Microbial Phytase and Nutrient Utilization in Low Phosphorus Chicken Diets

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Experiments were conducted with male growing chicken (Cobb 500) to examine effects of added microbial phytase on growth performance and nutrient utilization in corn-soybean meal (CSM) and wheat-soybean meal (WSM) diets. Low P basal diets (CSM1: 0.15 / 0.11% NPP in DM of starter/grower diets; WSM1: 0.16/0.12% NPP in DM of starter/grower diets) were supplemented with 250 or 500 U/kg of an experimental microbial phytase (CSM2, CSM3; WSM2, WSM3) or 1.5 g/kg inorganic phosphorus (CSM4, WSM4). Growth studies were conducted (7–42 d) to establish nutrient deposition (6 pens per diet, 10 birds per pen). Separate balance studies ($n=8$) yielded individual N and P balance data for the age period 21–25 d. Additionally, studies with CSM diets also provided tibia composition data.

Phytase supplementation in CSM diets yielded significant effects on growth and deposition data. Additionally, the mortality rate declined significantly and tibia composition (ash, P, Ca) was improved. WSM diets provided more pronounced effects on deposition data, but growth responded only numerical. Balance studies delivered significant effects on P utilization due to phytase addition in both CSM and WSM diets, respectively. However, lower P utilization was observed in CSM diets. Significant effects of phytase addition on N utilization were established only in CSM diets. Consequently, generalized conclusions due to improved N utilization following phytase addition are not supported by the current study.

Abbreviation key: CSM=corn-soybean meal; iP=inorganic P; NPP=non-phytate P; WSM=wheat-soybean meal

Key words: growing chicken, growth performance, nutrient utilization, phytase, P utilization

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Introduction

Lack of endogenous phytase activity limits phytate degradation in the gut of growing chicken (Nelson, 1967; Davies *et al.*, 1970). Improved P utilization is achieved following supplementation of microbial phytase, as reported extensively (Simons *et al.*, 1990; Kornegay, 2001; Shirley and Edwards, 2002; Augspurger and Baker, 2004; Snow *et al.*, 2004; Payne *et al.*, 2005; Ebrahimnezhad *et al.*, 2008). Contradictory reports are published in laying hens (Peter *et al.*, 1992; van der Klis *et al.*, 1997; Carlos and Edwards, 1998; Boling *et al.*, 1997, 2000a, b; Gordon and Roland, 1997, 1998; Rama Rao *et al.*, 1999; Jalal and Scheideler, 2001; Shan *et al.*, 2002; Ceylan *et al.*, 2003; Keshavarz, 2003a, b; Lim *et al.*, 2003; Liebert *et al.*, 2005). Different sources of microbial phytase may also affect phytate degradation (Augspurger *et al.*, 2003; Zyla *et al.*, 2004; Liebert and Portz, 2005, 2007). Furthermore, effects of added phytase on nutrient utilization in growing chicken are possible. Phytate-

protein-bindings in the gut (O'Dell and De Boland, 1976; Gifford and Clydesdale, 1990) could play a significant role and effects of phytates on endogenous amino acid losses are reported (Cowieson and Ravindran, 2007). Consequently, improved phytate hydrolysis could yield enhanced protein digestibility. Inhibiting effects of phytates on several enzyme activities were mostly observed *in vitro* (Deshpande and Cheryan, 1984; Knuckles *et al.*, 1989; Caldwell, 1992). Marginal effects on improvement of digestibility were expected *in vivo* (Reddy *et al.*, 1988; Vaintraub and Bulmaga, 1991; Zhang *et al.*, 1999). According to several reports (Sebastian *et al.*, 1997; Zhang *et al.*, 1999; Peter *et al.*, 2000; Augspurger and Baker, 2004), Bedford (2000) concluded inconsistent secondary effects of microbial phytase in growing chickens. In contrast, other studies confirmed significant responses (Farrell *et al.*, 1993; Yi *et al.*, 1994, 1996a; Kornegay *et al.*, 1996; Sebastian *et al.*, 1996; Namkung and Leeson, 1999; Zhang *et al.*, 1999; Lan *et al.*, 2002; Juanpere *et al.*, 2004; Rutherford *et al.*, 2004; Onyango *et al.*, 2005). Several factors could be of importance, like type of applied microbial phytase, solubility of phytates, and native phytase activity. Dietary calcium supply is an additional factor (Plumstead *et al.*, 2008).

