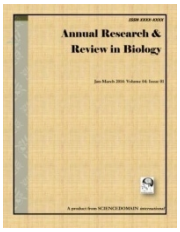

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Effects of Phytase Supplementation of Low Protein Diets on Performance, Egg Quality Traits and Blood Biochemical Parameters of Laying Hens

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Authors' contributions

Major contribution for this study came from author MT and he carried out this study with the other assistants. Author AM took part in planning of the study, did the poultry examination, discussed the results and commented on the draft and final manuscript. Author AM carried out this study and collected the data. Author SK took part in the data collection. Author MH took part in preparation of the final manuscript.

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ABSTRACT

Effects of phytase supplementation of low protein diets on performance, egg quality traits and blood biochemical parameters of laying hens were evaluated by using 216 Lohmann LSL-Lite hens. Birds were randomly divided in 36 cages (n=6). Based on a 3×2 factorial arrangement of treatments, 6 iso-caloric experimental diets consisting three levels of crude protein (CP, 150, 138, and 126 g/kg) and phytase (0 and 300 FTU/kg) were formulated and fed to hens with 6 replicates per diet. Collected data of feed intake (FI), egg production (EP), egg mass (EM) and calculated feed conversion ratio (FCR), egg quality traits and blood parameters during 7-wk trial period were analyzed based on completely randomized design. Decreasing dietary crude protein significantly decreased EP, EM and FI and increased FCR ($P < .05$). In the first egg sampling (wk 3) egg index, yolk index, yolk color, egg gravity, shell weight and shell thickness were not significantly affected by dietary treatment ($P > .05$). Decreasing dietary CP significantly increased Haugh unit compared to the control group. In the second egg sampling period (wk 7), Haugh unit significantly

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decreased in the hens fed low protein diets compared to the control group ($P < .05$). Phytase supplementation did not have any beneficial effect on productive performance of laying hens and egg quality traits ($P > .05$). There was no interaction between protein level and phytase on egg traits except for egg index ($P < .05$). There was no interaction between CP levels and phytase on blood parameters except for Heterophil count ($P < .01$). Interaction between protein levels and phytase on lymphocyte as well as heterophil to lymphocyte (H/L) ratio was significant ($P < .05$). In conclusion, feeding low CP diets significantly decreased blood levels of cholesterol, triglycerides and LDL in compared to the control group ($P < .05$).

Keywords: Low protein, phytase, laying hens, performance, egg quality, blood parameters.

1. INTRODUCTION

The cost of protein and energy has fluctuated dramatically for the feed industry during the last few years. Therefore, feed formulation practices are directed toward economic analysis rather than optimal bird performance [1]. The excretion of nitrogen (N) originating from dietary protein is largely responsible for the environmental issues arisen from intensive livestock production [2]. In response, dietary means to decrease the impact on the environment of intense livestock production have successfully been implemented; one of which is the partial replacement of intact protein (e.g., soybean meal) with crystalline, free amino acids (AA). Through this replacement, excesses of dietary AA are minimized in relation to their requirement, bringing the dietary protein closer to ideal protein and, in turn, decreasing the dietary CP content. The use of low-protein, AA-fortified diets for various classes of poultry has been the subject of numerous investigations. However, these investigations, for the most part, had limited practical application at the time because, with the exception of methionine and lysine, other essential amino acids were not available to the feed industry. Due to technological advances, threonine and tryptophan have become economically available in recent years, and there is a good possibility that others will be commercially available in the future. These advances, combined with recent emphasis on reducing the excretion of N and phosphorous (P) by farm animals to protect the environment, have revived interest in the use of low-protein diets for various kinds of poultry. Recent reports indicated that promising results can be obtained by the use of low-protein, AA-supplemented diets for laying hens. In a majority of these reports, however, productive performance still remained inferior to the control groups that were fed diets with conventional crude protein levels [3,4]. The phytate-protein complexes may reduce the utilization of the protein and AA [5]. Dietary supplemental phytase improved the utilization of protein [6] and nitrogen retention [7] in broiler chicks.

