Performance and Nutrient Utilization of Laying Hens Fed Low-Phosphorus Corn-Soybean and Wheat-Soybean Diets Supplemented with Microbial Phytase

F. Liebert,[1] J. K. Htoo, and A. Sünder

Institute for Animal Physiology and Animal Nutrition, Georg-August-University, Kellnerweg 6, 37077, Goettingen, Germany

ABSTRACT Two experiments were conducted with laying hens (Lohmann Brown) in an individual cage system and with single feeding conditions. Experiment 1 (n = 24) was a performance trial (22 to 61 wk) to evaluate phytase effects on performance and nutrient utilization in corn-soybean meal (CSM1) and wheat-soybean meal (WSM1) basal diets (0.12% NPP; 3.1% Ca) supplemented (300 U/kg) with an experimental microbial phytase (CSM2 and WSM2) or 1.5 g/kg inorganic P (CSM3 and WSM3). Experiment 2 (n = 16) was also conducted as a performance trial (22 to 61 wk) only using CSM diets with dietary treatments similar to those in experiment 1. In addition, parallel N and P balance experiments in 2 age periods (26 and 33 wk, respectively) were conducted. In experiment 1, no significant ($P < 0.05$) differences in mortality, feed intake, egg production, egg weight, or body weight were observed. Tibia bone mineral composition was significantly affected by microbial phytase. Microbial phytase in the low-P CSM diet significantly ($P < 0.05$) improved the feed conversion ratio. In experiment 2, only feed conversion ratio was significantly improved by microbial phytase. The phytase supplementation had no significant effect on P excretion, P balance, P utilization, N balance, N utilization, or AME_n in the balance experiments.

(*Key words*: phytase, laying hen, performance, phosphorus utilization)

2005 Poultry Science 84:1576–1583

INTRODUCTION

Only nonphytate P (NPP), about one-third of the total P in plant-based diets, can be used by poultry due to insufficient endogenous phytase activity to degrade phytate effectively (Nelson, 1967). Consequently, significant improvement of P use due to microbial phytase is demonstrated in numerous studies with growing chickens (Simons et al., 1990; Kornegay, 2001; Augspurger and Baker, 2004; Snow et al., 2004). The efficiency of supplementation of microbial phytase in layer diets is still under discussion because of an open debate about the NPP requirement of laying hens and the factors influencing the phytate degradation by supplemented phytase in the gut of layers.

Previous studies with laying hens fed corn-soybean meal (CSM) diets have demonstrated that microbial phytase supplementation improved performance (Van der Klis et al., 1997; Lim et al., 2003), tibia ash, Ca and P contents in the tibia ash (Peter et al., 1992), P use (Jalal and Scheideler, 2001; Shan et al., 2002), and ileal digestibility of amino acids (Jalal et al., 1999). Microbial phytase completely alleviated the adverse effect of low NPP (0.20%) CSM diets (Keshavarz, 2003a). However, the interrelationship between NPP supply in the growing and laying period as well as strain effects were clearly pointed out (Keshavarz, 2003b). In addition, the corn genotype was an important factor for the efficiency of microbial phytase in low-NPP CSM diets (Ceylan et al., 2003). In contrast, no significant effect on performance was observed after phytase supplementation to CSM diets containing 0.15% NPP (Boling et al., 1997, 2000a; Rama Rao et al., 1999) and 0.2% NPP (Gordon and Roland, 1997; Scott et al., 1999a). Furthermore, phytase addition to low-P wheat-soybean meal (WSM) layer diets had no effect on performance (Peter et al., 1992; Jeroch, 1994; Scott et al., 1999b) or tibia ash content (Jeroch, 1994). Effects of phytase supplementation on availability of energy in layer diets are only partly documented (Pan et al., 1998). Snow et al. (2003b) observed no phytase effect on ileal amino acid digestibility.

Summers (1995) reported that 0.20% NPP in CSM diets was not adequate for optimal performance of laying hens between 32 and 64 wk of age, supporting the NRC (1994)

Received for publication March 2, 2005.
Accepted for publication June 30, 2005.
[1]To whom correspondence should be addressed: flieber@gwdg.de.

Abbreviation Key: CSM = corn-soybean meal; iP = inorganic P; NPP = nonphytate P; WSM = wheat-soybean meal.

Table 1. Experimental design

	Corn-soybean meal (CSM) diets			Wheat-soybean meal (WSM) diets[1]		
Supplementation	CSM1	CSM2	CSM3	WSM1	WSM2	WSM3
Phytase (U/kg of diet)[2]	0	300	0	0	300	0
Inorganic P (g/kg of diet)[3]	0	0	1.5	0	0	1.5
Phytase activity (U/kg)[4]	20	352	23	267	521	268

[1]WSM diets were used in experiment 1 only.

[2]Experimental microbial phytase (SP1002, Roche Vitamins, Ltd., Basel, Switzerland).

[3]Monosodium phosphate (22% P).

