

Effects of a nanostructure of phytic acid absorber on the performance and protein digestibility in laying hens

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Abstract A ten-week experiment was conducted to study the effect of a nanostructure of phytic acid absorber (NPAA) on the performance and protein digestibility in laying hens. A total of 100 Bovans laying hens were randomly assigned to 5 treatments, with 4 replicates, 5 hens each. The experiment was a completely randomized design with 5 levels of NPAA; including zero (T1, as a basal diet (BD)); T2 (BD+ 0.5% NPAA); T3 (BD + 0.25% NPAA); T4 (BD + 0.0625% NPAA) and T5 (BD + 0.03125% NPAA of total P) in diet. Water and feed were offered *ad libitum*. Inclusion of NPAA to the diet had significant effects ($P<0.05$) on egg production (%) at 25 weeks of age and over the whole experiment. Dietary treatments significantly affected the egg weight ($P<0.001$) at 29th weeks of age, and hens receiving T5 produced heavier eggs compared to T1, T2 and T3 treatments. NPAA also affected ($P<0.05$) the egg mass and feed conversion ratio (FCR) at weeks 29 and 30. Egg shell weight was significantly ($P<0.05$) affected at 29th week of age; however, dietary treatments had no effect on the relative egg shell thickness. Egg shell phosphorus and calcium contents were increased by 14.6 and 14.2% respectively at 29th week of age in T5 compared to the control. At 33rd week of age, hens in T4 group had significantly higher protein digestibility than T1 birds ($P<0.05$). It was concluded that inclusion of NPAA to the diet at the level of 0.0625%, may reduce the anti-nutritional effects of phytic acid and improve production performance and protein digestibility in laying hens.

Keywords: egg production, phytate phosphorus, egg shell, digestibility, Bovans

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Introduction

Feed accounts for about 75 percent of production cost for laying hens. In conventional corn-soybean meal based diets for birds, additional sources of phosphorus (P) must be supplied to fulfil the maintenance and production requirements. This supplementation is typically performed using inorganic phosphates with high biological availability, such as mono-calcium and di-calcium phosphate, but the costs are high (Casartelli et al., 2005). Also, corn contains phytic acid (an anti-nutritional substance) that decreases availability of minerals especially P.

Approximately two-thirds of P in plant feedstuffs is present as phytic acid in the form of myo-inositol-hexa phosphates (Cromwell, 1980). Phytate P has low P-bio-availability, which leads to the need for an inorganic P source to meet the P requirement of laying hens (Lim et al., 2003; Peeler, 1972). Peter (1992) reported that laying hens fed a low non-phytate P (NPP) diet containing

phytase had significantly higher egg production, egg weight and feed consumption than hens consuming the low NPP diet without supplemental phytase. Phytase supplementation decreased the percentage of broken and soft-shell eggs. Phytase increased availability of dry matter, fiber, and P (Lim et al., 2003). Negative influences of phytic acid on the solubility of proteins and pepsin activity can be expected because of the ionic binding between basic phosphate groups of phytic acid and protonized amino acids, such as Lys, His, and Arg residues (Okubo et al., 1976). Phytic acid can form insoluble salts with Ca, Mg, Fe, Zn, Cu, and Mn (Oberleas, 1973; Morris, 1986; Bedford and Schulze, 1998; Liu et al., 1998). When phytic acid is hydrolyzed by microbial phytase, it may release all phytate-bound minerals such as Ca, P, Mg, Cu, Zn, Fe and K (Sebastian et al., 1996). Phytase also improved N absorption in laying

hens (Vander Klis and Versteegh, 1991) and improved nitrogen and amino acid digestibility in broilers (Ravindran et al., 2001).

Nano processing increases the surface area to volume ratio and, as a result, increases absorption and decreases the need for supplements. However, there are few studies on nanotechnology in animal nutrition. For instance, use of 90 ppm nano-zinc oxide in a broiler diet led to body, breast and thigh muscle enhancement (Ebrahimnezhad et al., 2013). Supplementing the broiler diet with nano-chromium resulted in a decrement of serum cortisol and insulin and increment of immunoglobulins. Also, it increased the performance and carcass parameters of heat-stressed broilers (Ravindran, 2013). It has been reported that use of 0.3 % nano-selenium could improve protein digestibility in broilers (Peng et al., 2009). Therefore, this study was conducted to determine the effect of using a nanostructure of phytic acid absorber (NPAA) in the diet to inactivate this anti-nutritional compound and improve performance and protein digestibility in laying hens.

Materials and methods

The experiment was conducted at a research laying hen house in Kerman, Iran. The protocol of management and

and experimental design was reviewed and approved by the Animal Science Department of the University of Jiroft.

Preparation of NPAA

The NPAA was prepared by a sono-chemical procedure (Murayama et al., 2012). The TEM (Transition electron microscopy) and SEM (Scanning electron microscopy) images (Figures 1 and 2) show that the product was composed of porous nanoparticles. This analyses were used by Fazelirad et al. (2015) to confirm the nano-particle's structure. The product was a burned brown powder and the size of the NPAA was 500 nm. Limestone was used as a carrier to dilute the concentration of NPAA to a suitable level for blending with the diet to achieve the desired levels. The desired amount of NPAA was calculated for each experimental diet and thoroughly mixed before feeding to the hens.

