3.1		3.0		2.9		2.6		2.5		2.4		2.4		1.8		1.7		1.7		1.6	
L-Threonine	1.0		1.0		1.0		0.9		0.5		0.6		0.5		0.6		-		-		-		-	
Premix[4]	4.0		4.0		4.0		4.0		4.0		4.0		4.0		4.0		4.0		4.0		4.0		4.0	
Sodium bicarbonate	6.5		6.5		6.5		6.5		6.0		6.0		6.0		6.0		6.0		6.0		6.0		6.0	
Sodium chloride	2.0		2.0		2.0		2.5		2.0		2.0		2.0		2.0		2.0		2.0		2.0		2.0	
Noxyfeed[5]	0.2		0.2		0.2		0.2		0.2		0.2		0.2		0.2		0.2		0.2		0.2		0.2	
Phytase, FTU/kg[6]	-	1,500	-	1,500	-	1,500	-	1,500	-	1,500	-	1,500	-	1,500	-	1,500	-	1,500	-	1,500	-	1,500	-	1,500
Filler[7]	-	10.3	-	10.4	-	10.2	-	10.2	-	8.5	-	8.8	-	9.2	-	9.6	-	7.4	-	7.7	-	8.1	-	8.5
MCP	9.9	1.9	10.2	2.2	10.5	2.5	10.8	2.8	6.7	-	7.0	-	7.3	-	7.7	-	6.1	-	6.5	-	6.7	-	7.1	-
Limestone	10.9	8.6	10.9	8.5	10.7	8.5	10.7	8.5	8.6	6.8	8.6	6.8	8.6	6.7	8.6	6.7	7.0	5.7	6.9	5.7	6.9	5.5	6.9	5.5
Calculated composition, g/kg																								
AME_N, MJ/kg	12.0		12.0		12.1		12.2		12..2		12..3		12.3		12.4		12.4		12.4		12.5		12.5	
CP	210		206		202		198		200		196		192		188		190		186		183		179	
Lys	13.8		13.5		13.3		13.0		12.5		12.3		12.0		11.8		11.3		11.1		10.9		10.6	
Met+Cys	10.2		10.0		9.8		9.6		9.5		9.3		9.1		8.9		8.6		8.4		8.3		8.1	
Thr	9.0		8.8		8.6		8.4		8.3		8.1		8.0		7.8		7.5		7.4		7.2		7.1	
Calcium	9.2	6.8	9.2	6.8	9.2	6.8	9.2	6.8	7.8	5.5	7.8	5.5	7.8	5.5	7.8	5.5	7.0	5.0	7.0	5.0	7.0	5.0	7.0	5.0
Phosphorus	6.6	4.9	6.6	4.9	6.6	4.9	6.6	4.9	5.8	4.1	5.8	4.1	5.8	4.1	5.8	4.1	5.6	3.9	5.6	3.9	5.6	3.9	5.6	3.9
Calcium/Phosphorus ratio	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3

Abbreviations: MCP, monocalcium phosphate; PC, positive control with nutrient concentrations according to FEDNA (2018) recommendations; RSM, rapeseed meal; SBM, soybean meal with 44 and 48% CP; SFM, sunflower meal; −2%, AA/CP level reduced by 2% compared to the positive control in the respective phase; −4%, AA/CP level reduced by 4% compared to the positive control in the respective phase; −6%, AA/CP level reduced by 6% compared to the positive control in the respective phase; −, without added phytase; +, with added phytase.

[1]Starter phase from d 1 to 10.

[2]Grower phase from d 10 to 25.

[3]Finisher phase from d 25 to 35.

[4]P-free vitamin/mineral premix provided per kg of diet: 10,000 IU vitamin A (3a672a); 4,800 IU vitamin D3 (3a671); 45 mg vitamin E (3a700); 3 mg vitamin B1 (3a821); 9 mg vitamin B2 (riboflavin 80%); 45 mg vitamin B6 (3a831); 40 μg vitamin B12 (cyanocobalamin); 3 mg vitamin K3 (3a710); 16.5 mg calcium panthotenate (3a841); 51 mg niacin (3a314); 1.8 mg folic acid (3a316); 0.15 mg biotin (3a880); 54 mg iron (3b103); 1.2 mg iodine (3b201); 12 mg copper (3b504); 90 mg manganese (3b503); 66 mg zinc (3b603); 0.18 mg selenium (E8); 25 mg butylhydroxytoluene (E321); 5 mg calcium formate (E328); 25 mg silicic acid (E551a); 4 g calcium carbonate.

[5]Antioxidant (ITPSA, Barcelona, Spain) that contains butylated hydroxytoluene + 56% propyl gallate and 14% citric acid.

[6]Phytase added on top of the diets.

[7]CLARCEL® DICB obtained by the means of calcination/activation of purified diatomite.

CP (**AA/CP level**) were used in each phase. The AA/CP levels of each phase comprised a positive control (**PC**) diet that met or exceeded the nutrient requirements according to the recommendations of the Fundación Española para el Desarrollo de la Nutrición Animal (**FEDNA**) (2018) for the respective age and diets with AA/CP level reduced by 2, 4, and 6% compared to the PC diet. The AA/CP level was reduced by substituting the oilseed meals with corn, leading to a slight increase in calculated energy levels. The diets were not calculated to be isoenergetic to exclude an effect of varying levels of oil to adjust the energy level among diets. Each AA/CP level was fed with and without 1,500 FTU phytase/kg feed (Natuphos E 5000 G, BASF SE, Germany). This phytase level was chosen because feeding a phytase dosage higher than 1,500 FTU phytase/kg did not further increase the pcAAD in a previous study (Siegert et al., 2019b). In contrast, a lower phytase level than 1,500 FTU phytase/kg was not used because 500 FTU phytase/kg did not increase pcAAD for most of the AA studied by Sommerfeld et al. (2018a). In the phytase-supplemented diets, the P concentration was reduced by 1.7 g/kg, as recommended by the phytase supplier, by reducing the inclusion of monocalcium phosphate, resulting in grower and finisher diets without mineral phosphate supplements. Accordingly, the dietary calcium (**Ca**) concentrations were adjusted by reducing the inclusion of limestone to achieve a similar total Ca/total P ratio among diets within each phase. The diets were mixed at the feed mill of IRTA and pelleted through a 3-mm die by using steam and a temperature of 65 to 70°C. Pelleted diets were provided as crumbles in the starter phase. Chemical analyses confirmed the calculated nutrient concentrations (Table 2) and the calculated reduction of dietary AA and CP (Supplementary Table 1). However, due to a mixing error, the animals receiving the PC diet without phytase supplementation were fed a diet with higher dietary AA and CP concentrations than calculated (Supplementary Table 2) during the first 6 d.