[4]Analyzed activity.

recommendation of 250 mg/d. In contrast, Boling et al. (1997, 2000a,b) reported that 0.15% NPP (159 mg/d) in CSM diets supported optimal performance between 20 and 70 wk of age. German recommendations (Gesellschaft für Ernährungsphysiologie, 1999) are 0.31 to 0.33% NPP for layer diets with 11.4 MJ of AME_n/kg. No general agreement concerning the dietary NPP requirement of laying hens is indicated. The objective of the present study with laying hens was to evaluate the effects of microbial phytase on performance, tibia bone ash mineralization, and N, P, and energy use of hens fed a low-P CSM or WSM diet with different phytase activities from native origins.

MATERIALS AND METHODS

Birds and Housing

Hens, 18 wk of age (Lohmann Brown), from a commercial farm were kept in individual cages (cage size: 0.4 × 0.36 m) under controlled climate conditions and a 14-h lighting period schedule as recommended (Lohmann Management Guide B 103 E). During the preexperimental period (up to 22 wk of age) a crumbled commercial prelay diet (17.4% CP) was offered ad libitum. Two experiments were conducted in an individual cage system with single feeding conditions.

Experiment 1 was designed as a performance trial (24 birds/treatment; 22 to 61 wk of age) with low-P corn-soybean (CSM) and wheat-soybean (WSM) diets. The birds were randomly assigned to 6 dietary treatments (Table 1). Experiment 2 was a combination of a performance trial (16 birds/treatment; 22 to 61 wk of age) and 2 balance studies (8 birds/treatment; 10-d collection period) within the performance trial at different age periods (26 and 33 wk of age, respectively). The birds were randomly assigned to 3 dietary treatments with CSM diets similar to those in experiment 1 (Table 1).

Experimental Diets

Two low-P basal diets (CSM1, WSM1) were supplied in mash form (Table 2) to meet or to exceed the nutritional recommendations (NRC, 1994), except for P (0.39% total P and 0.12% NPP). The Ca supply (3.1% of the diets) was equal for all dietary treatments in both experiments. The experimental microbial phytase (SP 1002[2]) used for phytase supplementation (CSM2, WSM2) is a consensus phytase produced by a genetically modified strain of the yeast *Hansenula polymorpha.* The production strain carries a synthetic gene coding for 3-phytase (IUB no. 3.1.3.8.) with elevated pH optimum (>5) and higher stability against proteolytic activity and thermal treatments (Igbasan et al., 2000). Monosodium phosphate was used as source of inorganic P (iP) at the expense of wheat starch (CSM3, WSM3). Diets and drinking water were supplied ad libitum. During balance studies a controlled feeding regimen (110 g/d) was used.

[2]Roche Vitamins Ltd., Basel, Switzerland.

Measurements

Laying performance, individual egg weight, and mortality were daily recorded. Individual feed consumption was determined weekly. Individual body weight of hens

Table 2. Composition of the basal corn-soybean meal (CSM) and wheat-soybean meal (WSM) diets

Ingredients (%)	CSM1	WSM1
Corn	59.0	—
Wheat	—	60.4
Soybean meal	24.9	22.0
Soybean oil	3.2	4.75
Wheat starch	2.6	2.57
Calcium carbonate	8.8	8.8
Salt	0.4	0.35
Premix[1]	1.0	1.0
DL-Methionine	0.1	0.13
Analyzed composition	(DM %)	
Crude protein	17.3	17.8
Crude fat	7.9	7.4
Lysine	0.96	0.94
Methionine	0.43	0.44
Methionine + cystine	0.93	0.94
Total P	0.39	0.39
Nonphytate P	0.12	0.12
Ca	3.1	3.1
AME_n (MJ/kg of DM)[2]	12.85	12.77

[1]Provided (per kilogram of diet): vitamin A, 6,000 IU; vitamin D_3, 1,000 IU; vitamin E, 18.5 mg; thiamin, 1.6 mg; riboflavin, 4.8 mg; vitamin B_6, 3 mg; vitamin B_{12}, 20 µg; vitamin K_3, 2 mg; nicotinic acid, 28 mg; calcium pantothenate, 10 mg; folic acid, 0.6 mg; biotin, 100 µg; choline chloride, 800 mg; Se, 25 mg; Zn, 80 mg; Mn, 80 mg; Cu, 16 mg, Fe, 25 mg; I, 1.2 mg; Co, 0.55 mg; canthaxanthin, 3.5 mg; and BHT, 100 mg.

[2]Calculated.

Table 3. Laying performance of hens fed low-P corn-soybean meal (CSM) and wheat-soybean meal (WSM) diets (22 to 61 wk) supplemented with microbial phytase or inorganic P (n = 24) and the results (*P*-value) of univariate ANOVA, experiment 1[1]