Birds, diets and experimental design

A total of 100 Bovans laying hens were randomly assigned to 5 treatments, with 4 replicates of 5 hens each. The experiment was a completely randomized design with 5 levels of NPAA including zero (T1, as a basal diet

Table 1. Feed ingredients and calculated chemical compositions (%) of the basal diet

	T1	T2	T3	T4	T5
Ingredients (%)					
Corn grain	61.5	61.5	61.5	61.5	61.5
Soya bean meal	26.64	26.64	26.64	26.64	26.64
Vegetable oil	1.29	1.29	1.29	1.29	1.29
Methionine	0.09	0.09	0.09	0.09	0.09
Dicalcium phosphate	1.64	1.64	1.64	1.64	1.64
Calcium carbonate	8.06	8.06	8.06	8.06	8.06
NPAA	0	0.5	0.25	0.0625	0.03125
Vitamin supplement ¹	0.25	0.25	0.25	0.25	0.25
Mineral supplement ²	0.25	0.25	0.25	0.25	0.25
NaCl	0.28	0.28	0.28	0.28	0.28
Sand	0	0	0	0	0
Total	100	100	100	100	100
Calculated nutritive value					
ME (kcal/kg)	2800				
Crude protein (%)	17				
Calcium (%)	3.66				
Phosphorus (%)	0.5				

¹Provided per kg of diet: 8,989,200 mg vitamin A from retinyl acetate; 880,000 µg cholecalciferol; 35,200 mg vitamin K from menadione sodium biosulphite; 1320 mg vitamin B12; 798,336 mg riboflavin; 498,960 mg niacin from nicotinic acid; 323,855.4 mg pantothenic acid from calcium pantothenate; 99,792 mg folic acid; 821,286.4 mg vitamin B6; 58,666.67 mg vitamin E from DL-tocopheryl acetate; 598,400 mg choline; 88,000 mg thiamin from thiamine mononitrate; 2,200 mg of biotin.

²provided per kg of diet: copper, 8.75 mg from copper sulfate, Zn 35 mg from zinc sulfate; iodine, 0.035 mg from organic iodine; Mn, 20 mg from manganese sulfate, iron 45 mg from iron sulfate.

NPAA: Nanostructure of Phytic Acid Absorber.

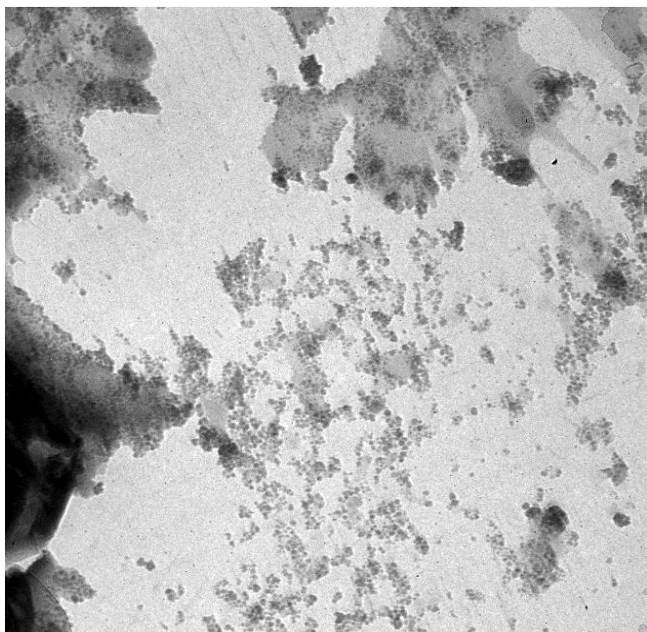


Figure 1. The SEM image of NPAA.

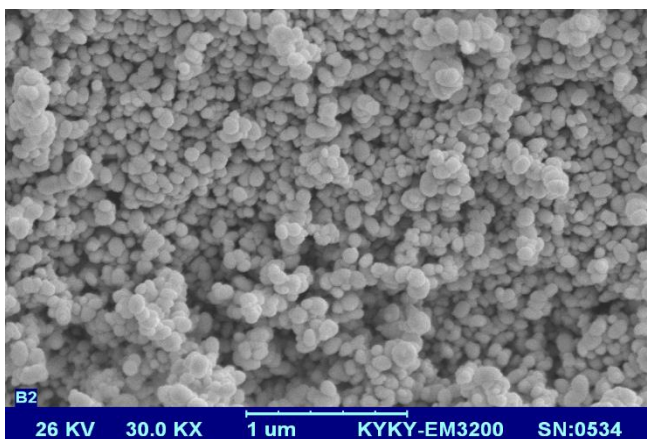


Figure 2. The TEM image of NPAA.