Experimental Procedures

Total bird weight and feed in each pen were recorded at the beginning and end of each phase on d 1, d 10, d 25, and d 35 of age to calculate ADG, ADFI, and FCR. The animals were inspected at least twice daily. Dead birds were removed and weighed.

On d 36, six animals were selected randomly from each pen and weighed, slaughtered, bled, and defeathered in a commercial slaughter house. After chilling the bodies at 5°C for 24 h, the left legs (including femur, tibia, fibula, metatarsus, and feet) were excised and frozen at -18°C until further preparation for tibia ash measurements. In addition, from 3 of the 6 slaughtered animals per pen, the carcass, eviscerated carcass (without feathers, viscera, heart, and abdominal fat), breast meat, and abdominal fat pad were weighed.

Chemical Analyses

Samples of all diets were ground with a centrifugal mill (ZM 200; Retsch GmbH, Haan, Germany) through a 0.5-mm sieve and analyzed for CP (method no. 4.1.1) and fiber fractions (methods no 6.5.1 and 6.5.2) according to the official methods for nutrient analyses in Germany (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, 2007). Following pulverization in a vibrating cup mill (Pulverisette 9; Fritsch GmbH, Idar-Oberstein, Germany), concentrations of AA were analyzed according to Rodehutscord et al. (2004) with slight laboratory modifications, as described by Sommerfeld et al. (2018b) in an L-8900 AA analyzing system (VWR; Hitachi Ltd, Tokyo, Japan). Methionine and Cys were determined as methionine sulfone and cysteic acid, respectively. Dietary Ca, P, and $InsP_6$ concentrations in pulverized diets were determined using the methods outlined by Zeller et al. (2015) with modifications for $InsP_6$ analysis, as described by Sommerfeld et al. (2018a). Dietary Ca and P were analyzed in an inductively coupled plasma optical emission spectrometer (VISTA PRO; Varian Inc., Palo Alto, CA) and dietary $InsP_6$ concentrations were analyzed using an ICS-3000 system (Dionex; Idstein, Germany). Phytase activity was analyzed using the method ISO EN 30024:2009.

The frozen legs were defrosted, cleaned of all adhering tissue including cartilage caps, and then the femur, fibula, metatarsus, and feet

Table 2. Analyzed nutrient composition and phytase activity of the experimental diets (g/kg as fed).

	Starter phase[1]								Grower phase[2]								Finisher phase[3]							
AA/CP level	PC		−2%		−4%		−6%		PC		−2%		−4%		−6%		PC		−2%		−4%		-6%	
Phytase	−[4]	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
DM	898		888		889		889		888		883		885		889		884		886		887		886	
CP	211		206		201		199		204		199		196		190		193		189		185		182	
aNDFom	143		141		138		137		138		133		132		133		134		136		130		129	
ADFom	85		83		84		81		84		82		73		68		65		67		64		62	
Ala	10.2		10.0		9.7		9.6		9.8		9.6		9.5		9.3		9.5		9.3		9.2		9.2	
Arg	14.4		13.9		13.5		13.1		13.1		12.8		12.5		12.2		12.2		11.9		11.7		11.5	
Asx[5]	20.9		20.3		19.5		19.1		18.9		18.5		18.0		17.6		17.8		17.5		17.1		17.0	
Cys	3.3		3.2		3.2		3.1		3.4		3.4		3.3		3.3		3.3		3.3		3.2		3.1	
Glx[4]	38.7		37.4		36.1		35.6		35.2		34.9		33.8		33.5		33.7		33.6		32.9		32.7	
Gly	10.1		9.8		9.4		9.2		9.5		9.2		9.1		8.8		9.0		8.7		8.6		8.5	
His	5.9		5.7		5.6		5.5		5.7		5.6		5.4		5.3		5.4		5.3		5.2		5.2	
Ile	8.8		8.6		8.3		8.1		8.3		8.0		7.9		7.7		7.9		7.7		7.5		7.5	
Leu	16.5		16.1		15.6		15.5		15.9		15.6		15.3		15.1		15.3		15.2		15.0		14.9	
Lys	13.9		13.5		13.0		12.8		12.6		12.3		11.9		11.8		11.1		11.0		10.7		10.6	
Met	6.9		6.9		6.7		6.5		6.2		6.0		5.8		5.9		5.1		5.1		5.0		5.0	
Phe	10.0		9.7		9.4		9.2		9.3		9.1		8.8		8.7		8.8		8.7		8.5		8.5	
Pro	11.0		10.6		10.2		10.2		11.1		10.9		10.7		10.6		10.4		10.4		10.3		10.2	
Ser	10.1		9.8		9.5		9.3		9.4		9.3		9.1		8.8		8.9		8.9		8.7		8.6	
Thr	9.2		9.0		8.6		8.5		8.3		8.3		8.0		8.0		7.5		7.4		7.3		7.2	
Tyr	6.7		6.5		6.3		6.2		6.4		6.4		6.2		6.0		6.1		6.0		5.9		5.9	
Val	10.0		9.8		9.4		9.3		9.7		9.4		9.2		9.1		9.2		9.0		8.8		8.8	
Phytase (FTU/kg)	<60	1,595	<60	1,641	<60	1,530	<60	1,452	<60	1,582	<60	1,572	<60	1,437	<60	1,515	<60	1,670	<60	1,970	<60	1,650	<60	1,720
Calcium	9.6	7.2	9.9	7.1	9.6	7.2	9.5	7.6	8.5	6.4	8.3	6.3	7.9	5.7	8.4	6.5	7.2	5.7	7.7	5.6	7.3	5.5	7.1	5.3
Phosphorus	7.0	4.8	6.7	4.9	6.7	4.7	6.6	4.8	6.0	4.3	6.0	4.4	5.8	4.2	5.8	4.2	5.5	4.2	5.6	4.1	5.7	4.0	5.6	4.0
$InsP_6$ (μmol/g)	15.4		15.4		15.2		15.0		11.5		10.9		10.9		10.5		11.2		11.0		10.7		12.3	
$InsP_6$-P	2.9		2.9		2.8		2.8		2.1		2.0		2.0		2.0		2.1		2.0		2.0		2.3	