Diets	Supplementation		Egg production (%)	Egg mass production (g/d)	Egg weight (g)	Body weight gain (g)	Feed intake (g/d)	FCR (g/g)
	Phytase (U/kg)	iP (g/kg)						
CSM1	0	0	$83.8^{a} \pm 11.4$	$51.2^{a} \pm 6.6$	$59.7^{a} \pm 4.2$	$129^{a} \pm 166$	$111.2^{ab} \pm 8.7$	$2.27^{b} \pm 0.30$
CSM2	300	0	$86.8^{a} \pm 11.7$	$53.7^{ab} \pm 4.7$	$60.5^{ab} \pm 3.9$	$233^{ab} \pm 184$	$111.9^{ab} \pm 7.4$	$2.12^{a} \pm 0.14$
CSM3	0	1.5	$87.3^{a} \pm 8.2$	$54.6^{b} \pm 5.4$	$62.0^{b} \pm 2.9$	$98^{b} \pm 166$	$114.1^{b} \pm 10.5$	$2.15^{a} \pm 0.16$
WSM1	0	0	$82.2^{a} \pm 12.5$	$52.0^{ab} \pm 4.8$	$60.8^{ab} \pm 4.4$	$227^{ab} \pm 188$	$108.1^{a} \pm 6.6$	$2.20^{ab} \pm 0.19$
WSM2	300	0	$84.7^{a} \pm 17.0$	$54.0^{ab} \pm 4.1$	$60.6^{ab} \pm 3.2$	$258^{b} \pm 220$	$109.1^{a} \pm 7.9$	$2.11^{a} \pm 0.19$
WSM3	0	1.5	$85.3^{a} \pm 3.9$	$53.1^{ab} \pm 4.3$	$61.4^{ab} \pm 3.6$	$292^{b} \pm 212$	$107.7^{a} \pm 8.0$	$2.13^{a} \pm 0.18$
Factor								
Phytase			0.245	0.033	0.764	0.092	0.642	0.006
iP			0.166	0.036	0.075	0.004	0.468	0.025
Basal diet			0.330	0.727	0.966	0.413	0.004	0.400
Basal diet × phytase			0.909	0.821	0.508	0.358	0.943	0.424
Basal diet × iP			0.921	0.263	0.272	0.190	0.333	0.569

[a,b]Means within the same column with different superscript letters are significantly different ($P < 0.05$).

[1]FCR = feed conversion ratio; iP = inorganic P.

was recorded in 4-wk periods. At the end of the performance trials (61 wk of age) 6 hens per treatment (experiment 1) and 12 hens per treatment (experiment 2) were randomly selected and killed by CO_2 inhalation for preparation of the left tibia. After removal of adhering tissue, drying at 105°C for 48 h, and overnight combustion at 550°C the concentrations of crude ash, Ca, and P were analyzed. For the balance trials, excreta were collected twice, and the samples were frozen at –18°C. After the 10-d individual egg collection, shell and egg content were separated, weighed, and individually pooled. Shell samples were dried at 105°C for 48 h and homogenized. Egg contents were also homogenized and freeze-dried. According to the determined individual ratio, shells and egg contents were remixed and finally homogenized for further analysis. The use of N and P was calculated based on the results of N and P balance studies. Digestibility of N was determined due to the α-amino method of Pahle et al. (1985) and assuming that the only N excretion via feces was in the form of α-amino N. Nitrogen use is calculated as the difference between N intake and total N excretion (N balance) related to the N intake. The N quantity of the daily egg mass was given separately. The AME_n content of the experimental diets was determined by calorimetric measurements in feed and excreta after the balance trials and included the N correction according to the recommendations of Titus et al. (1959).

Chemical Analyses

Diets and freeze-dried excreta were analyzed for gross energy[3] and N (Dumas method)[4] for determination of AME_n. Total P in feed, excreta, egg and tibia ash were analyzed according to Naumann and Bassler (1997). Phytate P analysis in feed was based on AOAC (1990). The Ca in feed and tibia samples was determined according to the methods of Naumann and Bassler (1997), who used an atomic absorption spectrophotometer.[5] The α-amino N content in feed and excreta were quantified according to the method of Pahle et al. (1983, 1985). The dietary phytase activity was determined according to the method of Engelen et al. (1994).

Statistical Analyses

Results are presented as means ± standard deviation. Data analysis was based on 2-way and 3-way ANOVA[6] using an incomplete factorial design. Fisher's least significant difference or Games-Howell posthoc test was used according to equality or nonequality of variances (verified by the Levene test).

RESULTS AND DISCUSSION

Experiment 1

Supplemented and analyzed total activities of phytase in the experimental diets were in good conformity (Table 1). The results of the performance trial are summarized based on 1-way ANOVA (Table 3). In addition, the general effects were evaluated by multivariate ANOVA. With the CSM diets, laying performance over the total experimental period (22 to 61 wk) was not significantly influenced by microbial phytase and iP supplementation. Daily egg mass production was significantly improved only in diet CSM3 with iP addition. Supplemented microbial phytase (CSM2) affected daily egg mass production only numerically corresponding to a slight increase in egg production and individual egg weight in this group. In case of diet CSM3, the egg weight was significantly improved. Due to observed high individual variation in group CSM1, the body weight gain was only numerically affected by diet CSM2. However, the increase in body weight gain was significant due to iP supplementation

[3]AC 350 LECO Instrumente GmbH, Moenchengladbach, Germany.
[4]FP 2000 LECO Instrumente GmbH.
[5]SpectrAA55, Varian Deutschland GmbH, Darmstadt, Germany.
[6]SPSS for Windows, 2002, SPSS, Inc., Chicago, IL.