Phosphorus is a critical and expensive mineral in poultry nutrition. Animal protein supplements are rich in P whose availability is generally considered as 1.0 while the availability of P from vegetable protein supplements is only about 0.30 [8]. Vegetable protein supplements are being currently used in increased quantity in place of animal protein supplements due to high cost, non-availability and presence of *E. coli* and Salmonella in animal protein supplements. The major portion of P in plant feed ingredients is found in the form of phytate which is largely unavailable to monogastric animals. The interest in the use of microbial feed enzymes such as phytase arises from the need to improve the availability of phytate bound P and reduce the P levels in effluent from intensive livestock operations. The effectiveness of microbial phytase in releasing a significant portion of bound P and

improving P bioavailability in poultry diets is well documented [9]. Over the past 15 years, phytase enzymes [10] have been introduced to the poultry feed industry to increase the availability of P from phytate to the bird, thus reducing the environmental costs of poultry production [11]. A number of studies have demonstrated that use of microbial phytase supplementation in feeding poultry has the ability to hydrolysis, releasing phytic acid in phosphate form [8] and adding microbial phytase in laying hens diet improves phytate P utilization and productive performance [12–17]. The results of a number of research studies with laying hens have shown that a diet with 0.1-0.13% available P (AP) in the presence of 100-300 units phytase can result in comparable performance to the control group which was fed a normal level of 0.4-0.45% AP. Much of the 16 to 18 g P per kg rice bran occurs as phytic acid P. Not only is the availability of P very low (< 18%) in rice bran [18,19] but some other minerals can be complexed with phytate and their availability reduced [20]. Farrell et al. [7] reported the significant beneficial responses when a microbial food phytase is added to chicken and duckling diets based on sorghum and soybean meal. They reported increased availability of dietary P. In addition, the apparent metabolizable energy of the diet and in nitrogen retention increased. Although it appears that poultry can utilize plant P in a bound form to a limited extent, this is unlikely to be of significance in the young bird [20]. As reviewed by Selle and Ravindran [11], the ramifications of phytate in poultry diets are numerous but essentially, phytases liberate phytate-bound phosphorus (P) and reduce P excretion, which is of ecological importance. Also, Francesch et al. [21] and Jalal and Scheideler [14] saw an improvement in egg production (EP), hen weight gain, feed conversion (FCR), egg mass (EM) and feed intake (FI) in hens that were fed a diet low in non phytate P (NPP) with supplementary phytase when compared to hens fed a low non-phytate phosphorous (NPP) diet without supplemental phytase. Wu et al. [22] reported that Phyzyme or Natuphos supplementation into diets containing 0.11% NPP reduced significantly the excreta P with no adverse effects on egg production and egg mass. Plumstead [23] studied the effects of varying dietary NPP level with or without added phytase enzyme on performance of broiler breeders from 29 to 64 wk of age.

The objectives of the present study were to investigate effects of phytase supplementation of low protein diets on performance of laying hens.

2. MATERIALS AND METHODS

All procedures used in this 7-wk experiment were approved by the Animal Ethics Committee of Razi University and complied with the "Guidelines for the Care and Use of Animals in Research".

2.1 Birds and Experimental Diets

A total number of 216 56-wk-old Lohmann LSL-Lite hens with an average egg production rate of 90.6±4.8% (late laying phase) and 1460±24 g live body weight, were obtained from a commercial supplier. After a wk of adaptation, the hens were allocated randomly to one of four experimental diets. Hens were semi-randomly distributed between 36 cages (n=6). The hens were placed in individual wire-floored cages (0.3 m wide×0.4 m length×0.4 m height) arranged in a single tier within a conventional open-sided house. The cages were located in a windowless and environmentally controlled room with the room temperature kept at 21-23°C and the photoperiod set at 16 h of light (incandescent lighting, 10 lx) and 8 h dark. Each cage had a nipple watered. Water was available *ad libitum* throughout the experiment. Feed intake was measured on a weekly basis.

