JPSA
Japan Poultry Science Association

http://www.jstage.jst.go.jp/browse/jpsa
doi:10.2141/jpsa.2023019

A Novel Bacterial 6-Phytase Improves Productive Performance, Precaecal Digestibility of Phosphorus, and Bone Mineralization in Laying Hens Fed a Corn-Soybean Meal Diet Low in Calcium and Available Phosphorus

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Exogenous phytases are commonly added to low-phosphorus and low-calcium diets to improve P availability and reduce P excretion by poultry. This study investigated the effect of supplementation with a novel bacterial 6-phytase on egg production, egg quality, bone mineralization, and precaecal digestibility of P in laying hens fed corn-soybean meal-based diets. A total of 576 Hy-Line brown laying hens were used in a completely randomized block design at 25–45 weeks of age (woa). The three treatments included a positive control (PC) adequate-nutrient diet with 2840 kcal metabolizable energy/kg, 0.77% digestible lysine, 3.5% Ca, and 0.30% available P (avP); a negative control (NC) diet with 0.16% points less Ca and avP; and an NC diet supplemented with a novel bacterial 6-phytase at 300 phytase units/kg diet. Hen performance and the percentage of damaged eggs were measured every 4 weeks. Body weight, precaecal digestibility of P, and bone parameters at 45 woa were also measured. The reduction in avP and Ca in the NC diet did not compromise performance or egg quality. However, it decreased ($P < 0.001$) body weight, tibial dry matter, tibial ash and P content, and precaecal digestibility of P. Importantly, all these parameters were significantly improved ($P < 0.001$) and essentially restored to the levels measured in PC diet-fed hens upon supplementation with phytase. In summary, the present study demonstrates that the new bacterial 6-phytase could effectively counteract the negative effects of P and Ca deficiencies on body weight, bone mineralization, and P availability, thereby supporting high productivity without compromising the welfare of laying hens.

Key words: Keywords:available phosphorus, calcium, digestibility, laying hens, mineralization, phytase

J. Poult. Sci., 60: jpsa.2023019, 2023

Introduction

Phosphorus and calcium are essential nutrients for the growth and development of monogastric animals. Laying hens require P to replace tissue metabolites, such as nucleotides and phospholipids, as well as to maintain skeletal integrity and egg production. Hence, improving the utilization of P derived from plant-based feed ingredients could reduce the requirement for Ca phosphates by monogastric animals. In corn and soybean meal, P is stored primarily in the form of phytic acid (myo-inositol 1,2,3,4,5,6-hexakisphosphate), also known as phytate or phytin[1,2]. Phytate requires hydrolyzing enzymes to make its P available to animals.

Thus, exogenous phytases are commonly used as feed additives for non-ruminant nutrition. In broilers, the efficacy of phytase in laying hens depends on dietary nutrient content, including Ca and P levels, source and amount of phytate, strain and age of hens, and phytase dosage[3–6]. Taylor et al.[7] demonstrated that 300 phytase units (FTU)/kg diet was sufficient to improve egg production and egg mass in laying hens fed a diet containing only 0.17% available P (avP) between 22 and 46 weeks of age (woa).

Recently, a new biosynthetic bacterial 6-phytase from *Trichoderma reesei* was developed, and its efficacy has been demon-

Received: May 24, 2023, Accepted: July 18, 2023
Available online: August 5, 2023
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strated in broilers[8]. The phytase functions at pH 2.5–6.5, has high affinity for phytic acid and other myo-inositol phosphate esters, and displays high intrinsic thermostability. In broilers, phytase addition to a corn-soybean meal-based diet reduced Ca and avP, while significantly improving the average daily gain, feed conversion ratio (FCR), and precaecal digestibility of P[9]. The aim of the present study was to investigate the effect of reducing avP and Ca through supplementation with 300 FTU/kg diet on productive performance, egg quality, bone mineralization, and precaecal digestibility of P in laying hens fed corn-soybean meal-based diets between 25 and 45 woa.

Materials and Methods

The study was conducted on an experimental farm (Mas Bové; Ctra. Reus-El Morell km. 3.8, Constantí, Spain). All experimental procedures were approved by IRTA (Institute of Agrifood Research and Technology)'s Ethics Committee for Animal Experimentation.