Table 4. Tibia composition (% of DM) of laying hens fed low-P corn-soybean meal (CSM) and wheat-soybean meal (WSM) diets (22 to 61 wk) supplemented with microbial phytase or inorganic P (iP; n = 6) and the results (*P*-value) of univariate ANOVA, experiment 1

	CSM1	CSM2	CSM3	WSM1	WSM2	WSM3
Supplementation	0	300 U/kg	1.5 g of iP/kg	0	300 U/kg	1.5 g of iP/kg
Tibia ash (DM %)	40.7 ± 1.8^{a}	43.1 ± 1.6^{ac}	46.6 ± 2.7^{bc}	42.9 ± 2.0^{a}	46.8 ± 4.7^{b}	47.9 ± 3.0^{b}
P in tibia (DM %)	6.4 ± 0.3^{a}	6.7 ± 0.3^{ab}	7.3 ± 0.4^{b}	6.7 ± 0.3^{ab}	7.3 ± 0.7^{ab}	7.2 ± 0.7^{ab}
Ca in tibia (DM %)	15.1 ± 0.8^{a}	16.3 ± 1.0^{abc}	17.3 ± 1.1^{bc}	15.9 ± 0.9^{ab}	17.4 ± 1.6^{c}	17.3 ± 1.2^{bc}

Factor	Phytase	iP	Basal diet	Basal diet × phytase	Basal diet × iP
Tibia ash (DM %)	0.019	<0.0001	0.030	*0.570*	*0.702*
P in tibia (DM %)	0.035	0.002	0.147	0.599	0.227
Ca in tibia (DM %)	0.009	0.001	0.166	0.788	0.448

[a–c]Means within the same line with different superscript letters are significantly different ($P < 0.05$).

(CSM3). The dietary treatments in CSM diets had no significant effects on feed intake. These results are in general agreement with observations of Boling et al. (1997, 2000a), based on short-term (wk 20 to 36) and long-term (wk 20 to 70) performance trials. It was reported that egg mass production, egg weight, body weight, and feed intake of hens were not significantly improved by adding microbial phytase (300 U/kg) or iP to CSM diets with 0.15% available P (Rama Rao et al., 1999; Boling et al., 2000b). In contrast, daily egg mass production increased significantly in the actual study. The significant effect of phytase supplementation on the feed conversion ratio is in agreement with the results of Kim et al. (2001), concluding that the adverse effect of a low-P CSM diet on feed conversion ratio can be alleviated by phytase supplementation. According to Muji et al. (2002), the dietary level of protein may play an important role for the nutritional effect of supplemented phytase, which was only observed in low-protein CSM diets. Moreover, the effect of supplemental phytase in layer diets can be markedly influenced by the dietary supply of Ca and Ca:P ratio (Lei et al., 1994).

When fed WSM diets, laying performance, daily egg mass production, individual egg weight, body weight gain, feed intake, and FCR were not significantly influenced by supplementation of microbial phytase and iP (Table 3). This observation agrees with the results of Peter et al. (1992) and Jeroch (1994) when feeding WSM diets (0.11% NPP) supplemented with phytase (500 U/kg) or iP (1 g/kg), which suggests that WSM diets have a basic activity of native phytase (Table 1). In addition, because of multivariate ANOVA, general effects ($P < 0.05$) of microbial phytase were observed only on daily egg mass production and feed conversion ratio. The iP addition significantly influenced daily egg mass production, body weight gain, and feed conversion ratio. The basal diet had significant effects on daily feed intake.

The results of tibia analysis (Table 4) following 1-way ANOVA show the lowest tibia ash, P, and Ca concentrations in laying hens after being fed diet CSM1. Due to iP supplementation (CSM3), the ash, P, and Ca contents of the tibia bone were significantly increased. Phytase supplementation tended to increase these parameters. When WSM diets were supplemented with microbial phytase (WSM2) and iP (WSM3), the tibia ash content was significantly improved. The P concentration in tibia dry matter only tended to increase, which was also the case for iP supplementation. However, microbial phytase (WSM2) significantly improved the Ca content in the tibia. General effects on tibia ash content, based on multivariate ANOVA ($P < 0.05$), were observed for microbial phytase and iP supplementation. Additionally, the basal diet (CSM vs. WSM) was a significant factor, indicating the importance of the higher activity of native phytase in WSM diets. Based on general effects, the tibia P and Ca contents were significantly improved by microbial phytase and iP addition.

Table 5. Laying performance of hens fed low-P corn-soybean meal (CSM) diets (22 to 61 wk) supplemented with microbial phytase or inorganic P (n = 16) and the results (*P*-value) of univariate ANOVA, experiment 2[1]

	Supplementation							
Diets	Phytase (U/kg)	iP (g/kg)	Egg production (%)	Egg mass production (g/d)	Egg weight (g)	Body weight gain (g)	Feed intake (g/d)	FCR (g/g)
CSM1	0	0	$89.7^{a} \pm 5.1$	$58.6^{a} \pm 4.1$	$65.5^{a} \pm 4.0$	$222^{a} \pm 322$	$113.6^{a} \pm 7.7$	$1.98^{a} \pm 0.14$
CSM2	300	0	$89.4^{a} \pm 4.4$	$58.8^{a} \pm 3.3$	$65.6^{a} \pm 3.3$	$241^{a} \pm 211$	$110.0^{a} \pm 4.9$	$1.88^{b} \pm 0.16$
CSM3	0	1.5	$90.8^{a} \pm 3.9$	$59.2^{a} \pm 4.9$	$65.4^{a} \pm 4.9$	$378^{a} \pm 321$	$114.7^{a} \pm 7.8$	$1.93^{ab} \pm 0.13$
Factor								
Phytase			0.823	0.935	0.915	0.855	0.146	0.050
iP			0.517	0.725	0.973	0.146	0.657	0.339

[a,b]Means within the same column with different superscript letters are significantly different ($P < 0.05$).