Abbreviations: ADFom, acid detergent fibre, exclusive of residual ash; aNDFom, neutral detergent fibre assayed with a heat stable amylase, exclusive of residual ash; $InsP_6$ = phytate; $InsP_6$-P = phytate phosphorus; PC, positive control with nutrient concentrations according to FEDNA (2018) recommendations; −2%, AA/CP level reduced by 2% compared to the positive control in the respective phase; −4%, AA/CP level reduced by 4% compared to the positive control in the respective phase; −6%, AA/CP level reduced by 6% compared to the positive control in the respective phase; -, without added phytase; +, with added phytase.

[1]Starter phase from d 1 to 10.

[2]Grower phase from d 10 to 25.

[3]Finisher phase from d 25 to 35.

[4]Analyzed nutrient composition of the diet that was fed from d 6 to 10. During the first 6 d a diet with slightly higher CP and AA were fed due to a mixing error. The dietary composition of this diet is shown in Supplementary Table 2.

[5]Asn was determined together with Asp and Gln together with Glu because the amide residues in Asn and Gln are lost during acid hydrolysis, resulting in the formation of Asp and Glu, respectively (Fontaine, 2003).

bones were removed. The clean tibia were weighed and the DM content determined after drying overnight at 103˚C. Tibia ash weight was determined after incineration in a muffle furnace at 550˚C for 72 h.

Calculations and Statistical Analysis

The ADG, ADFI, and FCR were calculated from d 1 to 10 (starter phase), d 10 to 25 (grower phase), d 25 to 35 (finisher phase), and d 1 to 35 (overall growth phase). The FCR was corrected for mortality by taking the weight of dead birds into account.

Data were statistically evaluated by two-way ANOVA by using the MIXED procedure of the software package SAS (version 9.4, SAS Institute Inc., Cary, NC) after testing for normal distribution and homogeneity of variance. The pen was considered as the experimental unit for all measured traits. The following statistical model was used:

$$y_{ijk} = \mu + \text{phytase}_i + \text{AA/CPlevel}_j + \text{phytase}_i \times \text{AA/CPlevel}_j + \text{block}_k + e_{ijk} \quad (1)$$

where y_{ijk} is the dependent trait, μ is the overall mean, $phytase_i$ is the fixed main effect of phytase supplementation i (no phytase supplementation or 1,500 FTU/kg feed), $AA/CP\ level_j$ is the fixed main effect of AA/CP level j (according to FEDNA nutrient recommendations or 2%, 4%, or 6% lower AA/CP level), $phytase_i \times AA/CP\ level_j$ is the fixed interaction effect of phytase supplementation i and AA/CP level j, $block_k$ is the random effect of block k, and e_{ijk} is the residual error. Statistical significance was set at $P < 0.050$. Two-paired t tests were used to make comparisons between treatments.

RESULTS AND DISCUSSION

The objective of the present study was to investigate whether the growth-decreasing effect of stepwise reduction in dietary AA/CP can be compensated by phytase supplementation. The initial body weight of the animals averaged 38 g (SD ± 0.5 g) at the beginning of the experiment and was not different among treatments ($P = 0.180$). Mortality was low (3%) and was not related to any treatment (69 of 2240 birds died in all treatments).

Effect of AA/CP Level

Effects on ADG, ADFI, and FCR differed between the phases (Table 3). The reductions in the AA/CP level by 2% in the starter phase, by 6% in the finisher phase and by 4% overall led to reduced ADG compared to PC ($P \leq 0.008$) whereas no effect of AA/CP level on ADG was seen in the grower phase ($P = 0.534$). This may indicate that the recommended AA/CP level by FEDNA (2018) can be reduced by 6% in the grower phase. The overall ADFI was lower compared to PC when the AA/CP level was reduced by more than 2% ($P \leq 0.012$) and was not influenced by AA/CP level in the grower phase ($P = 0.532$). The overall FCR did not differ between PC and the 2% AA/CP reduction ($P = 0.191$) but increased when AA/CP was reduced by up to 6% ($P \leq 0.015$). No effect of AA/CP level on FCR was observed for the grower and finisher phases ($P \geq 0.097$). The final BW decreased when the AA/CP level was reduced by more than 2% ($P \leq 0.002$). The overall growth performance of the PC diets was similar to the performance objectives of the breeding company for unsexed broiler chickens (Aviagen, 2019), indicating that the birds of these treatments were abundantly supplied with all nutrients. Overall growth performance decreased with AA/CP level reduction, indicating that one or more AA limited growth.

The weight of carcass and breast, and the proportion of breast weight in eviscerated carcass weight were lower than PC when the AA/CP level was reduced by more than 2% ($P \leq 0.039$, Table 4). No effect of AA/CP level was found on eviscerated carcass weight but tended to be decreased upon AA/CP level reduction ($P = 0.056$). Carcass traits were measured from 3 randomly chosen animals per pen. Hence, as unsexed birds were used in the present study, it cannot be ruled out that sex of the slaughtered animals may have affected the measured traits. However, results of the carcass traits are consistent with the observed growth performance

Table 3. Effect of amino acid (AA)/CP level and phytase supplementation on growth performance of broiler chickens from d 1 to 10 (starter phase), d 10 to 25 (grower phase), d 25 to 35 (finisher phase), and d 1 to 35 (overall growth phase).