Birds, experimental design, and diets

A total of 576 Hy-Line brown laying hens at 16 woa were obtained from a commercial hatchery (Aviagen Terralta S. L., Vinallop, Spain) and housed in 72 cages with 8 hens per enriched cage (0.73 m^2 with one feeder and two nipple drinkers) located in an environmentally controlled facility. All birds were fed a common diet until 25 woa, after which they were shifted to the experimental diets. Birds were randomly assigned to three treatment groups based on laying rate and cage location, resulting in 24 replicates per treatment. The dietary treatment groups consisted of a positive control (PC) diet with 0.30% avP and 3.50% Ca, a negative control (NC) diet with 0.14% avP and 3.34% Ca, and an NC diet supplemented with a novel bacterial 6-phytase (Rovabio PhyPlus, Adisseo France SAS, France) at 300 FTU/kg diet (PHY). The composition and characteristics of the basal diet are listed in **Table 1**. In the last batch of feed, TiO_2 was included at 5 g/kg in all diets as to determine the exact digestibility of P. Feed and water were provided *ad libitum*, and all diets were presented in mash form. Throughout the experiment, birds were routinely monitored by a veterinarian for visual evidence of clinical signs, and animals showing health deficiencies or retarded growth were adequately treated.

Production performance, egg quality parameters, and sampling

Total egg production per replicate was measured and the eggs were weighed every second day during working days. Average hen-day production, egg weight, egg mass, average daily feed intake, and FCR (g feed/g eggs) were calculated every 4 weeks. Damaged eggs presenting broken, faulty (absent or misshaped), and dirty shells were recorded, and the damaged egg ratio was also calculated on a 4-week basis. Laying hens were weighed at the beginning and end of the experimental period. During the last week of the study, all eggs per replicate were laid on two consecutive days (12 replicate cages per treatment) and weighed. Liquid eggs were separated and pooled per replicate, and an aliquot (approximately 50 mL) was frozen, freeze-dried, and stored until analysis. At the end of the study, laying hens (12 replicates of eight birds per treatment) were euthanized, the intestines were removed, and the distal half of the ileum was gently flushed to remove its contents. The ileum was defined as the portion of the small intestine extending from Meckel's diverticulum to 2 cm anterior to the ileocecal-colonic junction[10]. Ileal digesta were pooled per replicate cage, resulting in 12 composite samples per dietary treatment, and stored at -18°C until further analysis.

Table 1 **Composition and characteristics of experimental diets.**

	Experimental diets	
	PC	NC
Ingredients (%)		
Corn	62.98	64.21
Soybean meal 48%	24.85	24.77
Calcium carbonate	7.83	8.02
Soybean oil	2.21	1.79
Dicalcium phosphate	1.13	0.21
Mineral and vitamin premix[a]	0.40	0.40
Sodium chloride	0.38	0.39
DL-methionine	0.17	0.17
Noxyfeed[b]	0.02	0.02
Capsantal FS-20[c]	0.03	0.03
Calculated nutrients (%)		
ME, kcal/kg	2840	2840
Crude protein	16.60	16.60
Crude fat	4.76	4.38
Crude fiber	2.31	2.33
Ash	11.76	11.06
Dig. lysine	0.77	0.77
Dig. threonine	0.56	0.56
Dig. methionine	0.41	0.41
Dig. Met+Cys	0.66	0.66
Dig. tryptophan	0.15	0.15
Dig. isoleucine	0.66	0.65
Dig. valine	0.73	0.73
Dig. leucine	1.35	1.36
Dig. histidine	0.41	0.41
Dig. arginine	1.01	1.01
Calcium	3.50	3.34
Total P	0.51	0.36
AvP[d]	0.30	0.14
Phytate phosphorus	0.21	0.22

[a]Premix provides per kg feed: vitamin A, 10,000 IU; vitamin D3, 2750 IU; vitamin E, 22.5 mg; vitamin B1, 1.8 mg; vitamin B2, 4 mg; vitamin B6, 3.25 mg; vitamin B12, 40 µg; vitamin K3, 2 mg; calcium D-pantothenate, 8 mg; nicotinic acid, 45 mg; folic acid, 0.66 mg; biotin, 66 µg; Fe, 60 mg; I, 1.85 mg; Cu, 7 mg; Mn, 70 mg; Zn, 60 mg; Se, 0.3 mg.
[b]BHT+ propyl gallate (56%) and citric acid (14%) were obtained from ITPSA (Barcelona, Spain).
[c]Provides 20 g of red natural pigments rich in capsanthin.
[d]AvP = non-phytate P.

Also, the complete left leg (including femur, tibia, metatarsus, and foot) from one laying hen per replicate cage (12 legs per treatment) was removed and frozen at -18°C.