Based on a 3×2 factorial arrangement of treatments, 6 iso-caloric experimental diets [8] consisting three levels of crude protein (150, 138, and 126 g/kg) and two levels of phytase (0 and 300 phytase unit (FTU)/kg of feed [of an *Escherichia coli*-derived phytase (Phyzyme XP, Danisco Animal Nutrition, Wiltshire, UK]) were formulated and fed to hens with 6 replicates per diet. Ingredients and composition of the experimental diets are shown in Table 1.

Table 1. Ingredients and composition of the experimental diets

Diets Label (CP %)	100	100	92	92	94	94
Feed ingredients (%)						
Corn	65.43	65.48	66.47	66.52	67.51	67.56
Fish meal	4.60	4.60	4.60	4.60	4.60	4.60
Soybean meal (48% CP)	13.25	13.27	9.76	9.78	6.27	6.28
Rice bran	6.34	6.22	8.72	8.61	11.11	10.99
Dicalcium phosphate	1.25	1.25	1.28	1.28	1.31	1.31
Lime stone	8.33	8.33	8.33	8.33	8.33	8.33
Common salt	0.22	0.22	0.22	0.22	0.21	0.21
Phytase	0	0.05	0	0.05	0	0.05
Vit. & Min. Premix[1]	0.50	0.50	0.50	0.50	0.50	0.50
DL-Methionine	0.08	0.08	0.12	0.12	0.15	0.15
Calculated analyses						
ME (Kcal/kg)	2720	2720	2720	2720	2720	2720
Crude protein (%)	15	15	13.80	13.80	12.60	12.60
Calcium (%)	3.79	3.79	3.79	3.79	3.79	3.79
Available phosphorus (%)	0.52	0.52	0.53	0.53	0.55	0.55
Sodium (%)	0.15	0.15	0.15	0.15	0.15	0.15
Methionine (%)	0.32	0.32	0.30	0.30	0.28	0.28
Methionine + cysteine (%)	0.45	0.45	0.45	0.45	0.43	0.43
Lysine (%)	0.83	0.83	0.73	0.73	0.63	0.63

[1] Mineral mix supplied the following per kg of diet: Cu, 20 mg; Fe, 100 mg; Mn, 100 mg; Se, 0.4; Zn, 169.4 mg. 'Vitamins mix supplied the following per kg of diet: Vitamin A, 18,000 IU; vitamin D3, 4,000 IU; vitamin E, 36mg; vitamin K; 4 mg; vitamin B\2, 0.03 mg; thiamine, 1.8 mg; riboflavin, 13.2 mg; pyridoxine, 6 mg; niacin, 60 mg; calcium pantothenate, 20 mg; folic acid, 2 mg; biotin, 0.2 mg; choline chloride, 500 mg.

2.2 Egg Quality Variables and Blood Parameters

Egg quality characteristics were measured twice on wk 3 and 7 of experiment and each time all eggs during three frequent d were used. At the end of the experiment (wk 7), four hens were selected randomly from each treatment (one hen per replicate) and blood samples were collected from the wing vein into a 5-ml syringe. Part of the blood which had been obtained having been centrifuged (3000×g for 15 min) immediately and serum collected for subsequent analysis, the rest was placed in tubes with heparin as anticoagulant in order to diacritical counts of white blood cells based on the procedures of Gross and Siegel [24]. Briefly, two drops of blood were placed on a slide, spin prepared and stained with May-Grünwald-Giemsa stain. All slides were coded and one hundred leukocytes, including granular (heterophils, eosinophils, and basophils) and nongranular (lymphocytes and monocytes) were counted on one slide per each bird, and the heterophil to lymphocyte (H/L) ratio was calculated. Serum triglycerides, high density lipoprotein (HDL)-cholesterol, low density lipoprotein (LDL)-cholesterol, and total cholesterol were analyzed using the diagnostic kit (Pars Azmun, Iran), and enzymatic methods.

2.3 Statistical Analysis

Data were analyzed based on 3×2 factorial arrangements in completely randomized design using GLM procedure of SAS [25]. All statements of significance are based a probability of less than 0.05. The mean values were compared by Duncan's least significance multiple-range test.

