Response of laying hens to diet inclusion of canola meal as influenced by dietary nonphytate phosphorus level and microbial phytase supplementation

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Abstract An experiment employing a factorial arrangement of three levels (0, 8 and 16%) of canola meal (CM), two levels (0.15 and 0.25%) of nonphytate phosphorus (NPP), and two levels (0 and 450 unit/kg; as fed basis) of microbial phytase was conducted using 216 Hy-Line W36 laying hens from 39 to 47 weeks of age. The birds receiving CM consumed more ($P < 0.05$) feed than birds receiving corn-soybean meal (SBM) diets. During the second 4-week of the experiment (44 to 47 weeks of age), egg production and egg mass were lower ($P < 0.05$) for birds receiving corn-SBM diet containing reduced NPP level; however, the adverse effects of reduced NPP were overcome by phytase supplementation ($P < 0.05$). During the second 4-week (44 to 47 weeks of age) and over the whole experiment (39 to 47 weeks of age), production of abnormal eggs was increased ($P < 0.05$) by feeding reduced NPP level; phytase supplementation decreased ($P < 0.05$) egg abnormality only when added to this diet. At the first egg sampling (43rd week of age), egg shape index and eggshell thickness were increased ($P < 0.05$) by phytase supplementation. Reduced NPP level caused a lower eggshell thickness in hens fed corn-SBM diet ($P < 0.05$). At the second egg sampling (47th week of age), birds fed corn-SBM diets or reduced NPP level produced eggs with lower ($P < 0.05$) shell thickness, whereas dietary phytase supplementation reversed these adverse effects ($P < 0.05$). Reduced NPP level increased serum thyroxine concentration in birds fed corn-SBM-CM diets ($P < 0.05$). The results showed that CM can be included in laying hen diets up to 16% during 39 to 47 weeks of age without any adverse effect on their health and productivity. Moreover, the results indicated that reduction of NPP level in corn-SBM-CM diets had little effect on performance and eggshell quality. The adverse effects of lowering NPP level in corn-SBM diets could be substantially reduced by phytase supplementation.

Keywords: bone ash, canola meal, eggshell quality, performance, phosphorus, phytase

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Introduction

The use of rapeseed meal in poultry diets is limited by the presence of glucosinolates and other minor anti-nutritional factors like erucic acid and tannins (Thomas et al., 1978; Ramesh et al., 2006). Two major problems associated with the feeding of high concentrations of rapeseed meal to laying hens are reduced egg production and increased mortality due to liver hemorrhages (Thomas et al., 1978). With the development of rapeseed cultivars low in glucosinolates and erucic acid contents, the inclusion of canola meal (CM) in poultry diets is increasing. Although CM is a good quality feed ingredient for laying hens, there are still occasional reports indicating unfavorable effects of low glucosinolate levels on thyroid function and circulating thyroxine (T4) accompanied by an appetite depression and increased leg problems and liver hemorrhages when CM is substituted for a significant amount of soybean meal (SBM; Ramesh et al., 2006). Moreover, the optimal utilization of CM in laying diets is often influenced by its high content of non-starch polysaccharides and presence of several anti-nutritive factors, including tannins, phytic acid and erucic acid (Khajali and Slominski, 2012).

The presence of phytic acid in CM negatively affects the protein and amino acid digestibilities by preventing the activities of the proteolytic enzymes such as pepsin and trypsin (Selle et al., 2012). Furthermore, phytic acid has higher phosphorus (P) content, and chelating ability with the phytate form of phytic acid diminishing the availability of calcium (Ca) and P. Poultry cannot make use of phytin-P due to lack of phytase enzyme in their

digestive tract and as a consequence, phytin-P is mostly excreted in the feces (Wu et al., 2006).

To meet P requirement of birds, inorganic P sources such as dicalcium phosphate and monocalcium phosphate or exogenous phytase enzymes are commonly added to diets of commercial laying hens. However, not only is inorganic P supplementation expensive, it also leads to environmental pollution as a result of over-supplementation (Wu et al., 2006).

Many researchers have demonstrated that phytase supplementation of the diets containing 0.10 or 0.15% nonphytate-P (NPP) had positive effects on egg production, egg mass, egg weight, egg specific gravity, eggshell quality and bone ash by improving P utilization (Boling et al., 2000; Keshavarz, 2003; Hughes et al., 2008). Phytase addition has also been shown to increase Ca availability probably due to increasing P availability (Ravindran et al., 1995). However, little information is available on the effect of phytase addition to the laying hen diets containing CM.

The aim of the present study was to investigate the effect of dietary phytase supplementation on the performance, eggshell quality, ash percentage, Ca and P content of the toe and eggshell, serum thyroxine (T4) concentration, mortality and liver hemorrhage in laying hens fed diets containing different levels of CM and NPP.