Chemical analysis

Samples from all experimental diets were analyzed for dry matter, crude protein, ether extract, ash, gross energy (GE), total P, phytate-P, Ca, and phytase activity. Additionally, freeze-dried ileal digesta samples were analyzed for total P and Ti content. Dry matter, crude protein, ether extract, and ash were determined according to AOAC methods (2000)[11] numbers 925.09, 968.06, 920.39, and 942.05, respectively. The GE of diet samples was determined using an adiabatic bomb calorimeter (DIN 51900, IKA C-400; Janke & Kunkel KG., Staufen I. Br., Germany). Total P was analyzed according to method 965.17[11], and Ca was analyzed using atomic emission spectrophotometry according to method 968.08[11]. Phytate-P in experimental diets was determined as described by Haug and Lantzsch[12]. TiO_2 content in freeze-dried ileal digesta was determined based on the protocol by Short et al.[13]. Phytase activity in experimental diets was analyzed according to ISO standard methodology[14]. One unit of phytase is defined as the amount of enzyme that liberates one micromole of inorganic orthophosphate from phytic acid per minute at pH 5.5 and 37°C. Frozen legs were thawed at room temperature before all adhering tissues were manually excised using laboratory scalpels. Clean tibias were obtained and weighed to calculate the tibial fresh weight. The tibias were then dried overnight at 103 ± 2°C, cooled in a dissector, weighed again to calculate dry matter, and placed in a muffle furnace at 550°C for 72 h to obtain ash. Tibia P content was then measured using the same method described for the feed and digesta samples[11].

Calculations and statistical analysis

The precaecal digestibility of P was calculated using TiO_2 as the inert marker:

Precaecal digestibility of P = $(1- [(Ti_{diet}/Ti_{ileal\ digesta}) \times (P_{ileal\ digesta}/P_{diet})]) \times 100$,

where, Ti_{diet} is the TiO_2 concentration in the diet, $Ti_{ileal\ digesta}$ is the TiO_2 concentration in the ileal digesta, $P_{ileal\ digesta}$ is the P concentration in the ileal digesta, and P_{diet} is the P concentration in the diet.

All data were analyzed using GLM procedures in SAS software (SAS version 9.1.3 Cray; SAS Institute, Inc., Cary, NC, USA), including treatment (n = 3) and block (n = 24) as fixed effects. Replications (n = 24 per treatment) were used as an experimental unit. Differences between treatments were detected using Tukey's multiple range test, and the results are presented as means and pooled standard error of the mean (SEM). Statistical significance was set at $P < 0.05$.

Results and Discussion

The analyzed values of dietary nutrients and phytase activity in the experimental diets of the four feeding batches are reported in **Table 2.** The measured crude protein, ether extract, ash, total P, and phytate-P were generally in agreement with the calculated values from **Table 1**. Phytase activity in the experimental diets supplemented with phytase was 319, 337, 297, and 321 FTU/kg in feed batches 1, 2, 3, and 4, respectively.

The effects of dietary treatments on productive performance parameters and body weight are summarized in **Table 3**. Productive performance did not differ significantly across dietary treatments. This result is in accordance with that of Jing et al.[15],

Table 2 **Analyzed nutrients and phytase activity of experimental diets.**

	Dry matter (%)	Crude protein (%)	Ether extract (%)	Ash (%)	GE, (kcal/kg)	Total P (%)	Phytate-P (%)	Ca (%)	Phytase activity (FTU/kg diet)
Batch 1									
PC	90.4	16.9	4.2	12.9	3666	0.46	0.19	4.04	67
NC	90.4	16.6	4.0	12.4	3670	0.31	0.19	3.56	68
PHY	90.2	16.9	3.9	12.1	3683	0.31	0.19	3.54	319
Batch 2									
PC	89.0	15.4	4.4	11.8	3667	0.45	0.17	3.57	82
NC	88.9	15.5	4.2	11.3	3692	0.35	0.18	3.25	82
PHY	88.7	15.7	3.9	10.5	3697	0.32	0.17	3.08	337
Batch 3									
PC	89.3	17.1	4.8	12.6	3648	0.50	0.19	3.64	80
NC	90.0	16.8	4.6	12.3	3673	0.32	0.18	3.38	86
PHY	89.3	17.0	4.6	12.2	3669	0.35	0.17	3.27	297
Batch 4									
PC	89.4	16.0	4.8	12.6	3683	0.45	0.19	3.82	72
NC	89.5	15.4	4.2	13.5	3593	0.29	0.18	4.18	62
PHY	89.4	15.7	4.3	12.0	3698	0.31	0.17	3.61	321