		ADG (g/d)				ADFI (g/d)				FCR (g/g)				Final BW (g)
Phytase	AA/CP level	Starter phase[1]	Grower phase[2]	Finisher phase[3]	Overall growth phase[4]	Starter phase[1]	Grower phase[2]	Finisher phase[3]	Overall growth phase[4]	Starter phase[1]	Grower phase[2]	Finisher phase[3]	Overall growth phase[4]	
-	PC	22.4	69.3	86.0	59.1	27.9^{a}	94.0	159.7^{ab}	92.5	1.25^{a}	1.36	1.86	1.56	2,108
	−2%	21.9	68.1	82.8	57.5	25.9^{c}	92.8	154.6^{bcd}	90.0	1.18^{bcd}	1.36	1.87	1.56	2,052
	−4%	21.6	66.2	80.2	55.9	25.8^{c}	91.2	150.0^{de}	88.0	1.19^{bc}	1.38	1.87	1.57	1,995
	−6%	21.7	67.2	76.7	55.4	26.2^{bc}	92.7	147.6^{e}	88.0	1.21^{b}	1.38	1.93	1.59	1,975
+	PC	23.1	71.0	81.5	58.8	26.8^{b}	96.2	155.5^{abc}	91.8	1.16^{e}	1.35	1.92	1.56	2,096
	−2%	22.0	71.1	87.0	60.1	25.9^{c}	94.9	160.5^{a}	92.6	1.18^{cde}	1.34	1.86	1.54	2,140
	−4%	22.5	69.3	80.7	57.6	26.2^{bc}	95.3	155.4^{abcd}	91.4	1.17^{de}	1.38	1.93	1.59	2,056
	−6%	22.3	70.6	76.6	57.0	26.5^{bc}	96.7	152.4^{cde}	91.2	1.19^{bcd}	1.37	1.99	1.60	2,033
	Pooled SEM	0.30	2.22	2.17	0.79	0.31	1.79	2.03	1.05	0.01	0.03	0.04	0.01	2,7.7
Main effects														
-		21.9^{B}	67.7^{B}	81.4	57.0^{B}	26.4	92.7^{B}	153.0	89.6^{B}	1.21	1.37	1.88	1.57	2,033^{B}
+		22.5^{A}	70.5^{A}	81.4	58.4^{A}	26.4	95.7^{A}	155.9	91.7^{A}	1.17	1.36	1.93	1.57	2,081^{A}
	Pooled SEM	0.16	1.64	1.09	0.55	0.19	1.32	1.19	0.69	0.01	0.02	0.02	0.01	19.5
	PC	22.7^{A}	70.2	83.8^{AB}	59.0^{A}	27.3	95.1	157.6	92.1^{A}	1.20	1.36	1.89	1.56^{C}	2,102^{A}
	−2%	22.0^{B}	69.6	84.9^{A}	58.8^{A}	25.9	93.8	157.6	91.3^{AB}	1.18	1.35	1.86	1.55^{C}	2,096^{A}
	−4%	22.1^{B}	67.7	80.5^{BC}	56.8^{B}	26.0	93.2	152.7	89.7^{B}	1.18	1.38	1.90	1.58^{B}	2,025^{B}
	−6%	22.0^{B}	68.9	76.6^{C}	56.2^{B}	26.4	94.7	150.0	89.6^{B}	1.20	1.37	1.96	1.60^{A}	2,004^{B}
	Pooled SEM	0.22	1.85	1.54	0.64	0.24	1.49	1.52	0.83	0.01	0.02	0.03	0.01	22.8
P-values														
Phytase		0.006	0.025	0.993	0.005	0.676	0.003	0.032	0.002	< 0.001	0.429	0.129	0.927	0.004
AA/CP level		0.029	0.534	0.002	< 0.001	< 0.001	0.532	< 0.001	0.020	0.022	0.487	0.097	< 0.001	< 0.001
Phytase × AA/CP level		0.637	0.961	0.266	0.170	0.041	0.819	0.033	0.097	< 0.001	0.942	0.747	0.064	0.171

Abbreviations: PC, positive control with nutrient concentrations according to FEDNA (2018) recommendations; −2%, AA/CP level reduced by 2% compared to the positive control in the respective phase; −4%, AA/CP level reduced by 4% compared to the positive control in the respective phase; −6%, AA/CP level reduced by 6% compared to the positive control in the respective phase; −, without added phytase; +, with added phytase.

[a-e]In case of significant ($P \leq 0.05$) interactions between main effects: labeled means in a column without a common lowercase letter differ significantly ($P \leq 0.05$).

[A-C]In case of nonsignificant ($P > 0.05$) interactions between main effects: labeled means in a column without a common capital letter differ significantly within the main effects CP/AA level or phytase supplementation ($P \leq 0.05$).

[1]Starter phase from d 1 to 10.

[2]Grower phase from d 10 to 25.

[3]Finisher phase from d 25 to 35.

[4]Overall growth phase from d 1 to 35.

Table 4. Effect of amino acid (AA)/CP level and phytase supplementation on carcass measurements of broiler chickens on d 36.[1]

Phytase	AA/CP level	Carcass weight (g)	Eviscerated carcass weight (g)	Breast weight (g)	Breast weight (% of eviscerated carcass weight)	Abdominal fat pad weight (g)	Abdominal fat pad (% of eviscerated carcass weight)
-	PC	1,737	1,307	430	32.9	18.7	1.4
	−2%	1,653	1,262	391	30.9	17.6	1.4
	−4%	1,589	1,211	378	31.2	18.6	1.6
	−6%	1,565	1,213	352	29.0	20.3	1.7
+	PC	1,745	1,333	412	30.9	17.4	1.3
	−2%	1,692	1,296	395	30.5	16.2	1.3
	−4%	1,680	1,295	385	29.7	19.6	1.5
	−6%	1,604	1,243	361	29.0	21.0	1.7
	Pooled SEM	47.5	33.6	15.5	0.67	1.33	0.09
Main effects							
-		1,636	1,248	388	31.0^{A}	18.8	1.5
+		1,680	1,292	388	30.0^{B}	18.6	1.4
	Pooled SEM	24.7	17.0	8.35	0.34	0.70	0.05
	PC	$1,741^{A}$	1,320	421^{A}	31.9^{A}	18.1^{AB}	1.4^{BC}
	−2%	$1,672^{AB}$	1,279	393^{AB}	30.7^{AB}	16.9^{B}	1.3^{C}
	−4%	$1,634^{B}$	1,253	382^{BC}	30.4^{B}	19.1^{AB}	1.5^{AB}
	−6%	$1,585^{B}$	1,228	356^{C}	29.0^{C}	20.6^{A}	1.7^{A}
	Pooled SEM	34.0	23.9	11.3	0.48	0.95	0.07
P-values							
Phytase		0.191	0.074	0.942	0.044	0.803	0.333
AA/CP level		0.015	0.056	0.001	0.001	0.045	0.002
Phytase × AA/CP level		0.845	0.805	0.788	0.366	0.713	0.749

Abbreviations: PC, positive control with nutrient concentrations according to FEDNA (2018) recommendations; −2%, AA/CP level reduced by 2% compared to the positive control in the respective phase; −4%, AA/CP level reduced by 4% compared to the positive control in the respective phase; −6%, AA/CP level reduced by 6% compared to the positive control in the respective phase; - ,without added phytase; + , with added phytase.