Materials and methods

Birds and experimental design

All procedures used in this experiment were approved by the Animal Ethics Committee of Razi University, Kermanshah, Iran. A total of 216 Hy-Line W36 laying hens of almost the same body weight (1,412 ± 14 g) and egg production rate (85.0 ± 3.5%) were randomly allotted to 36 cages (n = 6) at 39 weeks of age. The cages were located in an environmentally controlled room with the room temperature kept at 17 to 20°C and the photoperiod set at 16 h of light and 8 h dark. Water was available *ad libitum* throughout the experiment. Twelve iso-energetic and iso-nitrogenous diets (Table 1) including three levels (0, 8 and 16% diet) of dietary CM and two levels (0.15 and 0.25% diet) of dietary NPP without or with a commercial source of phytase (Ronozyme P-CT, DSM Nutritional Products Inc., Parsippany, NJ, 450 unit/kg diet) were fed to hens with three replicates per diet for 8 weeks. One unit of phytase is defined as the amount of enzyme that liberates 1 µmol of inorganic

Table 1. Ingredients and chemical composition of the experimental diets (%, unless stated otherwise)[1]

Canola meal[2]	0.00		8.00		16.00	
Nonphytate phosphorus	0.15	0.25	0.15	0.25	0.15	0.25
Ingredients						
Corn	62.80	62.80	62.50	62.60	62.40	61.80
Soybean meal	19.50	19.60	13.90	14.10	8.50	8.60
Wheat bran	4.30	3.80	2.20	1.70	–	–
Sunflower oil	3.70	3.70	3.70	3.70	3.70	3.70
Limestone	3.00	3.00	3.00	3.00	3.00	3.00
Oyster shell	5.20	4.90	5.20	4.90	5.10	4.80
Dicalcium phosphate	0.18	0.72	0.15	0.69	0.13	0.67
Common salt	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin premix[3]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[4]	0.25	0.25	0.25	0.25	0.25	0.25
Lysine-HCl	0.23	0.23	0.19	0.19	0.16	0.16
DL-Methionine	0.29	0.29	0.26	0.26	0.24	0.24
Calculated analysis						
Metabolizable energy (MJ/kg)	11.97	11.97	11.97	11.97	11.97	11.97
Crude protein	15.00	15.00	15.00	15.00	15.00	15.00
Calcium	3.25	3.25	3.25	3.25	3.25	3.25
Total phosphorus	0.41	0.51	0.44	0.54	0.48	0.58
Lysine	0.88	0.88	0.85	0.85	0.83	0.83
Methionine + Cysteine	0.74	0.74	0.74	0.74	0.74	0.74

[1] The composition is given as feed basis.

[2]The chemical composition (nutrient contents) of used canola meal: Crude protein = 35.0 %, Ether Extract = 1.0 %, Crude fiber = 12.0 %, Nitrogen free extract = 42.0 %, Ash = 6.3 %, Ca = 0.83%, P = 1.2%.

[3]Supplied per kg of the diet: retinol acetate 4.13 mg, DL-α-tocopherol acetate 42 mg, cholecalciferol 0.008 mg, menadione 2 mg, thiamine 2 mg, riboflavin 6.6 mg, pyridoxine 5 mg, cyanocobalamin 0.02 mg, niacin 99 mg, folic acid 1 mg, biotin 0.15 mg, calcium D-pantothenate 15 mg, choline chloride 0.7 g.

[4]Supplied per kg of the diet: Cu, 20 mg; Fe, 100 mg; Mn, 100 mg; Se, 0.4 mg; Zn, 169.4 mg.

P per min from sodium phytate at a pH of 5.0 and temperature of 37°C. The nutrient analysis of the CM source was carried out according to the AOAC (1995).

Performance production and egg quality traits

Production performance was measured from 39 to 47 weeks of age. Daily egg production per replicate was recorded, and at the end of each of the 4 experimental weeks, the total number of eggs laid per bird was calculated. Similarly, the eggs laid in each replicate were weighed and the mean egg weight per bird was calculated. Abnormal eggs, including the soft-shelled, cracked, and broken eggs, were also recorded daily. Feed intake and estimated NPP intake were measured on a weekly basis. Egg mass (g egg/hen/day) and feed conversion ratio (FCR; g feed/g egg) were calculated from egg production, egg weight, and feed intake. Body weights were recorded at the beginning and the end of the experiment.

The eggs laid during the last three days of each of the 4 weekly periods of the experiment were used to measure the egg quality traits. Egg specific gravity, eggshell weight, eggshell thickness, albumen height and egg shape index were measured on six eggs from each treatment (two eggs per replicate). Egg specific gravity was determined using 11 gradient saline solutions varying in specific gravity from 1.060 to 1.100 at 0.005-unit increments (Holder and Bradford, 1979). Eggshell thickness was measured using a digital micrometer (Mitutoyo, Japan). Haugh units were calculated as an indicator of interior egg quality. Albumen height was measured at three different sites by using a spherometer, and Haugh units were determined using the egg weight and albumen height (Eisen et al., 1962). Eggshell percentage was measured after breaking the egg and separating the eggshell from the liquid content. Eggshells were then dried overnight for 24 h in an oven at 100°C. Dry shell weight was expressed as percentage of the egg weight. The eggshells from the last collection were also used for determination of shell ash (after burning in a muffle furnace at 600°C for 8 h) and Ca contents (AOAC, 1995).

Organ weights, ash, Ca and P contents of the toe, and serum T4 concentration

There was no mortality, and all the birds appeared healthy during the experiment. At the end of the experiment (47 weeks of age), two birds from each replicate were slaughtered and the livers were excised, weighed and examined for the presence of any hemorrhages. The weights of the abdominal fat and pancreas were also measured, and all organ weights were expressed as percentage of the live body weight. The middle toes from the left side of the birds were then removed, dried to a constant weight at 100°C and ashed in a muffle furnace at 600°C for 6 h. Toe ash was expressed as a percentage of the dry weight. Subsequently, total P and Ca concentrations were determined (AOAC, 1995).