Table 3 **Effect of dietary avP, Ca, and phytase on productive performance, feed intake, and egg quality by laying hens between 25 and 45 woa.**

	Treatment			SEM	*P*
	PC	NC	PHY		
25–29 woa					
Egg production (%)	95.0	94.4	94.3	4.17	0.85
Egg weight (g)	59.1	59.2	58.9	1.19	0.64
Egg mass (g/day)	56.1	55.9	55.6	2.41	0.70
Feed intake (g/day)	107.6	105.6	105.7	3.40	0.08
FCR (g feed/g egg)	1.922	1.895	1.906	0.11	0.71
29–33 woa					
Egg production (%)	93.3	92.1	92.2	4.12	0.54
Egg weight (g)	58.8	58.9	58.7	1.20	0.85
Egg mass (g/day)	54.9	54.2	54.1	2.44	0.51
Feed intake (g/day)	107.9	105.6	107.0	4.08	0.14
FCR (g feed/g egg)	1.969	1.951	1.980	0.10	0.61
33–37 woa					
Egg production (%)	92.6	91.5	90.9	4.06	0.34
Egg weight (g)	59.7	59.9	59.5	1.31	0.57
Egg mass (g/day)	55.2	54.8	54.1	2.51	0.29
Feed intake (g/day)	114.2	112.5	113.2	4.10	0.36
FCR (g feed/g egg)	2.070	2.055	2.096	0.10	0.37
37–41 woa					
Egg production (%)	95.1	94.0	94.4	4.77	0.73
Egg weight (g)	62.3	62.9	62.7	1.59	0.46
Egg mass (g/day)	59.2	59.1	59.2	3.48	0.98
Feed intake (g/day)	118.4	117.8	118.4	5.72	0.91
FCR (g feed/g egg)	2.003	1.998	2.003	0.11	0.99
41–45 woa					
Egg production (%)	93.1	93.6	92.8	5.36	0.88
Egg weight (g)	64.4	64.7	64.6	1.48	0.86
Egg mass (g/day)	60.0	60.5	60.0	3.54	0.82
Feed intake (g/day)	118.2	116.7	117.5	5.03	0.61
FCR (g feed/g egg)	1.980	1.931	1.967	0.13	0.42
Overall (25–45 woa)					
Egg production (%)	93.8	93.1	92.9	3.42	0.64
Egg weight (g)	60.9	61.1	60.9	1.24	0.74
Egg mass (g/day)	57.1	56.9	56.6	2.17	0.71
Feed intake (g/day)	113.2	111.6	112.3	3.33	0.25
FCR (g feed/g egg)	1.985	1.964	1.987	0.08	0.56

who showed that laying rate, egg mass, feed intake, and FCR were not significantly different among hens fed diets with avP varying from 0.15% to 0.45% between 22 and 34 woa. Zhai et al.[16] reported that lower egg production due to P reduction depended on the severity and duration of P shortage. In the present study, phytase addition to a diet containing 0.14% avP and 3.34% Ca had no effect on laying hen performance compared to PC and NC diets. Previous studies have also reported that phytase addition did not influence the production performance of laying hens[17–19]. Rodehutscord et al.[20] suggested that, depending on the dose, phytase supplementation reduced avP concentrations to near complete omission of mineral P (e.g., monocalcium or dicalcium phosphate) in laying diets. Here, a significant decrease in body weight was observed in hens fed the NC diet relative to a PC diet (**Fig. 1**). This result may indicate that birds fed a diet reduced in Ca and avP maintain productive parameters similar to those of birds fed a PC diet, probably through mobilization of reserves. In the present study, no significant effect of

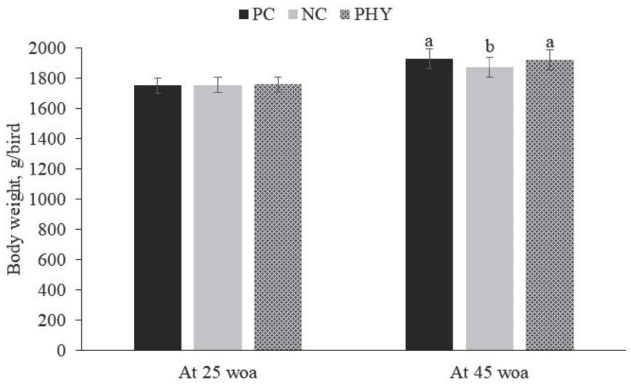

Fig. 1. **Effect of dietary avP, Ca, and phytase on body weight in laying hens at 25 and 45 woa.** Values correspond to least square means (n = 24 replicates per treatment) and vertical bars to the SEM.