3. RESULTS AND DISCUSSION

3.1 Productive Performance

The effects of dietary treatments on the performance of laying hens are presented in Tables 2 to 5. Dietary protein levels did significantly affect to EP, EM, FI and FCR ($P < .05$). Feeding low protein diets (138 and 126 g/kg) decreased EP, EM and FI but increased FCR ($P < .05$). These results are consistent with previous reports [26–28]. Calderon and Jensen [29] and Jensen et al [30] reported that performance of hens fed 13 or 14% protein diets were inferior to those fed higher levels of protein, although birds on low-protein diets consumed adequate amounts of the essential amino acids. These authors of the previously mentioned studies speculated that the inferior performance of birds on low-protein diets was due to the low availability of several essential amino acids in the protein sources used in the diets. Keshavarz and Austic [28] showed that adding phytase to the 130 g/kg CP diet containing 0.2 g/kg NPP and supplemented with methionine and lysine resulted in efficiency of feed conversion and egg size that were not different from those of the control (165 g/kg CP diet containing 0.4 g/kg NPP). Yonemochi et al. [31] also reported that dietary supplementation of phytase not only improved the utilization of phytate P but also reduced adverse effect of low protein diets on broilers performance. However, in the present study, phytase supplementation did not have any beneficial effect on EP, EM, FI, and FCR ($P > .05$). This may indicate that the level of decreasing of CP in the present study was high or the capability of phytase in overcoming this kind of adverse effects was not high enough. No significant interaction between dietary CP levels and phytase supplementation were seen on performance parameters ($P > .05$).

Table 2. Effects of dietary crude protein level (150, 138, and 126 g/kg) and phytase supplementation (0 and 300 FTU/kg) on hen-d egg production (%) of laying hens (wk 56-63 of age)[1]

Treatments	wk				
	1	2-3	4-5	6-7	1-7
Protein (P)					
150	78.97±6.41	86.61±4.98[a]	87.40±5.19[a]	89.58±3.33[a]	86.59±3.90[a]
138	77.18±8.52	78.07±5.71[b]	73.11±8.22[b]	77.18±6.69[b]	76.27±4.57[b]
126	74.40±8.86	73.02±9.54[b]	62.26±11.78[c]	63.09±8.39[c]	67.31±7.99[c]
Phytase (E)					
0	87.57±6.93	77.31±9.53	73.94±15.28	75.53±13.04	76.02±10.29
300	75.13±8.80	81.15±8.00	74.58±11.91	77.71±12.54	77.43±9.44
SEM	1.33	1.48	2.25	2.11	1.63
CV	10.06	8.99	11.76	8.73	7.73
Sources of variation	P values				
Protein (P)	.37	.001	.001	.001	.001
Phyatse (E)	.20	.11	.83	.34	.48
P × E	.28	.77	.21	.98	.72

[1]Means (±SD), [ab]Means within column (main effects) with different superscripts are significantly different (P < .05), Duncan's least significance multiple-range test were applied to compare means.

Table 3. Effects of dietary crude protein level (150, 138, and 126 g/kg) and phytase supplementation (0 and 300 FTU/kg) on egg mass (EM, g/hen/d) of laying hens (wk 56-63 of age)[1]

Treatments	wk				
	1	2-3	4-5	6-7	1-7
Protein (P)					
150	46.21±4.11	51.73±3.46[a]	52.64±3.51[a]	53.28±2.98[a]	51.64±2.93[a]
138	45.27±5.10	46.44±3.57[b]	43.17±5.08[b]	44.58±3.86[b]	44.81±2.72[b]
126	44.63±5.63	43.02±3.98[b]	36.55±7.23[c]	36.38±4.92[c]	39.50±4.91[c]
Phytase (E)					
0	46.63±4.53	46.00±6.32	44.02±9.65	44.14±8.32	44.99±6.66
300	44.11±5.03	48.12±4.89	44.22±7.59	45.35±7.88	45.64±5.80
SEM	0.81	0.95	1.43	1.33	1.03
CV	10.95	9.56	12.44	9.24	8.30
Sources of variation	P values				
Protein (P)	.74	.001	.001	.001	.001
Phyatse (E)	.14	.17	.92	.39	.61
P × E	.61	.66	.24	.97	.64

[1]Means (±SD), [ab]Means within column (main effects) with different superscripts are significantly different (P < .05), Duncan's least significance multiple-range test were applied to compare means.