[1]iP = inorganic P; FCR = feed conversion ratio.

Table 6. Tibia composition (% of DM) of laying hens fed low-P corn-soybean meal (CSM) diets (22 to 61 wk) supplemented with microbial phytase or inorganic P (iP; n = 12), experiment 2

Supplementation	CSM1	CSM2	CSM3
	0	300 U/kg	1.5 g of iP/kg
Tibia ash content	44.7 ± 4.7[a]	44.9 ± 5.4[a]	45.6 ± 4.7[a]
Tibia P content	6.4 ± 0.7[a]	6.6 ± 0.9[a]	6.7 ± 0.7[a]
Tibia Ca content	15.2 ± 1.4[a]	15.5 ± 2.2[a]	15.5 ± 1.6[a]

[a,b]Means within the same line with different superscript letters are significantly different ($P < 0.05$).

Experiment 2

Similar to the results of experiment 1, no significant effect on egg production was observed due to diets CSM2 and CSM3, respectively. Additionally, daily egg mass production, individual egg weight, body weight gain, and feed intake were not significantly influenced by phytase or iP supplementation (Table 5). However, according to experiment 1 the feed conversion ratio was significantly improved by supplemental microbial phytase. This general effect was supported by the results of 2-way ANOVA ($P = 0.05$). Tibia ash, P, and Ca contents of the tibia were not significantly influenced by supplementation with microbial phytase and iP (Table 6). Based on the results of tibia ash analyses in both experiments, no significant effect could be concluded for added microbial phytase in low-P CSM diets on tibia bone mineralization. This observation was surprising, mainly because of the low-P level in the diets. Even at high dietary phytase activity, negative effects of the Ca level on phytase activity in the gut are reported (Nelson, 1967). In addition, the formation of insoluble Ca phytate complexes could be a further important factor for the failed efficiency of added phytase by reducing the accessibility of the phytate molecule for phytase (Nelson et al., 1968; Scheuermann et al., 1988). Diets with graded Ca levels could bring out more detailed information related to the importance of this factor of influence.

Based on energy balance studies, AME_n values of the CSM diets were not significantly influenced by the dietary treatments (Table 7). This conclusion is in general agreement with observations for N digestibility and N use.

The results from the N and P balance studies with CSM diets are summarized in Tables 8 and 9, respectively. The N balance data (Table 8) in both balance periods were not significantly influenced by supplementation of microbial phytase (CSM2). This observation is in general agreement with both performance trials. The observed results for N digestibility and N use were similar in both balance periods and not significantly affected by microbial phytase. Otherwise, an elevated N excretion was observed due to diet CSM3 and mainly attributed to the slightly higher N intake. The negative influence of iP supplementation on N use was a trend in the first balance period and a general effect in the second balance period. No final explanation of this observation was possible based on our research. The results from the P balance study (Table 9) did not show significant effects of microbial phytase supplementation (CSM2) on P excretion. This observation indicated that no significant phytate degradation was achieved. In diets CSM1 and CSM2, the results of P balance and P use were similar. However, in both balance periods the iP addition (CSM3) significantly increased P balance as well as P excretion but also tended to reduce P use. In fact, nearly 70% of the surplus P intake due to diet CSM3 was excreted, indicating a very low efficiency of added iP. This observation could be attributed to a lower total P requirement of the laying hen. In the current study, hens fed the CSM diet containing 0.12% NPP (CSM1) had an average daily P intake of 130 mg of NPP during the entire period. Even in the absence of supplemental microbial phytase and 0.12% NPP (130 mg NPP/d), no significant negative effect on laying performance was observed, but feed conversion ratio was negatively affected. Results of Boling et al. (1997, 2000a, b) and Snow et al. (2005) support the assumption that the NPP requirement of layers could be overestimated. It was reported that daily NPP intake of 159 mg from CSM diets did not reduce laying performance. Keshavarz (2000) observed no adverse effects due to a step-down regimen of NPP (0.25, 0.20, and 0.15%) from 30 to 42, 43 to 54, and 55 to 66 wk of age, respectively. Further studies can be interpreted similarly (Vogt, 1992; Gordon and Roland, 1997; Rama Rao et al., 1999). However, Summers (1995) reported that 0.20% NPP could be inadequate, mainly with increasing

Table 7. The AMEn of low-P corn-soybean meal (CSM) layer diets supplemented with microbial phytase or inorganic P (iP; n = 8), experiment 2

Supplementation	CSM1	CSM2	CSM3
	0	300 U/kg	1.5 g of iP/kg
First balance period (26-27 wk)			
AME_n (MJ/kg)	13.21 ± 0.19[a]	13.39 ± 0.18[a]	13.31 ± 0.32[a]
Second balance period (33–34 wk)			
AME_n (MJ/kg)	13.61 ± 0.22[a]	13.65 ± 0.21[a]	13.64 ± 0.20[a]

[a]Means within the same line with different superscript letters are significantly different ($P < 0.05$).