dietary treatment was observed on the percentages of damaged (i.e., broken, faulty or dirty) eggs during the entire experimental period (**Table 4**), which may indicate that 0.14% avP and 3.34% Ca were sufficient to maintain the same egg quality as in birds fed a PC diet containing 0.30% avP and 3.50% Ca during 25–45 woa. This result may explain the absence of any effect of phytase supplementation on the percentage of damaged eggs ($P > 0.05$). Tibial ash has long been proposed as the most sensitive parameter for evaluating P nutrient status and the effects of dietary avP and Ca levels in poultry. Critically, phosphate deficiency may lead to bone defects. In the present study, Ca and P depletion decreased ($P < 0.05$) tibial dry matter, ash content, and P content compared to laying hens fed a nutrient-adequate diet (**Table 5**). This result confirms that birds fed the NC diet can maintain their performance by mobilizing minerals from skeletal tissue[21,22]. As shown in **Table 5**, phytase supplementation increased tibial dry matter, ash content, and P content by 7.9, 11.9%, and 11.4%,

Table 4 Effect of dietary avP, Ca, and phytase on egg quality by laying hens fed corn-soybean meal-based diets between 25 and 45 woa.

	Treatment			SEM	*P*
	PC	NC	PHY		
25–29 woa					
Broken eggs (%)	0.57	0.61	0.90	0.56	0.10
Faulty eggs (%)	0.16[a]	0.10[ab]	0.00[b]	0.23	$P < 0.05$
Dirty eggs (%)	0.07	0.08	0.04	0.19	0.72
29–33 woa					
Broken eggs (%)	1.12	1.44	0.96	0.94	0.21
Faulty eggs (%)	0.10	0.12	0.12	0.23	0.93
Dirty eggs (%)	0.10	0.02	0.06	0.16	0.28
33–37 woa					
Broken eggs (%)	0.40[b]	0.77[a]	0.60[ab]	0.51	$P < 0.05$
Faulty eggs (%)	0.08	0.02	0.05	0.17	0.48
Dirty eggs (%)	0.04	0.04	0.00	0.12	0.38
37–41 woa					
Broken eggs (%)	0.50	0.55	0.34	0.57	0.49
Faulty eggs (%)	0.10	0.02	0.02	0.17	0.17
Dirty eggs (%)	0.42	0.28	0.32	0.51	0.65
41–45 woa					
Broken eggs (%)	0.33	0.64	0.57	0.56	0.11
Faulty eggs (%)	0.06	0.15	0.06	0.20	0.32
Dirty eggs (%)	0.18	0.04	0.11	0.31	0.30
Overall (25–45 woa)					
Broken eggs (%)	0.59	0.80	0.68	0.36	0.18
Faulty eggs (%)	0.10	0.08	0.05	0.10	0.24
Dirty eggs (%)	0.16	0.09	0.11	0.14	0.16

[a,b]Within a row, least square means (n = 24 replicates per treatment) without a common superscript letter differ significantly ($P < 0.05$).

Table 5 **Effect of dietary avP, Ca, and phytase on bone mineralization of laying hens between 25 and 45 woa.**

	Treatment			SEM	*P*
	PC	NC	PHY		
Dry tibia weight (g)	7.10[a]	6.40[b]	7.20[a]	0.64	$P < 0.05$
Tibia dry matter (%)	66.8[a]	58.80[b]	66.70[a]	4.11	$P < 0.001$
Ash in dry tibia (%)	41.1	43.6	43.3	3.33	0.18
P in dry tibia (%)	6.40	6.90	6.80	0.55	0.14
Tibia ash content (g)	2.91[ab]	2.77[b]	3.10[a]	0.298	$P < 0.05$
Tibia P content (g)	0.45[ab]	0.44[b]	0.49[a]	0.046	0.05

[a,b]Within a row, least square means (n = 12 replicates per treatment) without a common superscript letter differ significantly ($P < 0.05$).