The present study was conducted to examine effects of

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an experimental microbial phytase in low P corn-soybean meal (CSM) and wheat-soybean meal (WSM) on growth, feed efficiency and nutrient utilization in growing chicken.

Materials and Methods

Animals and Housing

Day old male growing chickens (Cobb 500) from a commercial hatchery reared under controlled climate conditions in a cage system. Room temperature during rearing started with 32°C in the first week after hatching and declined by 2°C per week, lighting was 24 hours per day. Chicken were fed a standardized starter diet based on wheat and soybean meal (DM based: 266 g kg⁻¹ crude protein, 13 MJ kg⁻¹ AMEn) on free choice level up to six days. Two experiments were conducted, designed as growth trial (7–42 d) and balance study (21–25 d) using separate birds:

Experiment 1 utilized low P corn-soybean (CSM) diets (Table 1) with low native phytase activity, two steps of microbial phytase and a control diet with inorganic P addition (Table 2).

Experiment 2 examined low P wheat-soybean (WSM) diets (Table 1) with enhanced dietary native phytase activity from wheat, two steps of microbial phytase and a

control diet with inorganic P addition (Table 2).

As an example for low and high native phytase activity in the diet, the experiments utilized CSM and WSM diets, respectively. Each growth experiment was conducted as randomized design using totally 240 male chickens in 24 floor pens (average stocking density 7.6 chickens m⁻²), short cut straw bedding, controlled climate conditions and a continuous lighting regime, respectively. Diets were randomly assigned to six pens (10 birds per pen). Growth experiments were divided into starter (7–20 d) and grower period (21–42 d) with free choice level of feed and water supply.

Balance studies with individual metabolism cages (n = 8) utilized an adaptation period (16–20 d) and a period of quantitative excreta collection (21–25 d). Individual feeding adapted to body weight minimized feed losses and contamination of excreta by feed particles.

The experiments were carried out at the facilities of the Institute for Animal Physiology and Animal Nutrition in accordance with animal welfare legislations and were approved by the Ethics Committee of the Agricultural Faculty of Goettingen University.

Experimental Diets

Low P negative control diets (CSM1, WSM1) and

Table 1. Composition of the basal CSM and WSM diets

Diets	Corn-soybean meal (CSM)		Wheat-soybean meal (WSM)	
Ingredients (g/kg)	Starter	Grower	Starter	Grower
Corn	449	538	—	—
Wheat	—	—	462	549
Soybean meal	445	370	423	345
Soybean oil	33	20	43.5	35
Wheat starch	36	36	36	36
Calcium carbonate	22	22	21	22
Salt	5	4	4.5	3
Premix ¹⁾	10	10	10	10
Analyzed (DM%)				
Crude protein	26.5	24.1	26.9	23.8
Crude fat	7.4	5.9	6.8	5.5
Crude fiber	4.9	4.7	4.0	3.9
Crude ash	6.8	6.8	6.9	6.5
Starch	37.2	40.3	34.4	41.3
Sugar	5.9	6.6	6.7	6.3
Lysine	1.52	1.32	1.51	1.31
Met + Cys	1.05	0.99	1.05	0.99
Threonine	1.04	0.94	1.01	0.90
Total phosphorus	0.46	0.43	0.46	0.42
Non-phytate phosphorus	0.15	0.11	0.16	0.12
Calcium	1.28	1.14	1.23	1.24
AME _n (MJ/kg DM ²⁾	13.6	13.3	12.4	12.8

¹⁾ Provided (per kilogram of diet): vitamin A, 5000 IU; vitamin D₃, 1000 IU; vitamin E, 30 mg; vitamin B₁, 2.6 mg; vitamin B₂, 4.8 mg; vitamin B₆, 3.2 mg; vitamin B₁₂, 20 mcg; vitamin K₃, 3 mg; nicotinic acid, 50 mg; calcium pantothenate, 10 mg; folic acid, 0.9 mg; biotin, 100 mg; choline chloride, 1000 mg; Se, 25 mg; Zn, 80 mg; Mn, 120 mg; Cu, 30 mg; Fe, 50 mg; I, 1.2 mg; Co, 0.55 mg; Monensin-Na 125 mg; and BHT, 100 mg.