Table 4. Effects of dietary crude protein level (150, 138, and 126 g/kg) and phytase supplementation (0 and 300 FTU/kg) on feed intake (FI, g/hen/d) of laying hens (wk 56-63 of age)[1]

Treatments	wk				
	1	2-3	4-5	6-7	1-7
Protein (P)					
150	117.61±1.89[a]	109.69±4.75[a]	110.44±3.96[a]	117.05±3.02[a]	113.14±2.76[a]
138	111.63±3.61[b]	102.56±9.09[b]	103.21±7.57[b]	111.04±696[b]	106.46±5.52[b]
126	117.68±2.70[a]	108.29±6.28[a]	100.70±4.75[b]	110.08±4.62[b]	107.98±3.93[b]
Phytase (E)					
0	115.39±4.29	105.71±8.47	105.16±7.81	113.01±6.51	109.02±5.99
300	115.89±3.74	107.98±6.27	104.40±606	112.44±5.36	109.36±3.99
SEM	0.66	1.24	1.15	0.98	0.84
CV	2.49	6.42	5.52	4.79	4.02
Sources of variation	P values				
Protein (P)	.001	.03	.001	.001	.001
Phyatse (E)	.60	.32	.67	.75	.81
P × E	.36	.17	.50	.82	.46

[1]Means (±SD), [ab]Means within column (main effects) with different superscripts are significantly different (P < .05), Duncan's least significance multiple-range test were applied to compare means.

Table 5. Effects of dietary crude protein level (150, 138, and 126 g/kg) and phytase supplementation (0 and 300 FTU/kg) on feed conversion ratio (FCR, g feed: g egg) of laying hens (wk 56-63 of age)[1]

Treatments	wk				
	1	2-3	4-5	6-7	1-7
Protein (P)					
150	2.56±0.25	2.13±0.12[b]	2.11±0.12[c]	2.21±0.12[c]	2.21±0.10[b]
138	2.50±0.35	2.23±0.26[b]	2.43±0.21[b]	2.50±0.22[b]	2.40±0.17[b]
126	2.67±0.33	2.58±0.44[a]	2.87±0.61[a]	3.09±0.40[a]	2.82±0.39[a]
Phytase (E)					
0	2.50±0.25	2.36±0.43	2.50±0.54	2.66±0.52	2.50±0.40
300	2.66±0.35	2.27±0.26	2.43±0.44	2.54±0.39	2.45±0.31
SEM	0.052	0.059	0.081	0.076	0.059
CV	11.92	13.78	15.66	10.66	10.45
Sources of variation	P values				
Protein (P)	.39	.001	.00	.00	.00
Phyatse (E)	.11	.39	.60	.23	.54
P × E	.40	.82	.49	.63	.71

[1]Means (±SD), [ab]Means within column (main effects) with different superscripts are significantly different (P < .05), Duncan's least significance multiple-range test were applied to compare means.

3.2 Egg Quality Traits

Tables 6 to 10 show the results of egg quality traits. There was no statistically significant difference ($P > .05$) in egg abnormality percentage (the percentage of all broken, cracked and shellness egg of total laid eggs) among the dietary treatments (Table 6). No significant effects of dietary treatments were found on yolk color, egg gravity, shell weight and shell thickness ($P > 0.05$) during both the first and second sampling periods (Tables 8 and 10). The results of the present study are in accordance with those of Keshavarz [27], who reported no difference in eggshell strength as a result of different dietary CP levels in laying hens. Our results also are in agreement with the results of Novak et al. [32], which showed that feeding a low-protein diet to laying hens did not influence shell and internal egg quality measures. Conversely, Punna and Roland [33], Narahari and Jayaprasad [15] and Metwally [34] found a beneficial effect of phytase supplementation on shell quality.