On the same day, three birds from each replicate were bled by wing vein puncture and blood samples were collected in non-heparinized collection tubes. Serum was separated by centrifuging the blood samples for 20 min at 2,500 ×g and stored at –20°C until further analysis. Serum T4 concentration was measured by using a commercially available radioimmunoassay kit (Byk-Sangtec Diagnostica, Dietzenbach, Germany), which had been validated for poultry (Sciarrillo et al., 1999; Şahin and Küçük, 2001). The intra- and inter-assay coefficients of variation were 4.0% and 6.4%, respectively.

Statistical analysis

Data were subjected to ANOVA in a completely randomized design with a 3 × 2 × 2 factorial arrangement of treatments using the GLM procedure of SAS (2003). The means were compared by the Bonferroni tests ($P < 0.05$).

Results

Productive performance

The effects of dietary treatments on performance of laying hens are presented in Tables 2 and 3. There were increases in feed intake and NPP intake as a result of dietary inclusion of CM ($P < 0.05$). Increased dietary concentration of NPP also caused an increase in NPP intake ($P < 0.05$). No effect of CM inclusion was found on egg production, egg weight, egg mass and FCR ($P > 0.05$). Reducing NPP level from 0.25 to 0.15% had no effect on feed intake, FCR, body weight, egg weight, egg production and egg mass ($P > 0.05$). There was a CM by NPP by phytase interaction on egg production and egg mass from 43 to 47 weeks of age ($P < 0.05$). The nature of this interaction demonstrated that both egg production and egg mass were lower for hens given corn-SBM diets with reduced NPP level ($P < 0.05$). However, the adverse effects of reduced NPP were overcome by phytase supplementation ($P < 0.05$). Phytase supplementation, on the other hand, caused a decreased egg production and egg mass in laying hens fed diet containing 16% CM and reduced NPP level ($P < 0.05$). During the 44 to 47 weeks of age and overall period of the experiment (39 to 47 weeks of age), a NPP by phytase interaction was observed on production of

Table 2. Effects of different levels of dietary canola meal (%), nonphytate phosphorus (P, %) and phytase supplementation (unit/kg) on feed intake, FCR (39 to 47 weeks of age) and body weight of laying hens (47th weeks of age)

Items			Feed intake (g/hen/d)			NPP intake (g/hen/d)[1]			FCR (g egg/g feed)			Body weight (g)
Canola meal (CM)	Nonphytate P (NPP)	Phytase (Pz)	39-43 wk	44-47 wk	39-47 wk	39-43 wk	44-47 wk	39-47 wk	39-43 wk	44-47 wk	39-47 wk	47 wk
0	0.15	0	87.8^{b}	81.7^{c}	84.8^{c}	1.32^{d}	1.23^{c}	1.27^{c}	2.16	2.17	2.17	1447
0	0.15	450	88.3^{b}	91.3^{abc}	89.8^{abc}	1.32^{d}	1.37^{c}	1.35^{c}	1.98	2.11	2.04	1450
0	0.25	0	88.1^{b}	90.1^{abc}	89.1^{abc}	2.20^{bc}	2.25^{ab}	2.23^{ab}	1.90	1.90	1.91	1475
0	0.25	450	86.8^{b}	84.7^{bc}	85.8^{bc}	2.17^{c}	2.12^{b}	2.14^{b}	1.80	1.90	1.85	1665
8	0.15	0	93.7^{a}	91.4^{abc}	92.5^{abc}	1.41^{d}	1.37^{c}	1.39^{c}	2.07	1.95	2.01	1415
8	0.15	450	93.7^{a}	92.4^{ab}	93.1^{ab}	1.41^{d}	1.39^{c}	1.40^{c}	2.03	2.00	2.02	1650
8	0.25	0	93.0^{a}	95.8^{a}	94.4^{a}	2.32^{ab}	2.39^{a}	2.36^{a}	2.13	2.14	2.13	1375
8	0.25	450	95.7^{a}	94.8^{a}	95.2^{a}	2.39^{a}	2.37^{a}	2.38^{a}	2.16	2.06	2.11	1442
16	0.15	0	90.1^{a}	88.4^{abc}	89.3^{abc}	1.35^{d}	1.33^{c}	1.34^{c}	2.12	1.84	1.98	1535
16	0.15	450	93.2^{a}	91.7^{abc}	92.5^{abc}	1.40^{d}	1.37^{c}	1.39^{c}	2.21	2.23	2.22	1560
16	0.25	0	90.8^{a}	92.1^{ab}	91.4^{abc}	2.27^{abc}	2.30^{ab}	2.29^{ab}	2.13	1.96	2.04	1680
16	0.25	450	93.6^{a}	92.3^{ab}	92.9^{ab}	2.34^{ab}	2.31^{ab}	2.32^{a}	2.08	1.97	2.02	1635
Pooled SEM			0.71	0.98	0.78	0.079	0.083	0.081	0.033	0.031	0.029	
Sources of variation			*P* values									
CM			0.002	0.02	0.003	0.002	0.02	0.005	0.09	0.88	0.45	0.43
NPP			0.89	0.25	0.42	<.0001	<.0001	<.0001	0.36	0.29	0.27	0.67
Pz			0.33	0.47	0.36	0.35	0.81	0.59	0.54	0.41	0.92	0.36
CM × NPP			0.91	0.85	0.86	0.40	0.46	0.41	0.17	0.07	0.07	0.42
CM × Pz			0.58	0.88	0.87	0.53	0.96	0.80	0.57	0.23	0.36	0.73
NPP × Pz			0.94	0.07	0.25	0.74	0.12	0.39	1.00	0.22	0.50	0.92
CM × NPP × Pz			0.77	0.28	0.42	0.73	0.36	0.49	0.74	0.35	0.47	0.68