²⁾ N-corrected apparent metabolizable energy, calculated (WPSA, 1984).

Table 2. Experimental design

Experiment Diets Supplementation	1 Corn-soybean meal (CSM)				2 Wheat-soybean meal (WSM)			
	CSM1	CSM2	CSM3	CSM4	WSM1	WSM2	WSM3	WSM4
Phytase (U/kg diet) ¹	0	250	500	0	0	250	500	0
Inorganic P (g/kg diet) ²	0	0	0	1.5	0	0	0	1.5
Phytase activity (U/kg) ³	56	230	421	61	448	762	956	458

¹⁾ Experimental microbial phytase SP 1002.

²⁾ Monosodium phosphate (22% P).

³⁾ Analyzed activity due to Engelen *et al.* (1994).

positive control diets (CSM4, WSM4) supplemented with inorganic P (Monosodium phosphate) were prepared (Table 2). Nutrient composition (Table 1) ensured or exceeded recommendations of NRC (1994), except P supply (0.44% total P and 0.13% NPP). Two steps of microbial phytase (250 and 500 U/kg) were investigated (CSM2, CSM3 and WSM2, WSM3) without supplementation of inorganic P. Diets were pelleted under controlled mild conditions, temperatures near to the outlet of the 2 mm die did not exceed 50°C. The examined phytase SP 1002 (Former Roche Vitamins Ltd, Basel, Switzerland) was an experimental *Consensus* phytase produced by a genetically modified strain of the yeast *Hansenula polymorpha*. The production strain is carrying a synthetic gene coding for 3-phytase (IUB no. 3.1.3.8.) with elevated pH optimum (>5) and increased stability against thermal treatment and proteolytic activity.

Measurements

Growth Experiments

Growth and feed intake were weekly recorded for each pen. At the end of the experiment (42 d), for each diet totally six imposing birds for body weight (one bird per pen) were sampled for individual analysis of body nutrient composition. A pooled sample of five birds with average body weight was utilized for body analyses at the beginning of the growth experiments. Additionally, in experiment 1 the left tibia bone from six birds per diet was prepared. Following removal of adhering tissue, drying (105°C, 48 h) and overnight ashing at 550°C, crude ash, Ca and P were analyzed.

Balance Experiments

Following the adaptation period (16–20 d), a collection period (21–25 d) was conducted with quantitative excreta collection three times a day. Total excreta samples kept frozen at –18°C until further analysis. N utilization parameters were calculated from difference between N intake and total N excretion (=N balance) related to the total N intake. P utilization was calculated accordingly. N digestibility was calculated according to the α -amino-N procedure (Pahle *et al.*, 1985) assuming that N excretion via feces is mostly α -amino-N. The energy content of the experimental diets (AME_n) was determined by calorimetric measurements in feed and excreta, including N

corrections for individual N deposition (Titus *et al.*, 1959).

Chemical Analysis

Gross energy (AC 350 LECO Instruments) and N (Dumas method, FP 2000 LECO Instruments) were determined in the experimental diets and freeze-dried excreta samples. Total P in feed, excreta and tibia ash analyses run according to Naumann and Bassler (1997). In feed and tibia samples, Ca was determined according to Naumann and Bassler (1997) using an atomic absorption spectrophotometer (SpectrAA 55, Varian). Analysis of phytate P in feed samples was according to Association of Official Analytical Chemists (1990). The α -amino-N in feed and excreta samples was analyzed in line with the procedure of Pahle *et al.* (1983, 1985). In-feed activity of phytase was determined due to Engelen *et al.* (1994).

Statistical Analysis

Results are presented as means $\pm$ standard deviation ($\pm$ SD). Experimental unit was the pen (growth studies) and the individual bird (balance experiments), respectively. Data analysis in growth studies was based on one way ANOVA using Fisher-LSD or Games-Howell post-hoc test according to equality or non-equality of variances (verified by Levene-test). Data analysis of the balance experiments utilized univariate ANOVA. Univariate data analysis was utilized for balance studies to demonstrate possible effects of the basal diets under study on metabolic parameters. Under circumstance of standardized balance experiments, it could be assumed that the possible effect was lower compared to the growth studies.