Table 6. Effects of dietary crude protein level (150, 138, and 126 g/kg) and phytase supplementation (0 and 300 FTU/kg) on egg an abnormality (the percentage of all broken, cracked and shellness egg of total laid eggs) of laying hens (wk 56-63 of age)[1]

Treatments	wk		
	1-3	**4-7**	**1-7**
Protein (P)			
150	0.28±0.72	0.78±0.98	0.57±0.78
138	0.67±0.84	0.91±1.04	0.81±0.88
126	0.57±0.72	0.54±0.68	0.55±0.53
Phytase (E)			
0	0.38±0.64	0.64±0.86	0.53±0.68
300	0.63±0.86	0.85±0.94	0.76±0.79
SEM	0.13	0.15	0.12
CV	142.01	122.66	113.52
Sources of variation	P values		
Protein (P)	.39	.61	.63
Phyatse (E)	.29	.49	.35
P × E	.07	.31	.17

[1]*Means (±SD)*

In a previous study, Novak et al. [32] reported that Haugh unit was not affected during 20 to 44 wk of age but was increased by decreasing dietary protein during 44 to 63 wk of age. Leeson and Caston [35] reported similar responses for Haugh units when feeding low protein diets. In our present study and during the first sampling period (Table 7), Haugh unit was significantly higher ($P < .05$) for birds fed the 126 g/kg CP diet when compared with the other diets. However, in the second sampling period (Tables 9), Haugh unit was significantly lower ($P < .05$) for birds fed the 126 g/kg CP diet when compared with the other diets. On the other hand, Hamilton [36] observed no considerable change in Haugh units when feeding low protein diets to 4 different strains of laying hens. The reason for the discrepancy between our results and theirs as regard the changes of Haugh unit in response to dietary protein using even the relatively equal levels of CP is not yet understood.

There was interaction between dietary CP level and phytase supplementation on egg index ($P < .05$) and yolk index ($P < .05$) in the second sampling period. Statistical analysis based

on 6 separated dietary groups indicated that birds receiving 138 g/kg CP diet had significantly lower yolk index compared with control ($P < .05$). Similarly, birds receiving 138 g/kg CP diet supplemented with phytase had significantly lower egg index compared with control ($P < .05$). No significant difference between treatments was found on yolk color, egg gravity, shell weight and shell thickness ($P > 0.05$). These results are consistent with of those of Yildiz et al. [37], who showed no effect of phytase supplementation on egg index and egg yolk index in laying hens fed diet containing normal protein level (16% CP). More studies must be done to clarify the effects of dietary protein and phytase levels on egg quality traits of laying hens.

Table 7. Effects of dietary crude protein level (150, 138, and 126 g/kg) and phytase supplementation (0 and 300 FTU/kg) on egg quality characteristics of laying hens in first sampling period (wk 3 of the experiment, wk 59 of age)[1]

Treatments	Egg index	Yolk index	Haugh unit
Protein (P)			
150	75.87±1.64	40.44±1.87	67.04±5.39[b]
138	75.18±1.35	38.63±4.26	67.44±3.57[b]
126	75.46±1.81	35.73±7.74	73.20±7.54[a]
Phytase (E)			
0	75.72±1.68	38.46±5.11	69.39±6.99
300	75.29±1.51	38.08±5.88	69.07±5.64
SEM	0.26	0.90	1.04
CV	2.12	14.12	8.59
Sources of variation		P values	
Protein (P)	.58	.12	.03
Phyatse (E)	.42	.84	.87
P × E	.27	.70	.72

[1]Means (±SD), [ab]Means within column (main effects) with different superscripts are significantly different (P < .05), Duncan's least significance multiple-range test were applied to compare means.