Table 8. Nitrogen balance data (mg/$BW_{kg}^{-0.67}$ per day) of laying hens fed low-P corn-soybean meal (CSM) diets supplemented with microbial phytase or inorganic P (iP; n = 8) and the results (*P*-value) of univariate ANOVA, experiment 2

	First balance period (26–27 wk)			Second balance period (33–34 wk)		
Diets	CSM1	CSM2	CSM3	CSM1	CSM2	CSM3
Supplementation	0	300 U/kg	1.5 g of iP/kg	0	300 U/kg	1.5 g of iP/kg
N intake	2,122 ± 97[a]	2,071 ± 123[a]	2,144 ± 89[a]	1,971 ± 160[a]	1,922 ± 168[a]	2,021 ± 101[a]
N excretion	956 ± 77[a]	914 ± 84[a]	995 ± 74[a]	850 ± 56[a]	827 ± 84[a]	937 ± 54[b]
N egg mass	680 ± 63[a]	662 ± 40[a]	691 ± 54[a]	669 ± 68[a]	693 ± 65[a]	708 ± 71[a]
N balance	1,166 ± 73[a]	1,157 ± 69[a]	1,149 ± 58[a]	1,121 ± 129[a]	1,095 ± 131[a]	1,084 ± 98[a]
N digestibility (%)	84.3 ± 1.1[a]	85.2 ± 1.1[a]	84.1 ± 2.7[a]	85.1 ± 1.4[a]	85.4 ± 1.3[a]	84.1 ± 2.1[a]
N utilization (%)	55.0 ± 2.7[a]	55.9 ± 2.2[a]	53.6 ± 2.3[a]	56.8 ± 2.5[b]	56.9 ± 3.4[b]	53.6 ± 2.9[a]

Factor	Phytase	iP	Phytase	iP
N intake	0.341	0.676	0.511	0.498
N excretion	0.305	0.327	0.490	0.016
N egg mass	0.505	0.705	0.493	0.270
N balance	0.780	0.610	0.674	0.547
N digestibility (%)	0.299	0.857	0.715	0.267
N utilization (%)	0.442	0.281	0.914	0.044

[a,b]Means within the same line (based on separate statistical analysis within the respective balance period) with different superscript letters are significantly different ($P < 0.05$).

Table 9. Phosphorus balance data (mg/$BW_{kg}^{-0.67}$ per day) of laying hens fed low-P corn-soybean meal (CSM) diets supplemented with microbial phytase or inorganic P (iP; n = 8) and results (*P*-value) of univariate ANOVA, experiment 2

	First balance period (26–27 wk)			Second balance period (33–34 wk)		
	CSM1	CSM2	CSM3	CSM1	CSM2	CSM3
Supplementation	0	300 U/kg	1.5 g of iP/kg	0	300 U/kg	1.5 g of iP/kg
P intake	256 ± 12[a]	251 ± 12[a]	372 ± 13[b]	258 ± 21[a]	252 ± 22[a]	390 ± 20[b]
P excretion	165 ± 15[a]	157 ± 12[a]	248 ± 16[b]	151 ± 14[a]	142 ± 15[a]	236 ± 22[b]
P egg mass	59 ± 6[a]	57 ± 3[a]	60 ± 4[a]	63 ± 8[a]	64 ± 7[a]	65 ± 6[a]
P balance	91 ± 11[a]	94 ± 14[a]	124 ± 15[b]	107 ± 15[a]	110 ± 24[a]	154 ± 13[b]
P utilization (%)	35.7 ± 4.3[a]	37.3 ± 4.7[a]	33.3 ± 3.8[a]	41.5 ± 4.1[a]	43.4 ± 7.2[a]	39.7 ± 3.8[a]

Factor	Phytase	iP	Phytase	iP
P intake	0.375	<0.0001	0.546	<0.0001
P excretion	0.287	<0.0001	0.295	<0.0001
P egg mass	0.579	0.459	0.643	0.436
P balance	0.734	<0.0001	0.756	<0.0001
P utilization (%)	0.470	0.493	0.470	0.493

[a,b]Means within the same line (based on separate statistical analysis within the respective balance period) with different superscript letters are significantly different ($P < 0.05$).

age of the hens. At similar NPP levels Keshavarz and Austic (2004) observed performance data comparable to the positive control (0.4% NPP) when microbial phytase was added and feed protein quality was optimized by amino acid supplementation in low-protein diets. Sell et al. (1987) observed improved laying performance with iP supplementation in CSM diets containing 0.15% NPP. Diets with very low P supply (0.10% NPP) did not support optimal laying performance (Gordon and Roland, 1997, 1998; Punna and Roland, 1999). Snow et al. (2003a) reported that layer CSM diets with 0.19% NPP, based on corn with increased P availability, supported optimal performance. In general, the observations related to NPP supply and NPP requirement of laying hens for optimal performance, animal health, and egg quality are still inconsistent and do not allow final conclusions. In the present study, the supplementation of microbial phytase was not effective.

ACKNOWLEDGMENTS

The authors express their thanks to Roche Vitamins Ltd. for supplying the experimental microbial phytase SP1002 and analyses of in-feed phytase activity.