Results

Growth Data

CSM diets (Table 3) demonstrate significant effects of iP supplementation (CSM1 vs. CSM4) on zootechnical data and realize the marginal P supply in diet CSM1 in correspondence with very low native phytase activity (Table 2). Both factors were responsible for the observed increase of mortality (CSM1: 18.3%; CSM2: 11.7%; CSM3: 3.3%; CSM4: 1.7%; WSM1: 16.7%; WSM2: 13.3%; WSM3: 5%; WSM4: 0). Provided FCR data were not significantly affected. Adding phytase yielded significant dose depending response on growth data and feed intake, but insignificant effects on FCR. In addition,

Table 3. Growth performance of chicken fed low phosphorus CSM diets (7–42 d) supplemented with microbial phytase or inorganic phosphorus and results of ANOVA, Experiment 1 (6 pens, 10 birds per pen)

Diets	Supplementation		Initial body weight d7	Final body weight d42	Feed intake	Body weight gain	FCR
	Phytase	iP					
	(U/kg)	(g/kg)	(g)	(g)	(g/d)	(g/d)	(g/g)
CSM1	0	0	142.9±0.5	1362.9 ^a ±48.8	70.6 ^a ±3.2	34.9 ^a ±1.4	1.99±0.1
CSM2	250	0	142.5±0.6	1579.0 ^b ±43.6	81.6 ^b ±3.3	41.0 ^b ±1.3	1.94±0.1
CSM3	500	0	142.5±0.7	1830.3 ^c ±74.2	92.8 ^c ±3.9	48.2 ^c ±2.1	1.87±0.0
CSM4	0	1.5	142.8±0.8	2131.8 ^d ±54.3	116.0 ^d ±11.9	56.8 ^d ±1.6	1.97±0.2

^{a,b} Means within the same column with different superscript letters are significantly different ($p < 0.05$).

Table 4. Growth performance of chicken fed low phosphorus WSM diets (7–42 d) supplemented with microbial phytase or inorganic phosphorus and results of ANOVA, Experiment 2 (6 pens, 10 birds per pen)

Diets	Supplementation		Initial body weight d7	Final body weight d42	Feed intake	Body weight gain	FCR
	Phytase	iP					
	(U/kg)	(g/kg)	(g)	(g)	(g/d)	(g/d)	(g/g)
WSM1	0	0	124.0±1.3	1805.1 ^a ±91.6	91.3 ^a ±9.0	48.03 ^a ±4.9	1.85±0.1
WSM2	250	0	123.3±1.1	1853.2 ^a ±73.7	94.4 ^a ±5.2	49.43 ^a ±2.0	1.85±0.0
WSM3	500	0	124.0±0.8	1855.3 ^a ±80.6	95.7 ^a ±8.4	49.47 ^a ±4.2	1.88±0.0
WSM4	0	1.5	124.3±1.7	2255.6 ^b ±54.0	117.7 ^b ±8.8	60.89 ^b ±2.9	1.85±0.1

^{a,b} Means within the same column with different superscript letters are significantly different ($p < 0.05$).

mortality rate declined significantly (CSM3 vs. CSM1) but tended to be lowest following the positive control diet (CSM4). Growth data due to diet CSM3 were significantly below diet CSM4, indicating that the dietary P supply (CSM3) is still a limiting factor. Otherwise, it is not conclusive if the reachable effect of adding phytase is achieved with diet CSM3. Corresponding dietary P supply in WSM diets (Table 2) also yielded impaired zootechnical data (WSM1), but not so pronounced (Table 4). However, mortality rate was similar to diet CSM1 and decreased significantly by phytase supplementation (WSM3). According to CSM diets, the iP supplementation (WSM4) significantly improved growth, feed intake, feed efficiency and mortality rate.