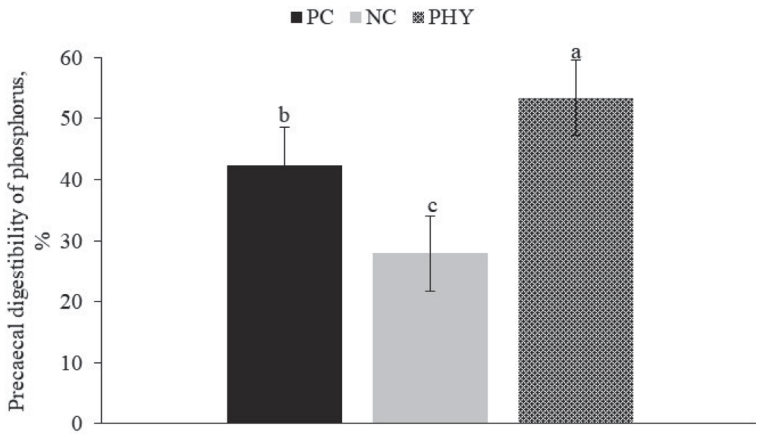

Fig. 2. **Effect of dietary avP, Ca, and phytase on precaecal digestibility of P in laying hens at 45 woa.** Values correspond to least square means (n = 12 replicates per treatment) and vertical bars to the SEM.

respectively, in laying hens fed the NC diet. These parameters were not statistically different from those of birds fed an adequate-nutrient diet. Moreover, the results are in accordance with those of Hughes et al.[23], who observed an increase in bone ash following phytase supplementation at 200 and 400 FTU/kg diet in laying hens at 61 woa. Because the NC diet used in the present study was formulated with reduced amounts of Ca and avP, the improvements on bone parameters observed upon phytase addition suggest better release of P and Ca from feed. This finding may also indicate that phytase supplementation is associated with an improved ability of laying hens to hydrolyze phytate, release inorganic P, and limit the interactions between phytate and other nutrients. As shown in **Fig. 2**, birds fed a diet with reduced avP and Ca exhibited lower ($P < 0.001$) precaecal digestibility of P than birds fed the PC diet. This observation relates to less mineral P in the NC diet compared to the PC diet. Liu et al.[24] observed a decrease in the ileal digestibility of P in Hy-Line brown layers fed a diet deficient in avP and Ca between 23 and 28 woa. Here, supplementation with phytase increased ($P < 0.001$) the precaecal digestibility of P by 25.5% relative to the NC diet. Bello and Korver[25] demonstrated that phytase supplementation with 300 FTU/kg diet improved the apparent ileal digestibility of P by 12.8% in a diet containing 0.23% avP and 3.6% Ca. However, Pongmanee et al.[26] observed a significant improvement in the ileal digestibility of P by *Escherichia coli* phytase in laying hens fed a 600 FTU/kg corn-soybean meal diet. The improvement in precaecal digestibility of P observed in the present study can be attributed to the ability of exogenous phytases to dephosphorylate the phospho-ester bonds of phytate and release phytate-bound P for use by the birds[27,28]. Using the same phytase at 300 FTU/kg diet, Hervo et al.[29] reported a significant improvement (21.1%) in phytate-P release in a corn-wheat-soybean meal-based diet fed to laying hens. However, further studies are required to elucidate the mode of action of this novel phytase in phytate degradation within the intestinal tract, as well as potential interactions between phytate and dietary nutrients in laying hens.

In conclusion, a reduction in avP and Ca in the diet did not adversely affect performance or egg quality between 25 and 45 woa; however, it resulted in lower body weight, tibial ash parameters, and precaecal digestibility of P. Phytase addition improved bone parameters and restored the precaecal digestibility of P and body weight of laying hens to the levels found in birds fed a

PC diet. This study demonstrates that addition of the new bacterial 6-phytase at 300 FTU/kg diet compensates for the negative impact of 0.16% lower avP and Ca on body weight, bone mineralization, and P availability, thereby saving on feed costs while maintaining the same productive performance.

Acknowledgments

The present study was supported by ADISSEO France S.A.S (Antony, France). The authors thank the staff of the breeding facilities at IRTA(Spain) for their valuable technical assistance.

Author Contributions

Maamer Jlali: Conceptualization, Methodology, Data analysis, Writing original draft. **Clémentine Hincelin:** Methodology, review & editing, **Maria Francesch:** Methodology, Data analysis, review & editing, **Tania Rougier:** review & editing, **Pierre Cozannet:** review & editing, **Sarper Ozbek:** review & editing, **Marcio Ceccantini:** review & editing, **Baris Yavuz:** review & editing. **Aurélie Preynat:** Methodology, review & editing. **Estelle Devillard:** Review & editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

This research was financially supported by Adisseo, France; Maamer, Clémentine, Tania; Cozannet Pierre; Sarper Ozbek; Marcio Ceccantini; Baris Yavuz; Aurélie Preynat and Estelle Devillard are employees at Adisseo.