[A-C]Labeled means in a column without a common capital letter differ significantly within the main effects CP/AA level or phytase supplementation ($P \leq 0.05$).

[1]n = 7 per treatment taken from 3 birds per pen.

traits in the overall growth phase, indicating that selection and sex of the slaughtered animals likely had no effect on the results and that carcass traits were affected in consequence of reduced growth. The amount of tibia ash was lower when the AA/CP level was decreased by 4% and 6% compared to PC ($P \leq 0.034$, Table 5). This was probably an effect of the overall smaller size of the birds as a consequence of reduced growth because no effect of AA/CP level was observed for tibia ash concentration ($P = 0.278$). Higher abdominal fat pad weight was found in animals receiving the diets with AA/CP level reduced by 6% compared to diets with AA/CP level reduced by 2% ($P = 0.007$). The proportion of the abdominal fat pad weight in the eviscerated carcass weight increased with lower AA/CP level and was higher at 6% AA/CP reduction compared to PC ($P = 0.002$). This is consistent with reports in the literature, where an increase in abdominal fat mass (Fancher and Jensen, 1989; Hilliar et al., 2020) and whole-body fat content (Aletor et al., 2000; Namroud et al., 2008) upon reduction in dietary AA/CP have been observed. Reducing the level of AA/CP decreased uric acid excretion (Hofmann et al., 2019, 2020), and energy is required for the formation of uric acid (Heldmaier et al., 2013). Hence, more energy is available to the animals when less uric acid is excreted, which could explain the increase in abdominal fat pad weight in the present study. In addition, the reductions in AA/CP levels herein were achieved by replacing oilseed meal with corn. This led to increased calculated AME_N contents because the AME_N content in corn is higher than in the oilseed meal used (Jeroch et al., 2019). An increase in the AME_N content in AA/CP-reduced diets was also found by Hofmann et al. (2019). Therefore, it is possible that higher AME_N contents in AA/CP-reduced diets contributed to the increased abdominal fat pad weight in the present study.

Influence of Phytase on the Effect of AA/CP Level

Significant interaction effects between phytase supplementation and AA/CP level were detected for ADFI and FCR in the starter phase, and for ADFI in the finisher phase ($P \leq 0.041$). In the starter phase, the ADFI of PC with phytase supplementation was lower compared to PC without phytase supplementation ($P = 0.009$), but phytase supplementation had no effect on ADFI in the diets with reduced AA/CP levels ($P \geq 0.077$). The FCR in the starter phase was reduced with AA/CP reduction at all steps of AA/CP reduction compared to PC when no phytase was supplemented ($P \leq 0.002$). With phytase supplementation, the FCR increased when the AA/CP level was reduced by 6% compared to PC ($P = 0.012$). The interaction effects of ADFI and FCR found in the starter phase should be treated with caution because the PC birds without phytase supplementation received a diet with slightly higher dietary AA and CP concentrations during the first 6 d of age. These higher dietary AA and CP concentrations most likely had no effect on the results because they exceeded the FEDNA (2018) recommendations; thus, animals were abundantly supplied with dietary AA and CP. However, it cannot be ruled out that the interaction effects observed for ADFI and FCR in the starter phase were caused by the diet change in PC without phytase supplementation after 6 d of life. In the finisher phase, the reductions in the AA/CP level by 4 and 6% without phytase supplementation decreased ADFI compared to PC ($P \leq 0.001$) and AA/CP level had no clear effect on ADFI in diets with phytase supplementation. No significant interaction effects between phytase supplementation and AA/CP level were observed for the growth performance traits in the other phases ($P \geq 0.064$), all carcass ($P \geq 0.366$) and tibia ash ($P \geq 0.118$) measurements.

Supplementation of phytase increased ADG in the starter, grower, and overall phases ($P \leq 0.025$) and had no effect on ADG in the finisher phase ($P = 0.993$). The ADFI was increased by phytase supplementation in the grower phase and overall ($P \leq 0.003$). No effect on FCR upon phytase supplementation was found in the grower, finisher, and overall phases ($P \geq 0.129$). The final BW was higher when phytase was supplemented ($P = 0.004$). This shows that the growth-limiting effect of decreased AA/CP level was diminished by phytase supplementation at all stages of AA/CP reduction. It is

Table 5. Effect of amino acid (AA)/CP level and phytase supplementation on tibia measurements in broiler chickens on d 36.[1]

Phytase	AA/CP level	Tibia weight (g)		Tibia ash concentration (% dry tibia)	Tibia ash weight (g)	Body weight (g)[2]
		Fresh	Dry			
-	PC	11.8	5.3	39.9	2.1	2,242
	−2%	11.3	5.1	39.5	2.0	2,189
	−4%	11.2	5.1	39.3	2.0	2,125
	−6%	10.9	5.0	39.7	2.0	2,071
+	PC	12.2	5.6	40.3	2.2	2,288
	−2%	11.9	5.4	40.2	2.2	2,223
	−4%	11.5	5.3	40.5	2.2	2,268
	−6%	11.2	5.2	39.2	2.0	2,138
	Pooled SEM	0.24	0.10	0.35	0.04	44.1
Main effects						
-		11.3^{B}	5.1^{B}	39.6	2.0^{B}	2,157^{B}
+		11.7^{A}	5.4^{A}	40.0	2.2^{A}	2,229^{A}
	Pooled SEM	0.12	0.05	0.20	0.02	22.0
	PC	12.0^{A}	5.4^{A}	40.1	2.2^{A}	2,265^{A}
	−2%	11.6^{AB}	5.3^{AB}	39.9	2.1^{AB}	2,206^{A}
	−4%	11.4^{BC}	5.2^{B}	39.9	2.1^{B}	2,197^{A}
	−6%	11.0^{C}	5.1^{B}	39.4	2.0^{B}	2,105^{B}
	Pooled SEM	0.17	0.07	0.26	0.03	31.2
P-values						
Phytase		0.023	<0.001	0.067	<0.001	0.025
AA/CP level		0.002	0.019	0.278	0.006	0.007
Phytase × AA/CP level		0.927	0.934	0.118	0.702	0.611

Abbreviations: PC = positive control with nutrient concentrations according to FEDNA (2018) recommendations; −2% = AA/CP level reduced by 2% compared to the positive control in the respective phase; −4% = AA/CP level reduced by 4% compared to the positive control in the respective phase; −6% = AA/CP level reduced by 6% compared to the positive control in the respective phase; - = without added phytase; + = with added phytase.