(BD)); T2 (BD+ 0.5% NPAA); T3 (BD + 0.25% NPAA); T4 (BD + 0.0625% NPAA) and T5 (BD + 0.03125% NPAA of total P) in the diet. Birds with similar mean egg production were randomly assigned to 1 of 5 dietary treatments which were fed for 70 days. Hens were reared under the same environmental condition (21°C and 16h light: 8h darkness) from weeks 23 to 33. Laying hens were housed in adjacent steel cages equipped with a nipple drinker, trough and egg collection plate. The diets were presented in mash form and formulated based on the recommendations for Bovans layer hens (Table 1). Feed and water were supplied *ad libitum* during the experiment.

Samples and procedures

Weekly records of egg production, egg weight and feed

consumption were kept throughout the experimental period. Feed conversion ratio (FCR) was expressed as kg of feed consumed per kg of egg produced (Catli et al., 2012). Egg production was calculated from the total number of eggs produced by each replicate in a week divided by the average number of birds (Pelicia et al., 2009). The average egg weight was calculated based on the total weekly egg weight divided by the number of produced eggs for each replicate. Egg mass was calculated by multiplying the egg weight by egg production. Feed intake and egg production were adjusted for the hen mortalities, which were recorded daily. After breaking the eggs, eggshell quality (weight/surface and thickness) was analyzed after each collection time every 14 days, from 27 to 33 weeks of age. Four eggs per replicate (16 eggs per treatment with the nearest weight to average egg weight) were analyzed at 27, 29, 31, and 33 weeks. For determination of egg shell weight/surface, the egg content was first extracted and then the eggshell was washed and air dried for 72 h before being weighed. Eggshell thickness was measured with a micrometer (Model 232, Zonechain Corp., Shanghai, China) as the average thickness of the rounded end, pointed end, and middle of the egg, excluding the inner membrane. At wk 29, sixteen eggshell samples from each treatment were digested by a dry ash-procedure at 600°C for 6 h to obtain eggshell ash, and digested by a wet-ash procedure using per-chloric acid and nitric acid. Phosphorus and calcium concentration were determined colorimetrically (AOAC, 1995).

To determine protein digestibility at week 33 of the experimental period, trays were placed beneath each cage and feces collected twice per day for the next 2 d. Feed consumption was also determined for these 2 d. Feces were pooled by replicate, frozen at -20°C, freeze-dried, mixed, and sub-sampled for determination of moisture and crude protein. Crude protein content was determined by the combustion method (AOAC, 1995-Method 984.13) using a Leco FP-528 protein analyzer.

Statistical analysis

Data were subjected to the analysis of variance using the GLM procedure in a completely randomized design (SAS, 2010). Treatment means were compared using the Duncan's multiple range test at $P < 0.05$.

Results and discussion

Egg production

Inclusion of NPAA at 0.5, 0.25 and 0.03125% in the diet increased egg production at week 25 of age, and over the

Table 2. Egg production performance (%) in Bovans laying hens from 24 to 33 weeks fed diets containing different levels of NPAA

Diets	Age (week)										Overall
	24	25	26	27	28	29	30	31	32	33	
T1	82.1	82.3 ^b	96.6	97.1	92.1	88.2	90.0	92.4	91.2	89.2	92.1 ^a
T2	87.1	94.9 ^a	95.7	97.1	96.4	95.7	95.7	93.5	94.2	95.7	94.6 ^{ab}
T3	86.4	95.7 ^a	98.5	95.7	96.4	97.1	97.1	97.1	96.4	96.4	95.7 ^a
T4	94.3	97.8 ^a	97.8	98.5	96.4	95.7	97.8	95.7	95.0	94.2	96.3 ^a
T5	82.9	88.5 ^{ab}	91.4	96.9	95.3	88.5	92.3	90.7	93.9	89.2	92.1 ^a
SEM	3.69	3.60	2.71	1.81	2.44	2.53	1.97	1.74	1.22	2.9	1.03
P-value	0.201	0.046	0.408	0.866	0.663	0.060	0.061	0.124	0.099	0.293	0.027

^{a-b}In each column, means with common superscript(s), do not differ (P > 0.05).

T1: Basal diet (BD) + 0 NPAA, T2: BD + 0.5% NPAA, T3: BD+ 0.25% NPAA, T4: BD + 0.0625% NPAA, T5: BD + 0.03125% NPAA.

NPAA: Nanostructure of Phytic Acid Absorber.

Table 3. Egg weight (g) in Bovans laying hens from 24 to 33 weeks of age fed diets containing different levels of NPAA

Diets	Age (week)										Overall
	24	25	26	27	28	29	30	31	32	33	
T1	53.2	54.2	55.0	55.6	56.1	53.8 ^c	55.3	56.0	56.3	56.3	55.2
T2	53.4	54.9	55.2	55.4	55.1	54.8 ^{bc}	54.7	56.0	56.0	56.0	55.2
T3	52.3	54.9	55.6	55.7	55.4	55.0 ^{bc}	55.8	56.5	56.5	56.2	55.4
T4	52.3	54.7	55.6	56.4	55.5	56.5 ^{ab}	57.0	57.2	57.2	58.3	55.7
T5	53.6	55.4	56.6	57.2	54.1	58.1 ^a	56.8	57.7	57.7	57.7	56.5
SEM	0.89	0.59	0.78	0.54	0.89	0.55	0.64	0.72	0.58	0.73	0.47
P- value	0.742	0.694	0.645	0.157	0.901	0.001	0.096	0.636	0.276	0.160	0.300

^{a-bc}In each column, means with common superscript(s) do not differ (P > 0.05).