[a, b] Means within column with different superscripts are significantly different ($P < 0.05$).
[1] Calculated based on dietary NPP concentration.
SEM = standard error of the mean.

Table 3. Effects of different levels of dietary canola meal (%), nonphytate phosphorus (P, %) and phytase supplementation (unit/kg) on egg production, egg weight, egg mass and production of abnormal eggs in laying hens (39 to 47 weeks of age)

Items			Egg production (%)			Egg weight (g)			Egg mass (g/hen/d)			Abnormal eggs (%)[1]		
Canola meal (CM)	Nonphytate P (NPP)	Phytase (Pz)	39-43 wk	44-47 wk	39-47 wk	39-43 wk	44-47 wk	39-47 wk	39-43 wk	44-47 wk	39-47 wk	39-43 wk	44-47 wk	39-47 wk
0	0.15	0	64.5	59.9^{b}	62.2	64.0	63.1	63.6	41.2	37.8^{c}	39.5	0.573	0.680^{a}	0.627^{a}
0	0.15	450	71.6	71.4^{a}	71.5	63.8	62.4	63.1	45.6	44.6^{ab}	45.1	0.140	0.063^{b}	0.100^{b}
0	0.25	0	75.8	75.8^{a}	75.8	61.4	62.9	62.1	46.5	47.6^{ab}	47.0	0.190	0.163^{b}	0.177^{b}
0	0.25	450	76.0	71.6^{a}	73.8	66.9	62.9	64.9	51.0	45.1^{ab}	48.0	0.350	0.257^{b}	0.300^{ab}
8	0.15	0	74.2	74.6^{a}	74.4	61.6	62.9	62.3	45.6	46.9^{ab}	46.3	0.407	0.220^{b}	0.313^{ab}
8	0.15	450	75.0	74.2^{a}	74.6	62.1	62.7	62.4	46.6	46.5^{ab}	46.5	0.257	0.093^{b}	0.173^{b}
8	0.25	0	72.2	73.6^{a}	72.9	61.3	61.7	61.5	44.2	45.4^{ab}	44.8	0.563	0.287^{b}	0.423^{ab}
8	0.25	450	73.6	73.8^{a}	73.7	60.5	63.0	61.7	44.6	46.4^{ab}	45.5	0.387	0.347^{b}	0.367^{ab}
16	0.15	0	68.1	75.0^{a}	71.5	63.0	64.4	63.7	42.8	48.3^{a}	45.6	0.487	0.087^{b}	0.283^{ab}
16	0.15	450	69.3	65.1^{ab}	67.2	61.6	63.5	62.6	42.6	41.3^{bc}	41.9	0.363	0.203^{b}	0.283^{ab}
16	0.25	0	69.4	74.4^{a}	71.9	58.7	63.6	61.2	43.0	47.3^{ab}	45.2	0.227	0.100^{b}	0.163^{b}
16	0.25	450	73.4	75.2^{a}	74.3	62.1	62.6	62.4	45.7	47.1^{ab}	46.4	0.337	0.153b	0.247^{ab}
Pooled SEM			0.97	1.10	0.94	0.64	0.22	0.36	0.73	0.67	0.60	0.0486	0.0383	0.0359
Sources of variation			*P* values											
CM			0.29	0.18	0.29	0.19	0.25	0.30	0.36	0.15	0.73	0.78	0.16	0.64
NPP			0.13	0.04	0.06	0.50	0.40	0.40	0.25	0.06	0.09	0.78	0.92	0.80
Pz			0.21	0.86	0.54	0.38	0.61	0.55	0.16	0.74	0.45	0.34	0.29	0.21
CM × NPP			0.14	0.18	0.11	0.80	0.72	0.71	0.17	0.12	0.09	0.50	0.15	0.22
CM × Pz			0.86	0.23	0.53	0.69	0.48	0.80	0.52	0.12	0.27	0.81	0.11	0.35
NPP × Pz			0.75	0.70	0.70	0.25	0.48	0.23	0.79	0.87	0.92	0.21	0.04	0.05
CM × NPP × Pz			0.55	0.02	0.11	0.51	0.76	0.70	0.88	0.02	0.25	0.49	0.06	0.16

[a, b] Means within column with different superscripts are significantly different ($P < 0.05$).
[1] The percentage of all cracked, broken soft-shelled and shellness egg of total laid eggs.
SEM = standard error of the mean.

abnormal eggs ($P < 0.05$). Production of abnormal eggs increased by feeding reduced NPP level ($P < 0.05$), and phytase supplementation reduced egg abnormality only when added to diets with reduced NPP level ($P < 0.05$). No effect of dietary CM was detected on abnormal eggs ($P > 0.05$).