$^{A-C}$Labeled means in a column without a common capital letter differ significantly within the main effects CP/AA level or phytase supplementation ($P \leq 0.05$).

[1]n = 7 per treatment taken from 6 birds per pen.

[2]Body weight of animals used for tibia ash measurements.

possible that this was caused by increased pcAAD, making higher amounts of AA available for the animals to increase growth in the AA/CP-reduced diets. This would be in line with previous studies where supplementation with the same phytase product as used herein increased the pcAAD of broiler chickens (Siegert et al., 2019b; Babatunde et al., 2020; Krieg et al., 2020). However, although findings from literature support the assumption that pcAAD was increased by phytase supplementation, digestibility was not measured in the present study.

Phytase supplementation had no effect on carcass weight or eviscerated carcass weight ($P \geq 0.074$) although the numerical increase was similar to the increase in detected final BW. The missing statistical significance was possibly due to the smaller number of animals used for the carcass measurements compared to the final BW measurements (3 vs. 40 animals per treatment).

The growth-increasing effect of phytase supplementation was similar among AA/CP levels. If this was caused by increased pcAAD this indicates that the extent of suggested pcAAD increase due to added phytase was similar among treatments. Statistical evaluation showed that overall ADG, ADFI, final BW, and FCR did not differ between PC and the AA/CP-reduction stage of 2%, suggesting that phytase had no effect on growth in these treatments. However, phytase supplementation numerically increased ADG, ADFI (each +2.6 g/d) and final BW (+88 g), and numerically decreased FCR (−0.02 g/g) in the overall growth period when AA/CP was lowered by 2%. This resulted in similar ADFI, higher ADG and final BW, and lower FCR at the −2% AA/CP level with phytase supplementation when compared to the PC diets. This shows that phytase supplementation might have compensated the reduced growth of animals fed diets with 2% lower AA/CP.

The reduced growth of birds fed diets with AA/CP level lowered by 4 and 6% probably was not compensated by phytase supplementation because the suggested increase in pcAAD was not high enough. Nevertheless, reduced growth at the −4 and −6% AA/CP levels were partly compensated by phytase supplementation, leading to growth performance similar to that found at the −2 and −4% AA/CP levels without phytase supplementation. Several factors like binary and ternary phytate-AA complexes (Selle et al., 2000), dietary ingredients (Krieg et al., 2020), endogenous AA losses (Selle et al., 2012), microbial activity (Siegert et al., 2021), and kosmotropic agents (Selle et al., 2012) were discussed as possible influencing factors for a phytase-induced increase in pcAAD, but it is not clear whether at least one of them was relevant in the present study.

The suggested increase in pcAAD in phytase supplemented diets might have been influenced by dietary Ca. Dietary Ca (together with dietary P) was reduced in the phytase-supplemented diets. Feed intake was lower in diets without phytase supplementation, and feed intake is known to influence pcAAD by affecting endogenous AA losses (Adedokun et al., 2011; Siegert et al., 2019a). Siegert et al. (2021) found that pcAAD, ADG, and ADFI decreased in broiler chickens fed diets containing 7.2 g Ca/kg without phytase compared to diets with 4.9 g Ca/kg and 1,500 FTU phytase/kg. The dietary P concentration in both treatments was 4.6 g/kg. The authors suggested that the lower feed intake caused by higher dietary Ca was responsible for these results. Reduced feed intake upon higher dietary Ca was also found by Amerah et al. (2014) and Wilkinson et al. (2014). Hence, varying dietary Ca in addition to phytase possibly contributed to the present results, highlighting the importance of Ca concentrations in feed formulation.

Breast weight was not influenced by phytase supplementation ($P = 0.942$), but the reduction in breast weight upon AA/CP reduction was numerically lower in phytase-supplemented diets. Phytase supplementation led to a reduction in the proportion of the breast weight in the eviscerated carcass weight ($P = 0.044$) showing that the increase in eviscerated carcass weight upon phytase supplementation was higher relative to the increase in breast weight. The proportion of the abdominal fat pad weight in the eviscerated carcass weight and the abdominal fat pad weight were not affected by phytase supplementation ($P \geq 0.333$). These outcomes indicate that higher breast weight and abdominal fat pad weight were not the reasons for the

increase in carcass weight upon phytase supplementation. Changes in the intestinal weight and feather weight upon phytase supplementation are unlikely to be a main reason for the increase in carcass weight because the observed increase was numerically still apparent in the eviscerated carcass weight (+44 g). Therefore, it is possible that other parts of the animals, such as the leg quarters, caused the carcass weight to increase. The supplementation of phytase increased the amount of tibia ash ($P \leq 0.001$). However, this increase in tibia weight (+0.3 g) was too low to explain the differences in carcass weight and eviscerated carcass weight (both +44 g) upon phytase supplementation. Hence, it is possible that the mass of the leg quarters might have been increased in the present study when phytase was supplemented. This would be in line with Scheideler and Ferket (2000) who reported an increase in the leg quarter weight of 7-wk-old broiler chickens upon phytase supplementation.