Nutrient Deposition and Nutrient Utilization

Growth data and nutrient analyses of body composition yielded the database for calculation of nutrient deposition and nutrient utilization (Table 5, Table 6). Nutrient deposition data were in line with the observed significant changes in growth performance. Phytase addition and iP supplementation in CSM diets yielded significantly improved deposition of crude protein, fat, ash, phosphorus and energy (Table 5). According to observed FCR data, the efficiency of protein and energy utilization was not significantly affected in CSM diets. In WSM diets (Table 6), phytase supplementation significantly improved crude protein deposition, but effects between WSM2 and WSM3 differed not significantly. Enhanced native phytase activity and consequently increased total phytase activity in WSM diets (Table 2) was indicated as a factor of infl-

uence. However, due to iP supplementation (WSM4) further significant response was observed on crude ash, P and energy deposition, respectively. Diet WSM4 also yielded improved protein utilization.

Tibia Composition

The tibia analyses (Table 7), only recorded for experiment 1 (CSM diets), demonstrated significantly improved tibia ash contents due to supplementation of microbial phytase (CSM2, CSM3) and iP (CSM4), respectively. Between phytase addition and iP supplementation, the tibia ash content differed not significantly. Tibia content of P and Ca was significantly improved by addition of microbial phytase, but evidently, iP supplementation further improved P and Ca content of tibia dry matter.

N Balance Data

Results with CSM diets indicated significantly improved daily N balance data following diet CSM3 (Table 8). N digestibility and N utilization responded in similar manner. Following diet CSM2, N digestibility only tended to be improved. Significant effects on N utilization were not observed both following iP supplementation (CSM4) and in WSM diets, respectively.

P Balance Data

In CSM diets, phytase supplementation at 500 U/kg (CSM3) significantly improved daily P balance and P utilization, respectively (Table 9). Observed P balance data between diets CSM3 and CSM4 differed not significantly. In WSM diets, iP supplementation provided further significant increase of daily P balance, but P utilization remained similar to diet WSM1. Supplementa-

Table 5. Nutrient deposition and nutrient utilization of chicken fed low phosphorus CSM diets (7–42 d) supplemented with microbial phytase or inorganic phosphorus and results of ANOVA, Experiment 1 (n=6)

Diets	Supplementation		Deposition					Utilization		
	Phytase	iP	Crude protein	Crude fat	Crude ash	P	Energy	Protein	P	Energy
	(U/kg)	(g/kg)	(g/d)	(g/d)	(g/d)	(g/d)	(kJ/d)	(%)		
CSM1	0	0	6.24 ^a ±0.4	4.06 ^a ±1.1	0.81 ^a ±0.0	0.12 ^a ±0.0	312 ^a ±52	43.1±3.0	39.6 ^a ±1.9	35.9±5.8
CSM2	250	0	7.60 ^b ±0.6	5.25 ^b ±1.1	1.01 ^b ±0.1	0.16 ^b ±0.0	392 ^b ±49	45.4±3.9	46.4 ^b ±4.3	39.1±5.4
CSM3	500	0	8.63 ^c ±0.5	6.18 ^b ±0.9	1.24 ^c ±0.1	0.21 ^c ±0.0	454 ^c ±43	45.3±1.9	52.6 ^c ±2.5	39.7±2.5
CSM4	0	1.5	10.48 ^d ±0.3	8.12 ^c ±0.5	1.53 ^d ±0.1	0.25 ^d ±0.0	575 ^d ±21	44.4±4.9	44.4 ^{ab} ±7.1	40.7±4.3

^{a,b} Means within the same column with different superscript letters are significantly different ($p < 0.05$).

Table 6. Nutrient deposition and nutrient utilization of chicken fed low phosphorus WSM diets (7–42 d) supplemented with microbial phytase or inorganic phosphorus and results of ANOVA, Experiment 2 (n=6)