Eggshell quality

Data on eggshell quality are shown in Table 4. At the first egg sampling (43rd weeks of age), shape index and shell thickness were increased by phytase supplementation ($P < 0.05$). A CM by phytase interaction was observed on eggshell weight ($P < 0.05$), indicating that the effect of phytase was dependent on the dietary CM level. A decreased eggshell weight was observed in birds fed corn-SBM diets ($P < 0.05$), but phytase supplementation reversed this adverse effect ($P < 0.05$). Additionally, there was a CM by NPP interaction on eggshell thickness ($P < 0.05$). Reduced NPP level caused a reduction in eggshell thickness in hens fed corn-SBM diet ($P < 0.05$), but not in those fed corn-SBM-CM diets. At the second egg sampling (47th weeks of age), a CM by phytase and a NPP by phytase interaction was observed on eggshell thickness ($P < 0.05$). Birds fed corn-SBM diets or diets with reduced NPP level produced eggs with lower shell thickness ($P < 0.05$), whereas dietary phytase supplementation compensate for these effects ($P < 0.05$). No effects of dietary treatments were detected on Haugh unit and egg specific gravity during the experiment ($P > 0.05$).

Eggshell ash and Ca and toe ash, Ca and P contents

As shown in Table 5, the ash and Ca contents of eggshell, and the ash, P and Ca contents of the toe were not affected by dietary treatments ($P > 0.05$).

Organ weights and serum T4 concentration

The effects of dietary treatments on organ weights and serum T4 concentration are presented in Table 5. No effect of dietary treatments was observed on organ weights ($P > 0.05$), with the exception of pancreas weight which was decreased by phytase supplementation ($P < 0.05$). Serum T4 concentration was similar for hens given diets containing adequate NPP level ($P > 0.05$), whereas reduced NPP level resulted in an increased serum T4 concentration in hens fed corn-SBM-CM diets as indicated by a CM by NPP interaction ($P < 0.05$). No abnormality was detected by histological comparison of the livers and in particular, there was no evidence of liver hemorrhages ($P > 0.05$).

Discussion

The results of the present study clearly showed that CM can be included in laying hen diets up to 16% during 39 to 47 weeks of age with no adverse effects on egg production, egg weight or egg mass. These results are similar to those reported by Janječić et al. (2009), who found that egg production and egg weight were not affected when CM was incorporated into laying hen diets at the rate of 8 or 16%. Perez-Maldonado and Barram (2004) reported that the replacement of SBM in the diets of laying hens with CM up to 15% had no negative effect on the hen performance, but when CM was substituted for SBM at 20%, a reduction in egg weight was observed. They speculated that the decrease in egg weight is due to a decrease in feed intake or more specifically, energy intake. However, in the present study, feed intake was significantly increased in birds fed the corn-CM-SBM diets, and this may explain the unchanged egg weight of these groups.

Dietary inclusion of CM and increased concentration of dietary NPP both resulted in increased NPP intake. Nonphytate-P intake of hens fed diets containing 0.25% NPP was around 2.4 g/hen/day, which was close to NRC (1994) value of 2.5 g/hen/day. However, when dietary NPP was 0.15%, NPP intake of hens ranged from 1.27 to 1.39 g/hen/day, which was much lower than dietary NPP requirement of laying hens (NRC, 1994). Nevertheless, reducing NPP level from 0.25 to 0.15% had no effect on measured performance criteria during the experiment, except for egg production which was significantly decreased when birds fed the diets containing reduced NPP level. Moreover, in the second 4-week of the experiment (44 to 47 weeks of age), both egg production and egg mass were lower in hens given corn-SBM diets with reduced NPP level. Our findings are in agreement with those of Lim et al. (2003) and Hughes et al. (2008), who reported decreased egg production in hens fed corn-SBM diets deficient in NPP. Phytase supplementation completely reversed this decline, in agreement with most previous reports (Boling et al., 2000; Keshavarz, 2003; Hughes et al., 2008). Ahmadi and Rodehutscord (2012) have used a meta-analytical approach to describe the relationship between dietary levels of NPP and phytase in laying hens. Their results revealed that diets containing 0.22% NPP without supplemental phytase produce high performance in regard to egg production, egg mass and FCR. In the presence of 150, 300, and 400 unit phytase/kg, the dietary NPP levels could be reduced, and optima were calculated as 0.18, 0.15 and 0.14%, respectively. Lim et al. (2003),

Table 4. Effects of different levels of dietary canola meal (%), nonphytate phosphorus (P, %) and phytase supplementation (unit/kg) on eggshell quality traits of laying hens