The growth-increasing effect of phytase supplementation in diets with lower AA/CP up to 6% may support the implementation of further AA/CP reduction in practical feed formulation even though reduced growth is not fully compensated. Assuming a CP concentration in weight gain of broiler chickens of 18% (Gesellschaft für Ernährungsphysiologie, 1999), calculated N-excretion of broiler chickens in phytase-supplemented diets decreased by 7, 8, and 11% compared to the PC diet when the AA/CP level was reduced by 2, 4, and 6%, respectively, in the present study. It is possible that the N-excretion of broilers in the present study was different from the calculated values if CP concentration in weight gain deviated from the assumed value of 18% CP in weight gain. However, the calculation shows that reduced dietary CP can decrease N-excretion to remarkable extent. Lowering the AA/CP levels by 4 and 6% was accompanied by slightly decreased growth. Currently, it seems economically unreasonable to accept reduced growth in broiler chickens. This could change in the future due to legislative restrictions on the use of animal excreta as fertilizer and rising costs for protein-rich feedstuffs (Siegert and Rodehutscord, 2019). If the primary goal in broiler production changes to minimizing N-excretion rather than maximizing growth performance, phytase could contribute to achieving this aim.

Effect of Phytase

The increased amount of tibia ash upon phytase supplementation indicates a higher P deposition in the bones. This was probably a result of a longer and/or thicker tibia in larger animals as the tibia ash concentration was not affected by phytase supplementation ($P = 0.067$). Bone ash is an indicator of bone mineralization and bioavailability of P in poultry (Shastak et al., 2012; Künzel et al., 2021), and the results indicate that mineralization of bones was not different among treatments, although dietary P was reduced in the phytase-supplemented diets. This suggests that the birds most likely were sufficiently supplied with digestible P in all treatments. An adequate P supply in all treatments indicates that increased growth upon phytase supplementation was not a result of higher P availability and underlines the suggestion that growth reduction was diminished upon phytase supplementation as a consequence of increased pcAAD.

Dietary P was adjusted in phytase-supplemented diets by reduction (starter phase) or omission (grower and finisher phases) in monocalcium phosphate addition. As the birds most likely were sufficiently supplied with digestible P in the present study, it can be concluded that broiler chickens can be fed without the inclusion of mineral phosphate supplements, at least in grower and finisher diets, at the given total P level and phytase addition. Assuming a P concentration in weight gain of broiler chickens of 0.47% (Khaksar et al., 2017), dietary P reduction in phytase-supplemented diets resulted in a 56% lower calculated P excretion of broiler chickens compared to the diets without phytase supplementation and with higher dietary P. Even if the P concentration in weight gain deviated from the assumed value, this calculation underlines the effects of phytase supplementation in diets with reduced dietary P. Hence, feeding reduced-P diets with phytase

supplementation can contribute to more sustainable broiler production by maintaining finite phosphate resources, reducing P excretion, and reducing the cost of feeding.

CONCLUSIONS AND APPLICATIONS

1. The results of the present study show that supplementation with 1,500 FTU phytase/kg feed diminished the growth-limiting effect of diets with reduced dietary AA/CP levels, possibly by increasing the pcAAD.
2. The growth performance traits indicated that phytase supplementation compensated the reduced growth from d 1 to 35 when the AA/CP level of the diet was reduced by 2%.
3. Tibia ash was not influenced, although dietary P was reduced in phytase-supplemented diets, indicating that the birds were sufficiently supplied with digestible P and that mineral phosphate supplements can be omitted in grower and finisher diets when phytase is used.
4. The increase in carcass weight upon phytase supplementation was not accompanied by higher breast or abdominal fat pad weight.
5. Phytase addition combined with reduced AA/CP and P in the feed enabled reductions in calculated N and P excretion of broiler chickens in comparison to a control diet at standard nutrient level without compromising growth performance or tibia ash.
6. In practical feed formulation, phytase supplementation enables the reduction of dietary AA/CP levels together with the omission of mineral phosphate supplements without decreasing growth.

ACKNOWLEDGMENTS

This study was financially supported by BASF SE, Ludwigshafen, Germany.

DISCLOSURES

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

SUPPLEMENTAL MATERIAL

Supplemental materials associated with this article can be found in the online version at https://doi.org/10.1016/j.japr.2022.100258.

REFERENCES

Adedokun, S. A., O. Adeola, C. M. Parsons, M. S. Lilburn, and T. J. Applegate. 2011. Factors affecting endogenous amino acid flow in chickens and the need for consistency in methodology. Poult. Sci. 90:1737–1748.

Aletor, V. A., I. I. Hamid, E. Nie, and E. Pfeffer. 2000. Low-protein amino acid-supplemented diets in broiler chickens: effects on performance, carcass characteristics, whole-body composition and efficiencies of nutrient utilisation. J. Sci. Food Agric. 80:547–554.

Amerah, A. M., P. W. Plumstead, L. P. Barnard, and A. Kumar. 2014. Effect of calcium level and phytase addition on ileal phytate degradation and amino acid digestibility of broilers fed corn-based diets. Poult. Sci. 93:906–915.

Aviagen. 2019. Ross 308/Ross 308 FF Broiler Performance Objectives. Aviagen, Midlothian, UK.

Babatunde, O. O., J. A. Jendza, P. Ader, P. Xue, S. A. Adedokun, and O. Adeola. 2020. Response of broiler chickens in the starter and finisher phases to 3 sources of microbial phytase. Poult. Sci. 99:3997–4008.

Bregendahl, K., J. L. Sell, and D. R. Zimmerman. 2002. Effect of low-protein diets on growth performance and body composition of broiler chicks. Poult. Sci. 81:1156–1167.

EU. 2010. Directive 2010/63/EU of 22 September 2010 on the protection of animals used for scientific purposes. Off. J. Eur. Union, Strasbourg, France L 276:33–79.

Fancher, B. I., and L. S. Jensen. 1989. Influence on performance of three to six-week-old broilers of varying dietary protein contents with supplementation of essential amino acid requirements. Poult. Sci. 68:113–123.

Fontaine, J. 2003. Amino acid analysis of feeds. Pages 15−40 in Amino Acids in Animal Nutrition. J. P. F. D'Mello, ed. (2nd ed.). CABI Publ., Wallingford, U K and Cambridge, MA.

Fundación Española para el Desarrollo de la Nutrición Animal. 2018. Necesidades Nutriticionales Para Avicultura: Normas FEDNA. 2nd ed. Fundación Española para el Desarrollo de la Nutrición Animal, Madrid, Spain.