References

[1] Eeckhout W, de Paepe M. Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. Anim Feed Sci Technol, **77**: 53e60, 1994. https://doi.org/10.1016/0377-8401(94)90156-2.

[2] Humer E, Schwarz C and Schedle K. Phytate in pig and poultry nutrition. J Anim Physiol Anim Nutr (Berl), **99**: 605–625. 2015. https://doi.org/10.1111/jpn.12258, PMID:25405653

[3] Kornegay ET. Nutritional, environmental and economic considerations for using phytase in pig and poultry diets. Pages 277-299 in Nutrient Management of Food Animals to Enhance and Protect the Environment. E. T. Kornegay, ed. Lewis Publishers, New York. 1996.

[4] Van der Klis JD, Versteegh HA, Simons PC and Kies AK. The efficacy of phytase in corn-soybean meal-based diets for laying hens. Poult Sci, **76**: 1535–1542. 1997. https://doi.org/10.1093/ps/76.11.1535, PMID:9355148

[5] Slominski BA. Recent advances in research on enzymes for poultry diets. Poult Sci, **90**: 2013–2023. 2011. https://doi.org/10.3382/ps.2011-01372, PMID:21844268

[6] Nie W, Yang Y, Yuan J, Wang Z and Guo Y. Effect of dietary nonphytate phosphorus on laying performance and small intestinal epithelial phosphate transporter expression in Dwarf pink-shell laying hens. J Anim Sci Biotechnol, **4**: 34. 2013. http://www.jasbsci.com/content/4/1/34. https://doi.org/10.1186/2049-1891-4-34, PMID:24028402

[7] Taylor AE, Bedford MR, Pace SC and Miller HM. The effects of phytase and xylanase supplementation on performance and egg quality in laying hens. Br Poult Sci, **59**: 554–561. 2018. https://doi.org/10.1080/00071668.2018.1483575, PMID:29855193

[8] Jlali M, Kidd MT, Gracia MI, Francesch M, Cozannet P, Yavuz B and Preynat A. Evaluation of a novel bacterial 6-phytase on growth efficiency of broilers at 35 days of age. 33rd Annual Australian Poultry Science Symposium, 7-9 February, Sydney, Australia. 2022.

[9] Khattak F, Jlali M, Rougier T, Houdijk J, Hincelin C. 2023. Bacterial 6-phytase supplementation can help improve bone mineralization, phosphorus digestibility and growth performance of broilers fed corn-soyabean meal-based diet. Br. Poult. Abstracts. 19, **1**:17. https://doi.org/10.1080/17466202.2023.2215131.

[10] Rodehutscord M, Adeola O, Angel R, Bikker P, Delezie E, Dozier WA III, Umar Faruk M, Francesch M, Kwakernaak C, Narcy A, Nyachoti CM, Olukosi OA, Preynat A, Renouf B, del Barrio AS, Schedle K, Siegert W, Steenfeldt S, van Krimpen MM, Waititu SM and Witzig M. Results of an international phosphorus digestibility ring test with broiler chickens. Poult Sci, **96**: 1679–1687. 2017. https://doi.org/10.3382/ps/pew426, PMID:27920192

[11] AOAC. Official Methods of Analysis (17th Ed.) Association of Official Analytical Chemists, Arlington, VA, USA, 2000.

[12] Haug W and Lantzsch HJ. Sensitive method for the rapid determination of phytate in cereals and cereal products. J Sci Food Agric, **34**: 1423–1426. 1983. https://doi.org/10.1002/jsfa.2740341217

[13] Short FJ, Gorton P, Wiseman J and Boorman KN. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim Feed Sci Technol, **59**: 215–221. 1996. https://doi.org/10.1016/0377-8401(95)00916-7

[14] ISO Standard 300242. Animal Feeding Stuffs-Determination of phytase Activity; International Organization for Standardization: Geneva, Switzerland, 2009.