Items			Shape index (%)		Haugh unit		Shell weight (%)		Shell thickness		Specific gravity	
Canola meal (CM)	Nonphytate P (NPP)	Phytase (Pz)	wk 43	wk 47	wk 43	wk 47	wk 43	wk 47	wk 43	wk 47	wk 43	wk 47
0	0.15	0	85.1^{abc}	83.6	96.3	96.0	7.49^{c}	10.6	0.320^{b}	0.315^{bc}	1.067	1.070
0	0.15	450	88.7^{a}	88.7	94.0	94.0	10.03^{ab}	10.0	0.417^{a}	0.417^{a}	1.077	1.077
0	0.25	0	84.6^{bc}	85.2	99.3	96.3	8.76^{bc}	10.8	0.412^{a}	0.320^{bc}	1.072	1.077
0	0.25	450	85.3^{abc}	85.7	93.5	95.1	9.88^{ab}	11.8	0.428^{a}	0.362^{b}	1.080	1.083
8	0.15	0	86.0^{abc}	85.8	97.1	98.3	9.35^{bc}	11.0	0.423^{a}	0.320^{bc}	1.075	1.078
8	0.15	450	83.7^{bc}	87.5	94.0	99.3	10.00^{ab}	10.2	0.422^{a}	0.320^{bc}	1.078	1.075
8	0.25	0	82.1^{c}	87.5	97.5	97.1	11.36^{a}	11.3	0.395^{a}	0.362^{b}	1.075	1.082
8	0.25	450	84.7^{bc}	86.6	93.0	94.4	9.03^{bc}	10.3	0.373^{ab}	0.315^{bc}	1.072	1.075
16	0.15	0	84.6^{bc}	85.9	94.4	95.5	9.49^{ab}	10.0	0.397^{a}	0.332^{bc}	1.078	1.075
16	0.15	450	84.9^{abc}	85.5	89.3	93.9	10.06^{ab}	10.9	0.412^{a}	0.325^{bc}	1.077	1.078
16	0.25	0	83.1^{bc}	85.7	95.4	98. 6	9.03^{bc}	11.1	0.372^{ab}	0.357^{b}	1.073	1.077
16	0.25	450	86.3^{ab}	83.6	94.7	95.6	10.00^{ab}	10.9	0.440^{a}	0.295^{c}	1.080	1.070
Pooled SEM			0.37	0.41	0.95	0.54	0.193	0.17	0.0072	0.0059	0.0011	0.00010
Sources of variation			*P* values									
CM			0.10	0.24	0.58	0.34	0.11	0.95	0.78	0.06	0.41	0.56
NPP			0.09	0.59	0.47	0.99	0.43	0.10	0.71	0.76	1.00	0.39
P			0.05	0.45	0.07	0.14	0.10	0.73	0.04	0.63	0.06	1.00
CM × NPP			0.51	0.76	0.77	0.12	0.57	0.60	0.03	0.21	0.32	0.12
CM × Pz			0.47	0.13	0.97	0.86	0.01	0.26	0.10	<0.0001	0.18	0.06
NPP × Pz			0.24	0.07	0.97	0.52	0.06	0.80	0.57	0.008	1.00	0.25
CM × NPP × Pz			0.06	0.73	0.70	0.69	0.15	0.26	0.14	0.96	0.32	0.56

[a, b] Means within column with different superscripts are significantly different ($P < 0.05$).
SEM = standard error of the mean

Table 5. Effects of different levels of dietary canola meal (%), nonphytate phosphorus (P, %) and phytase supplementation (unit/kg) on eggshell ash and calcium (Ca) content, toe ash, P and Ca content, organ weights and serum thyroxine (T4) concentration of laying hens (47 weeks of age)

Items			Eggshell (% of dry weight)		Toe (% of dry weight)			Organ weights (% of live body weight)			Serum T4
Canola meal (CM)	Nonphytate P (NPP)	Phytase (Pz)	Ash	Ca	Ash	P	Ca	Abdominal	Pancreas	Liver	(mmol/L)
0	0.15	0	91.0	33.1	54.1	29.8	13.1	6.21	0.210^{a}	2.42	30.0^{abc}
0	0.15	450	94.9	32.2	58.7	30.6	13.2	4.45	0.145^{c}	2.06	28.6^{bc}
0	0.25	0	93.1	32.7	56.7	31.1	13.1	3.94	0.200^{a}	2.22	30.0^{abc}
0	0.25	450	91.4	33.9	60.0	31.3	13.2	6.01	0.145^{c}	2.09	41.2^{a}
8	0.15	0	91.5	33.5	58.6	30.7	13.1	4.28	0.165^{b}	2.12	38.3^{ab}
8	0.15	450	91.7	33.2	55.5	27.6	12.8	4.59	0.155^{bc}	1.85	36.7^{abc}
8	0.25	0	91.0	32.6	54.2	30.9	13.2	4.29	0.210^{a}	2.16	31.6^{abc}
8	0.25	450	90.6	33.1	57.1	26.3	13.1	4.17	0.205^{a}	2.50	26.8^{c}
16	0.15	0	93.2	34.1	58.0	31.0	13.1	3.81	0.210^{a}	2.16	40.1^{a}
16	0.15	450	93.3	32.2	53.5	31.3	13.0	4.60	0.195^{a}	2.25	38.0^{ab}
16	0.25	0	94.0	31.6	59.5	31.4	13.1	4.50	0.210^{a}	1.90	30.3^{abc}
16	0.25	450	93.6	33.8	54.0	24.7	12.9	6.33	0.170^{b}	2.42	37.9^{abc}
Pooled SEM			0.41	0.37	0.68	0.65	0.05	0.239	0.0071	0.056	1.14
Sources of variation			*P* values								
CM			0.09	0.98	0.75	0.54	0.47	0.35	0.41	0.96	0.20
NPP			0.70	0.90	0.70	0.54	0.64	0.64	0.44	0.53	0.23
P			0.72	0.89	0.78	0.12	0.52	0.26	0.03	0.78	0.44
CM × NPP			0.77	0.81	0.60	0.46	0.69	0.32	0.15	0.25	0.01
CM × Pz			0.79	0.99	0.06	0.38	0.61	0.48	0.27	0.17	0.23
NPP × Pz			0.18	0.17	0.65	0.27	0.81	0.12	0.90	0.08	0.11
CM × NPP × Pz			0.39	0.73	0.48	0.57	0.87	0.18	0.83	0.78	0.22