Gesellschaft für Ernährungsphysiologie. 1999. Energie- und Nährstoffbedarf Landwirtschaftlicher Nutztiere. 7. Empfehlungen zur Energie- und Nährstoffversorgung der Legehennen und Masthühner (Broiler). DLG-Verlag, Frankfurt am Main, Germany.

Heldmaier, G., G. Neuweiler, and W. Rössler. 2013. Pages 49−86 in Vergleichende Tierphysiologie. Kapitel 2: Energiehaushalt von Tieren. 2nd ed. Springer Spektrum, Berlin, Heidelberg, Germany.

Hernández, F., M. López, S. Martínez, M. D. Megías, P. Catalá, and J. Madrid. 2012. Effect of low-protein diets and single sex on production performance, plasma metabolites, digestibility, and nitrogen excretion in 1- to 48-day-old broilers. Poult. Sci. 91:683–692.

Hilliar, M., G. Hargreave, C. K. Girish, R. Barekatain, S.-B. Wu, and R. A. Swick. 2020. Using crystalline amino

acids to supplement broiler chicken requirements in reduced protein diets. Poult. Sci. 99:1551–1563.

Hofmann, P., W. Siegert, Á. Kenéz, V. D. Naranjo, and M. Rodehutscord. 2019. Very low crude protein and varying glycine concentrations in the diet affect growth performance, characteristics of nitrogen excretion, and the blood metabolome of broiler chickens. J. Nutr. 149:1122–1132.

Hofmann, P., W. Siegert, V. D. Naranjo, and M. Rodehutscord. 2020. Effects of supplemented nonessential amino acids and nonprotein nitrogen on growth and nitrogen excretion characteristics of broiler chickens fed diets with very low crude protein concentrations. Poult. Sci. 99:6848–6858.

Jeroch, H., A. Simon, and J. Zentek. 2019. Geflügelernährung. 2nd ed. Ulmer, Stuttgart, Germany.

Khaksar, V., B. Meda, and A. Narcy. 2017. Updating the available P requirements of broilers. Pages 124−129 in Proceedings of the 21st European Symposium on Poultry Nutrition M. Francesch, D. Torrallardona and J. Brufau, eds..

Krieg, J., W. Siegert, D. Berghaus, J. Bock, D. Feuerstein, and M. Rodehutscord. 2020. 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. Poult. Sci. 99:3251–3265.

Künzel, S., D. Borda-Molina, T. Zuber, J. Hartung, W. Siegert, D. Feuerstein, A. Camarinha-Silva, and M. Rodehutscord. 2021. Relative phytase efficacy values as affected by response traits, including ileal microbiota composition. Poult. Sci. 100:101133.

Lemme, A., P. Hiller, M. Klahsen, V. Taube, J. Stegemann, and I. Simon. 2019. Reduction of dietary protein in broiler diets not only reduces n-emissions but is also accompanied by several further benefits. J. Appl. Poult. Res. 28:867–880.

Namroud, N. F., M. Shivazad, and M. Zaghari. 2008. Effects of fortifying low crude protein diet with crystalline amino acids on performance, blood ammonia level, and excreta characteristics of broiler chicks. Poult. Sci. 87:2250–2258.

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.

Scheideler, S. E., and P. R. Ferket. 2000. Phytase in broiler rations-effects on carcass yields and incidence of tibial dyschondroplasia. J. Appl. Poult. Res. 9:468–475.

Selle, P. H., A. J. Cowieson, N. P. Cowieson, and V. Ravindran. 2012. Protein−phytate interactions in pig and poultry nutrition: a reappraisal. Nutr. Res. Rev. 25:1–17.

Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 135:1–41.

Selle, P. H., V. Ravindran, W. L. Bryden, and T. Scott. 2006. Influence of dietary phytate and exogenous phytase on amino acid digestibility in poultry: a review. J. Poult. Sci. 43:89–103.

Selle, P. H., V. Ravindran, R. A. Caldwell, and W. L. Bryden. 2000. Phytate and phytase: consequences for protein utilisation. Nutr. Res. Rev. 13:255–278.

Selle, P. H., V. Ravindran, G. Ravindran, and W. L. Bryden. 2007. Effects of dietary lysine and microbial phytase on growth performance and nutrient utilisation of broiler chickens. Asian-Australas. J. Anim. Sci. 20:1100–1107.

Shastak, Y., M. Witzig, K. Hartung, W. Bessei, and M. Rodehutscord. 2012. Comparison and evaluation of bone measurements for the assessment of mineral phosphorus sources in broilers. Poult. Sci. 91:2210–2220.

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., J. Krieg, V. Sommerfeld, D. Borda-Molina, D. Feuerstein, A. Camarinha-Silva, and M. Rodehutscord. 2021. Phytase supplementation effects on amino acid digestibility in broiler chickens are influenced by dietary calcium concentrations but not by acid-binding capacity. Curr. Dev. Nutr. 5:nzab103.

Siegert, W., and M. Rodehutscord. 2019. The relevance of glycine and serine in poultry nutrition: a review. Br. Poult. Sci. 60:579–588.

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., S. Künzel, M. Schollenberger, I. Kühn, and M. Rodehutscord. 2018a. 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.

Sommerfeld, V., M. Schollenberger, I. Kühn, and M. Rodehutscord. 2018b. 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.

Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten. 2007. Handbuch der landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch), Vol. III. Die chemische Untersuchung von Futtermitteln. 1st ed. VDLUFA-Verlag, Darmstadt, Germany.

Walk, C. L., and S. V. R. Rao. 2019. High doses of phytase on growth performance and apparent ileal amino acid digestibility of broilers fed diets with graded concentrations of digestible lysine. J. Anim. Sci. 97:698–713.

Wang, J., R. Patterson, and W. K. Kim. 2021. Effects of phytase and multicarbohydrase on growth performance, bone mineralization, and nutrient digestibility in broilers fed a nutritionally reduced diet. J. Appl. Poult. Res. 30:100146.

Wilkinson, S. J., E. J. Bradbury, P. C. Thomson, M. R. Bedford, and A. J. Cowieson. 2014. Nutritional geometry of calcium and phosphorus nutrition in broiler chicks. The effect of different dietary calcium and phosphorus concentrations and ratios on nutrient digestibility. Animal 8:1080–1088.

Zeller, E., M. Schollenberger, I. Kühn, and M. Rodehutscord. 2015. 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.