REFERENCES

Augspurger, N. R., and D. H. Baker. 2004. High dietary phytase levels maximize phytate-phosphorus utilization but do not affect protein utilization in chicks fed phosphorus- or amino acid-deficient diets. J. Anim. Sci. 82:1100–1107.

Association of Official Analytical Chemists. 1990. Method No. 986.11. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Washington, DC.

Boling, S. D., M. W. Douglas, M. L. Johnson, X. Wang, C. W. Parsons, K. W. Koelkebeck, and R. A. Zimmerman. 1997. Supplemental phytase improves performance of laying hens consuming diets with low levels of available phosphorus. Poult. Sci. 76(Suppl. 1):20. (Abstr.)

Boling, S. D., M. W. Douglas, M. L. Johnson, X. Wang, C. W. Parsons, K. W. Koelkebeck, and R. A. Zimmerman. 2000a. The effects of dietary available phosphorus levels and phytase on performance of young and older laying hens. Poult. Sci. 79:224–225.

Boling, S. D., M. W. Douglas, R. B. Shirley, C. W. Parsons, and K. W. Koelkebeck. 2000b. The effects of various dietary levels of phytase and available phosphorus on performance of laying hens. Poult. Sci. 79:535–538.

Ceylan, N., S. E. Scheideler, and H. L. Stilborn. 2003. High available phosphorus corn and phytase in layer diets. Poult. Sci. 82:789–795.

Engelen, A. J., F. C. Van der Heeft, P. H. Randsdorp, and E. L. Smit. 1994. Simple and rapid determination of phytase activity. J. AOAC Int. 77:760–764.

Gesellschaft für Ernährungsphysiologie. 1999. Empfehlungen zur Energie- und Nährstoffversorgung der Legehennen und Masthühner (Broiler), DLG Verlag, Frankfurt, Germany.

Gordon, R. W., and D. A. Roland. 1997. Performance of commercial laying hens fed various phosphorus levels with and without supplemental phytase. Poult. Sci. 76:1172–1177.

Gordon, R. W., and D. A. Roland, Sr. 1998. Influence of supplemental phytase on calcium and phosphorus utilization in laying hens. Poult. Sci. 77:290–294.

Igbasan, F. A., K. Männer, G. Miksch, R. Borris, A. Farouk, and O. Simon. 2000. Comparative studies on the in vitro properties of phytases from various origins. Arch. Anim. Nutr. 53:353–371.

Jalal, M. A., and S. E. Scheideler. 2001. Effect of supplementation of two different sources of phytase on egg production parameters in laying hens and nutrient digestibility. Poult. Sci. 80:1463–1471.

Jalal, M. A., S. E. Scheideler, and C. Wyatt. 1999. Effects of phytase supplementation on egg production parameters and amino acid digestibilities. Poult. Sci. 78(Suppl. 1):74. (Abstr.)

Jeroch, H. 1994. The present knowledge about phytase application in poultry feeding. Arch. Geflugelkd. 58:1–7.

Keshavarz, K. 2000. Nonphytate phosphorus requirement of laying hens with and without phytase on a phase feeding program. Poult. Sci. 79:748–763.

Keshavarz, K. 2003a. Effects of continuous feeding of low-phosphorus diets with and without phytase during the growing and laying periods on performance of two strains of leghorns. Poult. Sci. 82:1444–1456.

Keshavarz, K. 2003b. The effect of different levels of nonphytate phosphorus with and without phytase on the performance of four strains of laying hens. Poult. Sci. 82:71–91.

Keshavarz, K., and R. E. Austic. 2004. The use of low-protein, low-phosphorus, amino acid and phytase-supplemented diets on laying hen performance and nitrogen and phosphorus excretion. Poult. Sci. 83:75–83.

Kim, S. H., W. J. Lee, S. J. Lee, D. J. Yu, S. Y. Park, B. S. Kang, J. C. Na, and K. S. Ryu. 2001. Effects of dietary supplemental microbial phytase and nonphytate phosphorus on performance, nutrient digestibility and egg quality of laying hens. Poult. Sci. 80(Suppl. 1):478. (Abstr.)

Kornegay, E. T. 2001. Digestion of phosphorus and other nutrients: the role of phytases and factors influencing their activity. Pages 237–271 in Enzymes in Farm Animal Nutrition. M. R. Bedford and G. G. Partridge, ed. CABI Publishing, Wallingford, UK.

Lei, X. G., P. K. Ku, E. R. Miller, M. T. Yokoyama, and D. E. Ullrey. 1994. Calcium level affects the efficacy of supplemental microbial phytase in corn-soybean meal diets of weanling pigs. J. Anim. Sci. 72:139–143.

Lim, H. S., H. Namkung, and I. K. Paik. 2003. Effects of phytase supplementation on the performance, egg quality, and phosphorus excretion of laying hens fed different levels of dietary calcium and nonphytate phosphorus. Poult. Sci. 82:92–99.

Muji, S., M. A. Kamberi, A. Gagic, G. M. Pesti, and R. I. Bakalli. 2002. The response of laying hens to phytase added to corn-soybean meal based diets containing two levels of crude protein. Poult. Sci. 81(Suppl. 1):141. (Abstr.)

National Research Council. 1994. Nutrient requirements of poultry. 9th rev. ed. National Academy Press, Washington, DC.