$^{a, b}$ Means within column with different superscripts are significantly different ($P < 0.05$).
SEM = standard error of the mean

however, reported in a study employing a factorial arrangement of NPP levels and phytase that feeding a 0.15% NPP without supplemental phytase decreased egg production, and phytase supplementation was not able to compensate for this effect. Reason for this difference is not clear; however, this may be related to the higher dietary content of Ca (4.0%) that employed in their study. The efficacy of phytase supplementation on egg production seems to be mediated not only by dietary NPP level but also by dietary Ca level (Roberts, 2004).

No significant effect of lowering NPP level was observed on production performance of hens fed corn-SBM-CM diets. In general, few studies have been done determining P requirement of laying hens fed diets containing CM. Thomas et al. (1983) in their early study observed no significant difference in either egg production or egg mass when laying hens were kept in floor pens and fed diets containing 10, 20 or 25% CM and 0.44, 0.49 or 0.50% total P, respectively. Although the diets which contained 20 and 25% CM had the lowest rate of production they were not significantly different from the controls. They concluded that a level of 0.47% total P in laying hen diets, with all of the P derived from plant sources, was sufficient to meet the dietary P requirements of laying hens, which is remarkably close to levels applied in the present study. Surprisingly, Phytase supplementation decreased egg production and egg mass of hens fed diet containing 16% CM and reduced NPP level. Such a response to dietary phytase is difficult to explain and this requires further study.

In the second 4-week (44 to 47 weeks of age) and overall experimental period (39 to 47 weeks of age), production of abnormal eggs were increased by feeding diets with reduced NPP level and phytase supplementation decreased egg abnormality when added to these diets. These results are in agreement with those of Lim et al. (2003) and Hughes et al. (2008) who reported that the addition of phytase to diets with 0.15% NPP decreased production of abnormal eggs. No significant effect of dietary CM was detected on production of abnormal eggs, in agreement with the results reported by other researchers (Riyazi et al., 2009).

Eggshell thickness was affected in a similar manner to that observed for production of abnormal eggs. At the first egg sampling (43rd weeks of age), egg shape index and shell thickness were increased by phytase supplementation. Reduced NPP level decreased eggshell thickness in hens fed the corn-SBM diets. Similarly, at the second sampling period (47th weeks of age), birds fed corn-SBM diets or reduced NPP level produced eggs with lower shell thickness, whereas dietary phytase supplementation reserved these adverse effects. Moreover, a decreased eggshell weight was observed in birds fed corn-SBM diets, but phytase supplementation offset this effect. No significant effects of dietary treatments were found on egg specific gravity and Haugh units during the experiment. These results seem contradictory. Specific gravity is an indirect measure of shell thickness and strength and, therefore, $CaCO_3$ deposition (Roberts, 2004). An eggshell could be considered thin with a specific gravity of 1.070 or less, while a shell with a specific gravity of 1.090 would be considered thick. A typical egg would have a shell with a specific gravity of at least 1.080 (Zeidler, 2001). The specific gravity values found in the present study were not significantly different from 1.080, which indicated that the strength of the shell was that of a typical egg. Moreover, dietary treatments did not have any significant influence on eggshell ash or Ca contents. Similar mixed results have been reported in the literature. Boling et al. (2000) showed that feeding diets containing 0.10, 0.15 or 0.45% NPP without or with phytase supplementation had no effect on specific gravity, whereas Zaghari (2009) showed no significant difference in Haugh unit, egg specific gravity, eggshell thickness and breaking strength or eggshell ash and Ca contents when diet of laying hens was supplemented by 300 unit phytase/kg. In contrast, Lim et al. (2003) reported that specific gravity and eggshell thickness were greater with low NPP, whereas percentage of broken and soft-shell eggs were higher with 0.15% than with 0.25% NPP.

Toe ash has been shown to be a good measurement of P status and accurate in determining P availability for poultry. Cabahug et al. (1999) demonstrated that less NPP is required for maximizing performance compared with increased toe and tibia ash. In the present study, dietary treatments had no significant effect on toe ash or toe P and Ca contents. This indicated that toe mineral content was not depleted even when the NPP concentration of the diets decreased up to 0.15%. Our results are in general agreement with the results of Thomas et al. (1983) who reported no difference in egg production and the degree of bone (toe and tibia) calcification when hens fed corn-SBM-CM diets containing different levels of P. In addition, as in the present experiment, Zaghari (2009) found no significant difference in toe ash, P and Ca contents when diet of laying hens was supplemented by 300 unit phytase/kg.

There was no significant effect of dietary CM on liver, pancreas and abdominal fat weights, which is in agreement with the report of Perez-Maldonado and Barram (2004) and contrary to that of Marangos and Hill (1976), who indicated that liver weight increased when CM were included in the diet. Likewise, NPP level had no

significant effect on organ weights, whereas pancreas weight was decreased by phytase supplementation. The decrease in pancreas weight could be related to a decrease in endogenous enzyme activities and secretion volume required to digest SBM and CM, as reported earlier (Butler et al., 1982).