T1: Basal diet (BD) + 0 NPAA, T2: BD + 0.5% NPAA, T3: BD+ 0.25% NPAA, T4: BD + 0.0625% NPAA, T5: BD + 0.03125% NPAA.

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the whole experimental period (Table 2), which is in agreement with the results of Mehment and Dalkilik (2005) who reported increased egg production in hens fed a phytase-supplemented diet. Other studies also showed that the negative effects of phytic acid might be reduced by phytase inclusion leading to increased egg production (Angel et al., 2000; Keshavarz, 2000; Liu and Run, 2010). Phytic acid is an anion and, therefore, can form double and triple bonds with cations like Cu²⁺,

Ni²⁺, Co²⁺, Mn²⁺, Ca²⁺ and Fe²⁺, forming insoluble complexes which decrease their availability (Angel et al., 2000). Phytic acid reduces amino acid and protein digestibility (Nelson et al., 1971) leading to a decrease in egg production in layers (Remus, 2005).

In the present experiment, NPAA reduced the negative effects of phytic acid resulting in increased bioavailability of Ca and P, which led to higher egg production; this was greater in birds fed with 0.0625% NPAA

Table 4. Egg mass (g/hen/d) in Bovans laying hens from 24 to 33 weeks of age fed diets containing different levels of NPAA

Diets	Age (week)										Overall
	24	25	26	27	28	29	30	31	32	33	
T1	43.8	46.1	44.9	54.8	51.8	47.2 ^b	51.3 ^b	53.4	51.6	51.7	50.4
T2	46.5	52.2	49.3	53.9	53.2	52.5 ^a	52.4 ^b	52.9	52.8	53.7	51.9
T3	45.3	52.6	48.9	53.7	53.4	53.5 ^a	54.3 ^{ab}	54.7	54.4	54.2	52.5
T4	49.4	52.3	50.0	55.6	53.5	54.1 ^a	55.8 ^a	54.6	55.1	54.9	53.8
T5	44.5	49.1	46.8	55.5	48.1	53.5 ^a	52.6 ^b	52.1	54.3	51.5	50.3
SEM	2.56	2.18	1.70	1.32	1.75	0.59	0.85	1.06	0.99	1.66	1.13
P- value	0.584	0.212	0.250	0.782	0.208	0.001	0.016	0.399	0.149	0.527	0.199

^{a-b}In each column, means with common superscript(s) do not differ (P > 0.05).

T1: Basal diet (BD) + 0 NPAA, T2: BD + 0.5% NPAA, T3: BD+ 0.25% NPAA, T4: BD + 0.0625% NPAA, T5: BD + 0.03125% NPAA.

NPAA: Nanostructure of Phytic Acid Absorber.

in the diet.

Mean egg weight

As shown in Table 3, egg weight showed significant differences between treatments at week 29 of age ($P < 0.05$), with the minimum egg weight recorded in the control group. Previously, Mehment and Dalkilik (2005) showed that increasing the amount of phytase in the diet (300 and 600 U/kg) significantly enhanced egg weight. Liu and Run (2010) and Tahir and Saleh (2008) showed that a reduction in the negative effects of phytic acid by phytase inclusion led to heavier eggs, but Liebert et al. (2005) reported that addition of phytase into the diet of old laying hens did not affect the egg weight. Our results demonstrated that supplementation of the diet with NPAA could increase the egg weight. The most important factor affecting the egg weight is the level of dietary protein; therefore, NPAA inclusion may have decreased the negative effect of phytic acid on protein availability.

Egg mass

Adding NPAA to the diet resulted in a greater egg mass ($P < 0.05$) compared to the control at weeks 29 and 30 (Table 4). Egg mass is a function of egg production and egg weight which are both related to the amount of nutrients the birds receive, demonstrating the ability of NPAA to make more nutrients available for the synthesis of egg. Liu (2006) reported that egg mass was improved by inclusion of 100 to 200 U/kg phytase in the diets. Increased egg mass production was also reported as a result of increased P availability (Frost and Roland, 1991). In contrast, Pelicia et al., (2009) found no effect of P availability on the egg mass.

Feed conversion ratio

The hens receiving the control diet had the highest FCR at weeks 29 and 30 ($P < 0.05$; Table 5), thus supplementing the diet of laying hen with NPAA reduced the negative effects of phytic acid and improved the utilization of available P and probably other minerals (cations) and compounds (amino acids) chelated to phytic acid.

The FCR is an index of feed intake in relation to egg production. If the diet does not supply enough Ca, the laying hen increases its feed intake to compensate for the deficiency of calcium; feeding diets containing phytase resulted in a better FCR in laying hens (Costa et al., 2003; Liebert et al., 2005; Mehment and Dalkilik, 2005). The FCR was improved in layers fed increasing levels of non-phytate P (0.1, 0.15 and 0.2%) (Keshavarz,

2000). In agreement with these reports, the present study demonstrated that NPAA was a suitable nano-absorber of phytic acid in improving the FCR.