Diets	Supplementation		Deposition					Utilization		
	Phytase	iP	Crude protein	Crude fat	Crude ash	P	Energy	Protein	P	Energy
	(U/kg)	(g/kg)	(g/d)	(g/d)	(g/d)	(g/d)	(kJ/d)	(%)		
WSM1	0	0	6.88 ^a ±1.1	5.35±1.8	1.08 ^a ±0.2	0.19 ^a ±0.0	379 ^a ±91	37.7 ^a ±3.0	54.2 ^{ab} ±5.9	33.7±6.3
WSM2	250	0	8.51 ^b ±0.7	5.31±0.9	1.19 ^a ±0.1	0.20 ^a ±0.0	417 ^a ±25	45.3 ^{ab} ±4.0	56.1 ^{ab} ±3.9	36.1±1.8
WSM3	500	0	8.50 ^b ±1.0	5.04±0.9	1.23 ^a ±0.1	0.22 ^a ±0.0	403 ^a ±40	44.2 ^{ab} ±3.9	61.4 ^b ±5.4	34.4±1.6
WSM4	0	1.5	10.69 ^c ±2.1	6.72±1.9	1.59 ^b ±0.4	0.29 ^b ±0.1	525 ^b ±122	46.0 ^b ±10.2	46.4 ^a ±10.4	36.9±9.7

^{a,b} Means within the same column with different superscript letters are significantly different ($p < 0.05$).

Table 7. Tibia composition (% of DM) of chicken fed low phosphorus CSM diets (7–42 d) supplemented with microbial phytase or inorganic phosphorus and results of ANOVA, Experiment 1 (n=6)

Diets	CSM1	CSM2	CSM3	CSM4
Supplementation	0	250 U/kg	500 U/kg	1.5 giP/kg
Tibia ash (DM%)	35.4 ^a ±1.7	39.4 ^b ±3.4	40.0 ^b ±1.7	41.9 ^b ±1.6
P in tibia (DM%)	5.6 ^a ±0.3	6.3 ^b ±0.5	6.6 ^{bc} ±0.4	7.0 ^c ±0.3
Ca in tibia (DM%)	12.7 ^a ±1.0	14.6 ^b ±1.4	14.5 ^b ±0.8	15.2 ^b ±0.8

^{a,b,c} Means within the same line with different superscript letters are significantly different ($p < 0.05$).

tion of 500 U/kg microbial phytase (CSM3, WSM3) improved P utilization significantly. The basal diet was a main factor of influence on P balance data including P utilization.

Effects on AME

Determination of metabolizable energy (AMEn) content in the CSM and WSM diets did not yield significant treatment effects (Table 10). Consequently, established AMEn content of the diets under study did not indicate significant effects of added phytase on availability of dietary energy.

Discussion

Following low phosphorus CSM diets, feed intake and

growth data responded significantly due to graded supply of microbial phytase (Yi *et al.*, 1996a; Qian *et al.*, 1997). This observation is in line with many reports (Schöner *et al.*, 1991; Kornegay *et al.*, 1996; Rama Rao *et al.*, 1999; Zhang *et al.*, 2000; Lan *et al.*, 2002). Earlier studies using CSM diets also observed strong effects of low P diets on zootechnical data (Sebastian *et al.*, 1996, 1997; Rama Rao *et al.*, 1999; Pillai *et al.*, 2002) and mortality rate (Simons *et al.*, 1990; Denbow *et al.*, 1995; Sohail and Roland, 1999). Supplementation of inorganic P yielded further improved zoo technical data, indicating that the enhanced P availability by added phytase was not equal to P supply following iP supplementation. Significant dose response effects due to added phytase lead to speculation that

Table 8. N balance data (mg/BW_{kg}^{0.67}/d) of chicken fed low phosphorus CSM and WSM diets (factor basal diet) supplemented with microbial phytase or inorganic phosphorus (n=8) and results (p) of univariate ANOVA, Experiment 1+2