[15] Jing M, Zhao S, Rogiewicz A, Slominski BA and House JD. Assessment of the minimal available phosphorus needs of laying hens: implications for phosphorus management strategies. Poult Sci, **97**: 2400–2410. 2018. https://doi.org/10.3382/ps/pey057, PMID:29617962

[16] Zhai HX, Wang JP, Zhang Q, Aureli R, Tschambser A and Faruk MU. Evaluation of the efficacy of a novel phytase in short-term digestibility and long-term egg production studies with laying hens. Poult Sci, **101**: 101894. 2022. https://doi.org/10.1016/j.psj.2022.101894, PMID:35508079

[17] Boling SD, Douglas MW, Johnson ML, Wang X, Parsons CM, Koelkebeck KW and Zimmerman RA. The effects of dietary available phosphorus levels and phytase on performance of young and older laying hens. Poult Sci, **79**: 224–230. 2000. https://doi.org/10.1093/ps/79.2.224, PMID:10735751

[18] Keshavarz K. Nonphytate phosphorus requirement of laying hens with and without phytase on a phase feeding program. Poult Sci, **79**: 748–763. 2000. https://doi.org/10.1093/ps/79.5.748, PMID:10824965

[19] Jing M, Zhao S, Rogiewicz A, Slominski BA and House JD. Effects of phytase supplementation on production performance, egg and bone quality, plasma biochemistry and mineral excretion of layers fed varying levels of phosphorus. Animal, **15**: 100010. 2021. https://doi.org/10.1016/j.animal.2020.100010, PMID:33515998

[20] Rodehutscord M, Sommerfeld V, Angel CR and Korver DR. Minimum phosphorus requirements for laying hen feed formulations. Poult Sci, **102**: 102344. 2023. https://doi.org/10.1016/j.psj.2022.102344, PMID:36476680

[21] Li X, Zhang D and Bryden WL. Calcium and phosphorus metabolism and nutrition of poultry: are current diets formulated in excess? Anim Prod Sci, **57**: 2304–2310. 2017. https://doi.org/10.1071/AN17389

[22] Sinclair-Black M, Garcia RA and Ellestad LE. Physiological regulation of calcium and phosphorus utilization in laying hens. Front Physiol, **14**: 1112499. 2023. https://doi.org/10.3389/fphys.2023.1112499, PMID:36824471

[23] Hughes AL, Dahiya JP, Wyatt CL and Classen HL. Effect of Quantum phytase on nutrient digestibility and bone ash in White Leghorn laying hens fed corn-soybean meal-based diets. Poult Sci, **88**: 1191–1198. 2009. https://doi.org/10.3382/ps.2008-00233, PMID:19439630

[24] Liu N, Liu GH, Li FD, Sands JS, Zhang S, Zheng AJ and Ru YJ. Efficacy of phytases on egg production and nutrient digestibility in layers fed reduced phosphorus diets. Poult Sci, **86**: 2337–2342. 2007. https://doi.org/10.3382/ps.2007-00079, PMID:17954583

[25] Bello A and Korver DR. Long-term effects of *Buttiauxella* sp. phytase on performance, eggshell quality, apparent ileal Ca and P digestibility, and bone properties of white egg layers. Poult Sci, **98**: 4848–4859. 2019. https://doi.org/10.3382/ps/pez220, PMID:31032860

[26] Pongmanee K, Kühn I and Korver DR. Effects of phytase supplementation on eggshell and bone quality, and phosphorus and calcium digestibility in laying hens from 25 to 37 wk of age. Poult Sci, **99**: 2595–2607. 2020. https://doi.org/10.1016/j.psj.2019.12.051, PMID:32359595

[27] Bougouin A, Appuhamy JADRN, Kebreab E, Dijkstra J, Kwakkel RP and France J. Effects of phytase supplementation on phosphorus retention in broilers and layers: A meta-analysis. Poult Sci, **93**: 1981–1992. 2014. https://doi.org/10.3382/ps.2013-03820, PMID:24902701

[28] Babatunde OO, Bello A, Dersjant-Li Y and Adeola O. Evaluation of the responses of broiler chickens to varying concentrations of phytate phosphorus and phytase. II. Grower phase (day 12–23 post hatching). Poult Sci, **101**: 101616. 2022. https://doi.org/10.1016/j.psj.2021.101616, PMID:34991035

[29] Hervo F, Létourneau-Montminy MP, Même N, Méda B, Duclos MJ and Narcy A. Effect of phytase and limestone particle size on mineral digestibility, performance, eggshell quality, and bone mineralization in laying hens. Poult Sci, **102**: 102613. 2023. https://doi.org/10.1016/j.psj.2023.102613, PMID:37001319