Egg shell weight/surface

The greatest value of egg shell weight/ surface was recorded for the die containing 0.0625 % NPAA (Table 6). Use of NPAA in the layer diet led to enhancement of the availability of minerals, especially Ca and P, and increased the content of minerals in the egg shell. Pelicia et al. (2009) found that increasing the dietary Ca from 0.3 to 4.5 % improved the egg shell weight/surface, and Remus (2005) showed that supplementing the diet with phytase led to better metabolism of Ca and P and shell quality. In contrast, Parsons (1999) showed that shell weight/surface was not affected by phytase supplementation.

Egg shell thickness

Dietary supplementation of NPAA had no significant influence on egg shell thickness, but the hens that received 0.0625 % NPAA had numerically highest egg shell thickness. Liu and Run (2010), Heinzl (1996) and Scott et al. (2001) reported an increase in egg shell thickness and a decrease in broken eggs in hens fed phytase-supplemented diets. Also, Tahir and Saleh (2008) showed that increasing dietary Ca from 2 to 3.5 % and P from 0.25 to 0.35 %, respectively, led to thickening of the egg shell.

Shell phosphorus and calcium

Inclusion of 0.03125 % NPAA had a positive effect on the concentrations of P and Ca in the egg shell ($P < 0.05$; Table 8). Although 80 % of P is found in poultry bones, egg shell contains some P. On the other hand, 95 % of dried egg shell is calcium carbonate. Therefore, the main role of P in layer diets is balancing the Ca metabolism. The dietary Ca:P ratio is one of the most important factors influencing Ca absorption and egg shell quality. In agreement with our results, Heinzl (1996) suggested that low P levels in the diet result in low egg shell P. Similarly, adding phytase to the diet reduced the negative effects of phytic acid and increased egg shell Ca (Lim and Namkung, 2003); and enhancement of Ca in layer diets resulted in a higher concentration of Ca in the egg shell (Roland and Zhang, 1998). In contrast, King et al. (2000) found that dietary phytase supplementation did not increase shell P content.

Protein digestibility

Protein digestibility measured at 33 weeks of age was

Table 5. Feed conversion ratio in Bovans laying hens from 24 to 33 weeks of age fed diets containing different levels of NPAA

Diets	Age (week)										Overall
	24	25	26	27	28	29	30	31	32	33	
T1	2.2	1.8	1.8	1.7	1.8	2.1 ^a	1.9 ^a	1.8	1.9	1.9	1.9
T2	2.0	1.7	1.7	1.8	1.9	1.9 ^b	1.9 ^{ab}	1.9	1.9	1.8	1.8
T3	2.2	1.8	1.7	1.8	1.9	1.9 ^b	1.8 ^{bc}	1.8	1.8	1.8	1.8
T4	2.0	1.8	1.7	1.8	1.9	1.8 ^b	1.8 ^c	1.8	1.8	1.8	1.8
T5	2.3	1.9	1.9	1.8	2.0	1.9 ^b	1.9 ^{abc}	1.9	1.8	1.9	1.9
SEM	0.5	0.05	0.04	0.05	0.04	0.03	0.85	0.03	0.06	0.04	0.14
P-value	0.597	0.143	0.540	0.708	0.075	0.001	0.016	0.386	0.139	0.495	0.298

^{a-bc}In each column, means with common superscript(s) do not differ (P>0.05).

T1: Basal diet (BD) + 0 NPAA, T2: BD + 0.5% NPAA, T3: BD+ 0.25% NPAA, T4: BD + 0.0625% NPAA, T5: BD + 0.03125% NPAA. NPAA: Nanostructure of Phytic Acid Absorber.

Table 6. Eggshell weight per unit area (g/cm²) in Bovans laying hens at 27, 29, 31 and 33 weeks of age fed diets containing different levels of NPAA

Diets	Age (week)			
	27	29	31	33
T1	0.9	0.8 ^b	0.9	0.9
T2	0.8	0.9 ^{ab}	0.9	0.8
T3	0.9	0.8 ^b	0.9	0.8
T4	0.9	0.9 ^a	0.9	0.9
T5	0.8	0.8 ^b	0.8	0.8
SEM	0.02	0.02	0.02	0.02
P-value	0.283	0.016	0.252	0.473

^{a-b}In each column, means with common superscript(s) do not differ (P > 0.05). T1: Basal diet (BD) + 0 NPAA, T2: BD + 0.5% NPAA, T3: BD+ 0.25% NPAA, T4: BD + 0.0625% NPAA, T5: BD + 0.03125% NPAA. NPAA: Nanostructure of Phytic Acid Absorber.