Diets Supplementation	CSM1 0	CSM2 250 U/kg	CSM3 500 U/kg	CSM4 1.5 g iP/kg	WSM1 0	WSM2 250 U/kg	WSM3 500 U/kg	WSM4 1.5 g iP/kg
N intake	3587 ± 184	3571 ± 266	3605 ± 144	3576 ± 90	3589 ± 103	3602 ± 142	3505 ± 87	3528 ± 152
N excretion	1678 ^a ± 123	1539 ^{ab} ± 220	1484 ^b ± 84	1674 ^a ± 78	1704 ^a ± 123	1616 ^a ± 175	1587 ^a ± 44	1618 ^a ± 112
N balance	1909 ^a ± 111	2032 ^{ab} ± 195	2122 ^b ± 123	1903 ^a ± 136	1885 ^a ± 125	1986 ^a ± 167	1918 ^a ± 91	1910 ^a ± 121
N digestibility (%)	80.6 ^a ± 1.9	81.0 ^a ± 1.2	83.2 ^b ± 0.9	82.8 ^b ± 1.6	81.8 ^a ± 2.5	82.5 ^a ± 1.9	81.6 ^a ± 0.7	81.8 ^a ± 1.8
N utilization (%)	53.2 ^a ± 2.1	57.0 ^b ± 4.6	58.8 ^b ± 2.0	53.2 ^a ± 2.8	52.3 ^a ± 3.2	55.2 ^a ± 4.3	54.7 ^a ± 1.6	54.2 ^a ± 2.5
<i>p</i> -value								
Factor	Basal diet	Phytase	iP	Basal diet x Phytase		Basal diet x iP		
N intake	0.407	0.811	0.526	0.480		0.655		
N excretion	0.398	0.005	0.347	0.714		0.389		
N balance	0.104	0.036	0.857	0.146		0.756		
N digestibility (%)	0.572	0.138	0.074	0.027		0.088		
N utilization (%)	0.123	0.002	0.484	0.297		0.440		

^{a,b} Means within the same line (based on statistical analysis within the respective balance period) with different superscript letters are significantly different ($p < 0.05$).

Table 9. P balance data (mg/LW_{kg}^{0.67}/d) of chicken fed low phosphorus CSM and WSM diets (factor basal diet) supplemented with microbial phytase or inorganic phosphorus (n=8) and results (p) of univariate ANOVA, Experiment 1+2

Diets Supplementation	CSM1 0	CSM2 250 U/kg	CSM3 500 U/kg	CSM4 1.5 g iP/kg	WSM1 0	WSM2 250 U/kg	WSM3 500 U/kg	WSM4 1.5 g iP/kg
P intake	462 ^a ± 25	460 ^a ± 34	463 ^a ± 18	538 ^b ± 13	436 ^b ± 12	418 ^{ab} ± 16	408 ^a ± 10	579 ^c ± 25
P excretion	253 ^a ± 28	224 ^a ± 45	220 ^a ± 35	304 ^b ± 26	146 ^b ± 26	122 ^{ab} ± 19	110 ^a ± 18	217 ^c ± 47
P balance	209 ^a ± 11	236 ^{ab} ± 33	243 ^b ± 37	234 ^{ab} ± 25	289 ^a ± 31	296 ^a ± 24	298 ^a ± 12	362 ^b ± 37
P utilization (%)	45.3 ^{ab} ± 3.7	51.6 ^{bc} ± 8.2	52.4 ^c ± 7.8	43.5 ^a ± 4.6	66.3 ^{ab} ± 6.3	70.8 ^{bc} ± 4.7	73.1 ^c ± 3.9	62.6 ^a ± 7.2
<i>p</i> -value								
Factor	Basal diet	Phytase	iP	Basal diet × Phytase		Basal diet × iP		
P intake	0.015	0.201	< 0.0001	0.160		< 0.0001		
P excretion	< 0.0001	0.012	< 0.0001	0.933		0.397		
P balance	< 0.0001	0.044	< 0.0001	0.626		0.033		
P utilization (%)	< 0.0001	0.007	0.210	0.903		0.644		

^{a,b} Means within the same line (based on statistical analysis within the respective balance period) with different superscript letters are significantly different ($p < 0.05$).

Table 10. AME_n of low phosphorus CSM and WSM chicken diets¹⁾ supplemented with microbial phytase or inorganic phosphorus (n=8), Experiment 1+2

Diets Supplementation	CSM1 0	CSM2 250 U/kg	CSM3 500 U/kg	CSM4 1.5 g iP/kg	WSM1 0	WSM2 250 U/kg	WSM3 500 U/kg	WSM4 1.5 g iP/kg
AME _n (MJ/kg)	13.25 ± 0.13	13.38 ± 0.40	13.74 ± 0.42	13.74 ± 0.24	13.39 ± 0.40	13.58 ± 0.25	13.35 ± 0.18	13.39 ± 0.25

¹⁾ Results of univariate ANOVA for all diets provided $p > 0.05$.

further increments of supplementation could provide additional effects on nutrient deposition. In contrast to several observations (Kornegay *et al.*, 1997; Zhang *et al.*, 2000; Lan *et al.*, 2002), no significant effect on FCR was observed. Following CSM diets, FCR tended to decline

according to phytase addition. However, WSM diets with added phytase did not provide a similar trend due to the enhanced native phytase activity in these diets.