Serum T4 level was similar for hens given diets containing adequate NPP level, in agreement with previous reports (Marangos and Hill, 1976; Ramesh et al., 2006). However, Marangos and Hill (1976) reported a significant thyroid enlargement when diet containing 12% CM was fed during the early laying period. They suggested that the requirements for T4 are greater for egg production than for growth and that they were likely to be more critical during the early months of production. The serum T4 concentration significantly increased when hens fed corn-SBM-CM diets with reduced NPP level. Such a change in serum T4 concentration due to reduced dietary NPP level has not been reported before.

Conclusions

The results of the present study showed that CM can be included in laying hen diets up to 16% during 39 to 51 weeks of age without any adverse effect on their health and productivity. Reduced NPP level decreased production performance and eggshell quality when hens fed corn-SBM diets, while the negative effects of reduced NPP level on performance and eggshell quality of laying hens were negligible when CM included in the diets. The adverse effects of reduced NPP level in corn-SBM diets could be substantially overcome by phytase supplementation.

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پاسخ مرغ‌های تخم‌گذار به استفاده از کنجاله کانولا در جیره با توجه سطح فسفر غیرفیتاتی جیره و استفاده از مکمل فیتاز میکروبی

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چکیده یک آزمایش فاکتوریل شامل سه سطح (۰، ۸ و ۱۶ درصد) کنجاله کانولا، دو سطح (۰/۱۵ و ۰/۲۵ درصد) فسفر غیرفیتاتی و دو سطح (۰ و ۴۵۰ واحد/کیلوگرم) فیتاز میکروبی با استفاده از ۲۱۶ مرغ تخم‌گذار سویه های-لاین دبلیو-۳۶ از سن ۳۹ تا ۴۷ هفتگی انجام شد. پرنده‌های دریافت‌کننده کنجاله کانولا در مقایسه با پرنده‌های دریافت‌کننده جیره ذرت-کنجاله سویا خوراک بیشتری مصرف کردند ($P < ۰/۰۵$). در چهار هفته دوم آزمایش (سن ۴۴ تا ۴۷ هفتگی)، تولید تخم‌مرغ و تولید توده‌ای تخم‌مرغ در پرنده‌های دریافت‌کننده جیره ذرت-کنجاله سویای با سطح پایین فسفر غیرفیتاتی کمتر بود ($P < ۰/۰۵$). اما اثرات نامطلوب سطح پایین فسفر غیرفیتاتی با استفاده از مکمل فیتاز برطرف شد ($P < ۰/۰۵$). در طول چهار هفته دوم آزمایش (سن ۴۴ تا ۴۷ هفتگی) و کل دوره آزمایش (سن ۳۹ تا ۴۷ هفتگی)، تولید تخم‌مرغ‌های نامطلوب با تغذیه سطح پایین فسفر غیرفیتاتی کاهش یافت ($P < ۰/۰۵$) و مکمل فیتاز تنها زمانی تولید تخم‌مرغ‌های نامطلوب را کاهش داد که به این جیره‌ها اضافه شد ($P < ۰/۰۵$). در نمونه‌برداری اول تخم‌مرغ (سن ۴۳ هفتگی)، شاخص شکل تخم‌مرغ و ضخامت پوسته تخم‌مرغ با استفاده از مکمل فیتاز افزایش پیدا کرد ($P < ۰/۰۵$). استفاده از سطح پایین فسفر غیرفیتاتی ضخامت پوسته تخم‌مرغ پرنده‌های دریافت‌کننده جیره ذرت-کنجاله سویا را کاهش داد ($P < ۰/۰۵$). در نمونه‌برداری دوم تخم‌مرغ (سن ۴۷ هفتگی)، پرنده‌های دریافت‌کننده جیره ذرت-کنجاله سویا و پرنده‌های دریافت‌کننده سطح پایین فسفر غیرفیتاتی تخم‌مرغ‌هایی با ضخامت پوسته کمتر تولید کردند ($P < ۰/۰۵$)، در حالی که مکمل فیتاز این اثرات را برطرف کرد ($P < ۰/۰۵$). استفاده از سطح پایین فسفر غیرفیتاتی غلظت تیروکسین را در پرنده‌های دریافت کننده جیره‌های ذرت-کنجاله سویا-کنجاله کانولا افزایش داد ($P < ۰/۰۵$). نتایج نشان داد که کنجاله کانولا می‌تواند تا ۱۶ درصد در جیره مرغ‌های تخم‌گذار ۳۹ تا ۴۷ هفته‌ای اضافه شود بدون آن که اثر نامطلوبی بر تولید و سلامت آنها داشته باشد. به علاوه، نتایج نشان داد که کاهش سطح فسفر غیرفیتاتی در جیره‌های ذرت-کنجاله سویا-کنجاله کانولا اثر نامطلوب اندکی بر عملکرد و کیفیت پوسته تخم‌مرغ مرغ‌های تخم‌گذار دارد. اثرات نامطلوب کاهش سطح فسفر غیرفیتاتی در جیره‌های ذرت-کنجاله سویا تا حد زیادی با استفاده از مکمل فیتاز برطرف شد.