Table 7. Relative egg shell thickness (%) in Bovans laying hens at 27, 29, 31 and 33 weeks of age fed diets containing different levels of NPAA

Diets	Age (week)			
	27	29	31	33
T1	0.4	0.3	0.5	0.5
T2	0.4	0.4	0.5	0.5
T3	0.4	0.4	0.5	0.5
T4	0.4	0.4	0.5	0.5
T5	0.4	0.3	0.5	0.5
SEM	0.01	0.02	0.01	0.01
P-value	0.851	0.076	0.297	0.305

T1: Basal diet (BD) + 0 NPAA, T2: BD + 0.5% NPAA, T3: BD+ 0.25% NPAA, T4: BD + 0.0625% NPAA, T5: BD + 0.03125% NPAA. NPAA: Nanostructure of Phytic Acid Absorber.

the highest in 0.0625% NPAA (T4) group (Table 9). Previous research were mostly concerned with the effect of phytase supplementation on nutrient digestibility in broiler chickens but data on laying hens are very limited. The efficacy of phytase supplementation in layer diets is still under discussion because of an open debate about the non-phytate P requirement of laying hens and factors influencing phytate degradation by exogenous phy-

Table 8. Phosphorus and calcium content of the egg shell (%) in Bovans laying hens at 29th week of age fed diets containing different levels of NPAA

Diets	Age (29 week)	
	Phosphorus	Calcium
T1	1.0 ^b	2.9 ^b
T2	1.0 ^b	2.9 ^b
T3	1.0 ^b	3.0 ^b
T4	1.1 ^{ab}	3.1 ^b
T5	1.2 ^a	3.4 ^a
SEM	0.04	0.09
P-value	0.050	0.026

^{a-b}In each column, mean with common superscript (s) do not differ (P>0.05). T1: Basal diet (BD) + 0 NPAA, T2: BD + 0.5% NPAA, T3: BD+ 0.25% NPAA, T4: BD + 0.0625% NPAA, T5: BD + 0.03125% NPAA. NPAA: Nanostructure of Phytic Acid Absorber.

Table 9. Protein digestibility (%) in Bovans laying hens at 33rd week of age fed diets containing different levels of NPAA

Diets	Protein digestibility
T1	64.9 ^{bc}
T2	56.9 ^c
T3	73.8 ^{ab}
T4	85.5 ^a
T5	71.0 ^{abc}
SEM	5.11
P-value	0.015

^{a-c}In each column, means with common superscript(s) do not differ (P > 0.05). T1: Basal diet (BD) + 0 NPAA, T2: BD + 0.5% NPAA, T3: BD+ 0.25% NPAA, T4: BD + 0.0625% NPAA, T5: BD + 0.03125% NPAA. NPAA: Nanostructure of Phytic Acid Absorber.

tase in the gastrointestinal tract of layers (Hughes et al., 2009), but negative influences of phytic acid on the solubility and digestibility of proteins and the activity of pepsinogen, due to ionic binding between phytic acid and protonized amino acids, are expected (Okubo et al., 1976). Therefore, any material which releases protein from this compound could improve the availability of protein (Juanperej et al., 2004), as did NPAA in the pre-

sent study. Similarly, 0.3 % nano-selenium improved protein digestibility (King et al., 2000).

Heinzl (1996) suggested that inclusion of phytase in poultry diets reduced N excretion and increased its retention by breaking the phytic acid-amino acid binding and improving the efficiency of digestive enzymes. Adding phytase caused higher protein digestibility in layers fed a wheat-corn based diet (Scott et al., 2001). Furthermore, supplementing the broiler diets with phytase resulted in better performance and protein digestibility (Cowieson and Adeola, 2005).

Conclusion

It was concluded that supplementation with nano-structure of phytic acid absorber at the level of 0.0625% in the diet of laying hens reduced the anti-nutritional effects of phytic acid and improved production performance and protein digestibility.