Following low phosphorus WSM diets, according to earlier reports (Huyghebaert, 1996; Ravindran *et al.*, 1999

a, b) the supplementation of microbial phytase did not improve growth data significantly. This observation was not in agreement with Zyla *et al.* (2000). However, iP supplementation further improved growth data significantly, indicating that the achieved P supply from WSM diets was still below the requirement of growing chicken.

P deposition established from body analyses was significantly increased by phytase addition to CSM diets. According to Schöner *et al.* (1991), the P utilization was significantly enhanced by microbial phytase. Following the WSM diets, these effects were observed only numerical, indicating that the phytase accessible part of the phytate phosphorus in WSM diets becomes mostly available by the native phytase activity. Consequently, graded supply of microbial phytase only tended to improve P deposition. Protein and energy deposition responded in similar manner. Protein and energy utilization based on results of body analyses were not significantly improved by supplementation of microbial phytase addition in CSM and WSM diets, respectively. The observed trends were more pronounced in the balance studies. In CSM diets, the protein utilization significantly responded to phytase supplementation. Surprisingly, this effect was not observed following WSM diets with enhanced native phytase activity coming from basal diet. Significant phytase effects on N utilization were observed in several studies (Yi *et al.*, 1994, 1996a, b; Kornegay *et al.*, 1996), indicating the occurrence of phytase accessible phytate-protein-complexes during digestion (Farrell *et al.*, 1993). Otherwise, direct phytate effects on digestive enzyme activity (Singh and Krikorian, 1982; Deshpande and Cheryan, 1984) and endogenous amino acid flow (Cowieson and Ravindran, 2007) were reported. Consequently, enhanced N utilization also could be the result of decreased endogenous amino acid losses. Established main effects from univariate ANOVA realize N balance, N excretion and N utilization as significantly influenced by phytase addition. The observed effects on N excretion in CSM diets were according to Yi *et al.* (1996c). Additionally, for N digestibility an interrelationship was observed between basal diet and phytase addition. This observation is supporting the growth data, which yielded a tendency of improved protein utilization only due to supplementation of microbial phytase.

P utilization derived from balance studies was influenced by the basal diet, indicating the importance of the native phytase activity. Additionally, the supplemented microbial phytase provided main effects on P excretion, P balance and P utilization, respectively. As expected, iP supplementation yielded main effects on P balance data. However, iP addition was not a main factor for the observed P utilization. According to Viveros *et al.* (2002), adding iP decreased P utilization. Additionally, P utilization data concluded from long-term deposition studies based on analysis of body composition and short-term P balance experiment are not directly comparable. Due to the prolonged exhausting of P depots during the

long-term study, suboptimal P supply provided effects that are more pronounced. Furthermore, interrelationships between dietary Ca supply and phytate degradation has to be taken into account. Reports in growing chicken (Plumstead *et al.*, 2008; Leytem *et al.*, 2008) indicate that our current investigations worked on Ca to NPP ratio where the efficiency of phytate hydrolysis could be partly depressed by dietary Ca supply. However, this statement is not free of speculation and verification is needed. Finally, caloric measurements in feed and excreta within the balance studies yielded no evidence of significant improvement of dietary AME by phytase addition, as reported in several studies (Ravindran *et al.*, 1999b, 2001).

In conclusion, current results with supplemented phytase in low phosphorus chicken CSM and WSM diets observed significant improvement of P utilization in both diets. The basal diet was a significant factor for the established level of P utilization. Phytase addition was also a main factor for N utilization, but only in CSM diets with marginal native phytase activity the N utilization responded significantly. The observed response was only partly explained by improved protein digestibility and this contrast needs further investigations to provide reliable conclusions due to conditions under which a significant effect of added phytase on N utilization can be expected. Finally, the added phytase source could be a further factor of influence on observed nutrient utilization as reported by Liebert and Portz (2005, 2007).

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