Naumann, K., and R. Bassler. 1997. Die Chemische Untersuchung von Futtermitteln. Methodenbuch, Bd. III. Verlag, Neumann-Neudam, Germany.

Nelson, T. S. 1967. The utilization of phytate phosphorus by poultry. A review. Poult. Sci. 46:862–869.

Nelson, T. S., L. W. Ferrara, and N. L. Storer. 1968. Phytate phosphorus content of feed ingredients derived from plants. Poult. Sci. 47:1372–1374.

Pahle, T., R. Köhler, I. Halle, H. Jeroch, and G. Gebhardt. 1983. Methodische Untersuchungen zur Bestimmung der Verdaulichkeit des Rohproteins beim Hhnergeflügel. Arch. Anim. Nutr. 33:363–370.

Pahle, T., R. Köhler, I. Halle, H. Jeroch, and G. Gebhardt. 1985. Die Bestimmung der Rohproteinverdaulichkeit beim Hühnergeflügel mit der α-NH_2-N-Methode. Arch. Anim. Nutr. 35:82–87.

Pan, C. F., F. A. Igbasan, W. Guenter, and R. R. Marquardt. 1998. The effects of enzyme and inorganic phosphorus supplements in wheat- and rye-based diets on laying hen performance, energy and phosphorus availability. Poult. Sci. 77:83–89.

Peter, W., R. Zachmann, and H. Jeroch. 1992. Zum Phytaseeinsatz bei Legehennen. Pages 78–83 in Internationale Tagung Schweine- und Geflügelernährung, Halle, Germany.

Punna, S., and D. A. Roland, Sr. 1999. Influence of supplemental microbial phytase on first cycle laying hens fed phosphorus-deficient diets from day one of age. Poult. Sci. 78:1407–1411.

Rama Rao, S. V., V. Ravindra Reddy, and V. Ramasubba Reddy. 1999. Enhancement of phytate phosphorus availability in the diets of commercial broilers and layers. Anim. Feed Sci. Technol. 79:211–222.

Scheuermann, S. E., H.-J. Lantzsch, and K. H. Menke. 1988. In vitro und in vivo Untersuchungen zur Hydrolyse von Phytat. I. Lslichkeit von Phytat. J. Anim. Physiol. A Anim. Nutr. 60:55–63.

Scott, T. A., R. Kampen, and F. G. Silversides. 1999a. The effect of phosphorus, phytase enzyme and calcium on performance of layers fed corn-based diets. Poult. Sci. 78:1742–1749.

Scott, T. A., R. Kampen, and F. G. Silversides. 1999b. The effect of phosphorus, phytase enzyme and calcium on performance of layers fed wheat-based diets. Can. J. Anim. Sci. 80:183–190.

Sell, J. L., S. E. Scheideler, and B. E. Rahn. 1987. Influence of different phosphorus phase-feeding programs and dietary calcium level on performance and body phosphorus of laying hens. Poult. Sci. 66:1524–1529.

Shan, A. S., L. J. Wang, J. C. Song, A. Wang, J. Du, Q. Y. Xu, and Z. Y. Zhang. 2002. Performance and nutrient utilization of layers fed diet supplemented with microbial phytase and cellulase. Poult. Sci. 80(Suppl. 1):138. (Abstr.) Simons, P. C. M., H. A. J. Versteegh, A. W. Jongbloed, P. A. Kemme, P. Slump, K. D. Bos, M. G. E. Wolters, R. F. Beudeker, and G. J. Verschoor. 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Br. J. Nutr. 64:525–540.

Snow, J. L., D. H. Baker, and C. M. Parsons. 2004. Phytase, citric acid, and 1 α-hydroxycholecalciferol improve phytate phosphorus utilization in chicks fed a corn-soybean meal diet. Poult. Sci. 83:1187–1192.

Snow, J. L., W. M. Douglas, A. B. Batal, M. E. Persia, P. E. Biggs, and C. M. Parsons. 2003a. Efficacy of high available phosphorus corn in laying hen diets. Poult. Sc. 82:1037–1041.

Snow, J. L., M. W. Douglas, and C. M. Parsons. 2003b. Phytase effects on amino acid digestibility in molted laying hens. Poult. Sci. 82:474–477.

Snow, J. L., A. Rafacz, P. L. Utterback, C. W. Utterback, R. W. Leeper, and C. M. Parsons. 2005. Hy-line W-36 and hy-line W-98 laying hens respond similarly to dietary phosphorus levels. Poult. Sci. 84:757–763.

Summers, J. D. 1995. Reduced dietary phosphorus levels for layers. Poult. Sci. 74:1977–1983.

Titus, H. W., A. L. Mehring, D. Johnson, L. L. Nesbitt, and T. Tomas. 1959. An evaluation of M.C.F. (Micro-Cel-Fat), a new type of fat product. Poult. Sci. 38:1114–1119.

Van der Klis, J. D., H. A. J. Versteegh, P. C. M. Simons, and A. K. Kies. 1997. The efficacy of phytase in corn-soybean meal-based diets for laying hens. Poult. Sci. 76:1535–1542.

Vogt, H. 1992. Dietary phosphorus levels for cage hens. Arch. Geflgelkd. 56:264–270.