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References

- Angel, R., Applegate T., Christman, M., 2000. Effect of dietary nonphytate phosphorus (npp) on performance and bone measurements in broilers fed on a four-phase feeding system. *Poultry Science* 79, 21-22.
- AOAC. 1995. Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists. Washington, DC.
- Bedford, M. R., Schulze, H., 1998. Exogenous enzymes for pigs and poultry. *Nutrition Research Reviews* 11, 91-114.
- Casartelli, E. M., Junqueira, O.M., Laurentiz, A.C., Filardi, R.S., Lucas, J., Juniorand, L.F., 2005. Effect of phytase in laying hen diets with different phosphorus sources. *Brazilian Journal of Poultry Science* 7, 93-98.
- Catli, A.U., Bozkurt, M., Kuchkyilmaz, K., Ginar, M., Bintas, E., Goven, F., Atik, H., 2012. Performance and egg quality of aged laying hens fed diets supplemented with meat and bone meal or oyster shell. *South African Journal of Animal Science* 42, 74-82.
- Costa, J., Almeida, C.E., Dotson, E.M., Lins, A., Vinhaes, M., Silveira, A.C., Beard, C. B., 2003. The epidemiologic importance of *Triatoma brasiliensis* as a chagas disease vector in Brazil: a revision of domiciliary captures during 1993-1999. *Memórias do Instituto Oswaldo Cruz*. 98, 443-449. <http://dx.doi.org/10.1590/S0074-02762003000400002>.
- Cowieson, A.J., Adeola, O., 2005. Carbohydrases, protease and phytase have an additive beneficial effect in nutritionally marginal diets for broiler chicks. *Poultry Science* 84, 1860-1867.
- Ebrahimnezhad, Y., Gheiasi, J., Maheri, N., Mohammadikhah, M., Ahmadi, F., 2013. Influence of zinc oxide nanoparticles on growth performance, carcass quality and growth index of immune organs of broiler chickens. *Poultry Science* 92, 98-107.
- Fazelirad, H., Ranjbar, M., Taher, M.A., Sargazi, G., 2015. Preparation of magnetic multi-walled carbon nanotubes for an efficient adsorption and spectrophotometric determination of amoxicillin. *Journal of Industrial Engineering Chemistry* 21, 889-892.
- Frost, J., Roland, D. A., 1991. The influence of various calcium and phosphorus levels on tibia strength and eggshell quality of pullets during peak production. *Poultry Science* 70, 963-969.
- Heinzl, B., 1996. Effect of phosphorus source and level on laying hen performance under varying temperature conditions. Proceedings of Georgia Nutrition Conference for the Feed Industry, PP. 74-87.
- Hughes, A. L., Dahiya, J. P., Wyatt, C. L., Classen, H. L., 2009. Effect of quantum phytase on nutrient digestibility and bone ash in White Leghorn laying hens fed corn-soybean meal-based diets. *Poultry Science* 88, 1191-1198.
- Juanperej, A., Perez-Vandrell, M., Brufau, J., 2004. Effect of microbial phytase on broilers fed barley-based diets in the presence or not of endogenous phytase. *Animal Feed Science and Technology* 115, 265-279.
- Keshavarz, K., 2000. Nonphytate phosphorus requirement of laying hens with and without phytase on a phase feeding program. *Poultry Science* 79, 748-763.
- King, D. E., Fan, M., Ejefta, G. E., Asem, E. K., Adeola, O., 2000. The effects of tannin on nutrient utilization in the white Pekin duck. *Journal of Poultry Science* 41, 630-639.
- Liebert, F., Htoo, J. K., Suner, A., 2005. Performance and nutrient utilization of laying hen fed low-phosphorous corn-soybean diets supplemented with microbial phytase. *Poultry Science* 84, 1576-1583.
- Lim, H. S., Numkung, H., Peik, I. K., 2003. Effects of phytase supplementation on the performance, egg quality, and phosphorous excretion of laying hens fed different levels of dietary calcium and non-phytate phosphorous. *Poultry Science* 82, 92-99.
- Liu, B. L., Rafiq, A., Tzeng, Y. M., Rob, A., 1998. The induction and characterization of phytase and beyond. *Enzyme Microbial Technology* 22, 415-424.
- Liu, N., Run, Y., 2010. Effect of phytate and phytase on the ileal flows of endogenous minerals and amino acids for growing broiler chickens fed purified diets. *Animal Feed Science and Technology* 156, 126-130.

- Liu, X., 2006. Effects of cysteamine on growth performance, digestive enzyme activities and metabolic hormones in broilers. *Poultry Science* 85, 1912-1916.
- Mehment, T.C., Dalkilik, B., 2005. Effect of microbial phytase supplementation on feed consumption and egg production of laying hens. *International Journal of Poultry Science* 410,758-760.
- Morris, E.R., 1986. Phytate and dietary mineral bioavailability. In: Graf, E. (Ed.), *Phytic Acid: Chemistry and Applications*. Pilatus Press, Minneapolis, MN, pp. 57-76.
- Murayama, N., Maekawa, I. H., Ushiro, T., Miyoshi, J., 2012. Synthesis of various layered double hydroxides using aluminum dross generated in aluminum recycling process. *International Journal of Mineral Processing*, 110, 46-52.
- Nelson, T. S., Shieh, T. R., Wodzinski, R. J., Ware, J. H., 1971. Effect of supplemental phytase on the utilization of phytate phosphorus by chicks. *Journal of Nutrition* 101, 1289-1293.
- Oberleas, D., 1973. *Phytates. Toxicants Occurring Naturally in Foods*. 2nd Ed. National Academy Press, Washington, DC. USA.
- Okubo, K., Myers, D. V., Iacobucci, G. A., 1976. Binding of phytic acid to glycine. *Cereal Chemistry* 53, 513-524.
- Parsons, C. M., 1999. The effect of dietary available phosphorus and phytase level on long-term performance of laying hens. BASF Corporation. Mt Olive. NJ. pp. 33-34.
- Peeler, H. T., 1972. Biological availability of nutrients in feeds: Availability of major mineral ions. *Animal Science* 77, 83-89.
- Pelicia, K., Garcia, E. A., Silva, A. P., Berto, D. A., Molino, A. B., Vercese, F., 2009. Calcium and available phosphorus for laying hens in second production cycle. *Brazilian Journal of Poultry Science* 11, 39-49.
- Peng, X., Cui, Y., Cui, W., Deng, J. L., Cui, H. M., 2009. The decrease of relative weight, lesions and apoptosis of bursa of Fabricius induced by excess dietary selenium in chickens. *Biological Trace Element Research* 131, 33-42.
- Peter, W., 1992. Investigations on the use of phytase in the feeding of laying hens. Proceedings of the 19th World's Poultry Congress, Amsterdam.
- Ravindran, D., 2013. Application of nanominerals in animal production system. *Research Journal of Biotechnology* 8, 1-3.
- Ravindran, V., Selle, P. H. G., Ravaindran, P. C. H., Morel, G., Kies, A. K., Bryden, W. L., 2001. Microbial phytase improves performance, apparent metabolizable energy, and ileal amino acid digestibility of broilers fed a lysine-deficient diet. *Poultry Science* 80, 338-344.
- Remus, J., 2005. Poultry and environment reap the benefits of new-generation phytase. *Feed Technology* 9, 22-25.
- Roland, G., Zhang, R. X., 1998. Effect of Natuphos phytase supplementation to feed on performance and ileal digestibility of protein and amino acids of broilers. *Poultry Science* 78, 15-67.
- SAS, 2010. SAS User's Guide: Statistics. Version 9.1.3. SAS Institute Inc. Cary, NC.
- Scott, T.A., Ampen, R. K., Silversides G., 2001. The effect of adding exogenous phytase to nutrient-reduced corn and wheat-based diets on performance and egg quality of two strains of laying hens. *Canadian Journal of Animal Science* 81, 393-401.
- Sebastian S, Touchburn, S. P., Chaveey, E. R., Lague, P. C., 1996. Efficacy of supplemental microbial phytase at different dietary calcium levels on growth performance and mineral utilization of broiler chickens. *Poultry Science* 75, 1516-1523.
- Tahir, M., Saleh, A., 2008. An effective combination of carbohydrases that enables reduction of dietary protein in broilers. *Poultry Science* 76, 1535-16.
- Vander Klis, J. D., Versteegh, H. I. J., 1991. Ileal absorption of P in light-weight white laying hens using microbial phytase and various calcium content in laying hen feed. Spelderholt Pub. No. 563. Het Spelderholt, Wageningen, The Netherlands.

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اثرات نانوساختار جاذب اسید فایتيک بر عملکرد و گوارش پذيری پروتئين در مرغ های تخمگذار

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چکیده این آزمایش به منظور بررسی اثرات نانوساختار جاذب اسید فایتيک بر عملکرد و گوارش پذيری پروتئين در مرغ های تخمگذار انجام شد. یکصد قطعه مرغ تخمگذار سویه بوئنز با میانگین تولید تخم مرغ یکسان در قالب یک طرح کاملاً تصادفی به پنج تیمار و هر تیمار دارای ۴ تکرار و ۵ قطعه مرغ در هر تکرار تقسیم شدند. این آزمایش دارای ۵ تیمار حاوی سطوح مختلف نانوساختار جاذب اسید فایتيک شامل: تیمار ۱ (جیره پایه + صفر درصد نانوساختار جاذب اسید فایتيک)، تیمار ۲ (جیره پایه + ۰/۵ درصد نانوساختار جاذب اسید فایتيک)، تیمار ۳ (جیره پایه + ۰/۲۵ درصد نانوساختار جاذب اسید فایتيک)، تیمار ۴ (جیره پایه + ۰/۰۶۲۵ درصد نانوساختار جاذب اسید فایتيک) و تیمار ۵ (جیره پایه + ۰/۰۳۱۲۵ درصد نانوساختار جاذب اسید فایتيک) بود. نتایج این آزمایش نشان داد که افزودن نانوساختار جاذب اسید فایتيک به جیره اثر معنی داری بر درصد تولید تخم مرغ در سن ۲۵ هفتگی و کل دوره آزمایش داشت ($P < 0/05$). تیمارهای غذایی اثر معنی داری بر وزن تخم مرغ در سن ۲۹ هفتگی داشت و مرغ هایی که تیمار ۵ را دریافت کرده بودند تخم مرغ سنگین تری را در مقایسه با تیمارهای ۱، ۲ و ۳ تولید کردند. تیمارهای غذایی اثر معنی داری بر جرم توده تخم مرغ و ضریب تبدیل خوراک در سن ۲۹ و ۳۰ هفتگی داشت ($P < 0/05$). وزن پوسته تخم مرغ به طور معنی داری ($P < 0/05$) تحت تاثیر تیمارهای غذایی در سن ۲۹ هفتگی قرار گرفت. ولی تیمارهای غذایی تاثیری بر ضخامت نسبی پوسته تخم مرغ نداشتند. در سن ۳۳ هفتگی، گوارش پذيری پروتئين در مرغ هایی که تیمار ۴ را دریافت کردند بیشتر از کنترل بود. یافته ها نشان دادند که افزودن نانوساختار جاذب اسید فایتيک به جیره در سطح ۰/۰۶۲۵ درصد آثار ضد تغذیه ای اسید فایتيک را کاهش داده و عملکرد تولید و گوارش پذيری پروتئين را در مرغ های تخم گذار افزایش داد.