Table 8. Effects of dietary crude protein level (150, 138, and 126 g/kg) and phytase supplementation (0 and 300 FTU/kg) on egg quality characteristics of laying hens in first sampling period (wk 3 of the experiment, wk 59 of age)[1]

Treatments	Yolk color	Gravity	Shell weight (g)	Shell
Protein (P)				
150	6.22±0.38	1.09±0.00	5.94±0.34	36.25±1.84
138	6.53±0.33	1.09±0.00	6.16±0.53	36.75±2.05
126	6.47±0.86	1.09±0.00	5.97±0.36	36.11±1.47
Phytase (E)				
0	6.30±0.50	1.09±0.00	6.00±0.35	36.48±1.45
300	6.52±0.64	1.09±0.00	6.04±0.49	36.26±2.09
SEM	0.10	0.00	0.07	0.30
CV	9.08	0.33	7.22	4.97
Sources of variation		P values		
Protein (P)	.40	.43	.42	.66
Phyatse (E)	.26	.70	.77	.71
P × E	.60	.82	.68	.27

[1]Means (±SD).

Table 9. Effects of dietary crude protein level (150, 138, and 126 g/kg) and phytase supplementation (0 and 300 FTU/kg) on egg quality characteristics of laying hens in second sampling period (wk 7 of the experiment, wk 63 of age)[1]

Treatments		Egg index	Yolk index	Haugh unit
Protein (P)				
150		75.06±1.72	42.06±1.39	67.65±2.51[a]
138		74.62±1.54	41.14±4.09	65.39±7.77[ab]
126		75.14±1.63	42.62±1.43	62.18±3.09[b]
Phytase (E)				
0		75.22±1.35	41.48±3.43	66.68±5.72
300		74.66±1.82	42.40±1.39	63.47±4.67
SEM		0.27	0.44	0.90
CV		1.96	5.67	7.16
Sources of variation			P values	
Protein (P)		.64	.32	.02
Phyatse (E)		.27	0.25	.05
P × E		.02	.02	.13
P	E			
1	0	75.86±1.56[a]	43.15±0.74[a]	
1	1	74.26±1.59[ab]	40.97±0.93[ab]	
2	0	75.43±7.33[ab]	39.37±5.35[b]	
2	1	73.81±1.74[b]	42.92±0.74[a]	
3	0	74.36±1.31[ab]	41.92±1.40[ab]	
3	1	75.93±1.62[a]	43.32±1.59[a]	
CV		1.96	5.67	
P value		.07	.05	

[1]Means (±SD), [ab]Means within column (main effects) with different superscripts are significantly different (P < .05), Duncan's least significance multiple-range test were applied to compare means.

Table 10. Effects of dietary crude protein level (150, 138, and 126 g/kg) and phytase supplementation (0 and 300 FTU/kg) on egg quality characteristics of laying hens in second sampling period (wk 7 of the experiment, wk 63 of age)[1]

Treatments	Yolk color	Gravity	Shell weight	Shell thickness
Protein (P)				
150	6.69±0.63	1.08±0.00	5.56±0.63	36.81±2.05
138	7.25±0.53	1.09±0.00	5.52±0.71	35.97±4.35
126	7.06±0.47	1.09±0.00	5.44±0.34	37.00±1.92
Phytase (E)				
0	7.11±0.50	1.09±0.00	5.46±0.71	36.50±3.45
300	6.89±0.65	1.08±0.00	5.56±0.40	36.68±2.42
SEM	0.10	0.00	0.09	0.49
CV	7.99	0.53	10.49	8.03
Sources of variation	P values			
Protein (P)	.06	.38	.88	.66
Phyatse (E)	.24	.42	.59	.85
P × E	.97	.94	.20	.15

[1]Means (±SD).

3.3 Blood Cell Counts and H/L Ratio

As it is presented in Table 11, there were interactions between dietary CP level and phytase supplementation on heterophil and lymphocyte differential counts and H/L ratio ($P < .05$). Statistical analysis based on 6 separated dietary groups revealed that birds receiving 150 g/kg CP diet supplemented with phytase had significantly higher lymphocyte counts but significantly lower heterophil counts and H/L ratio compared with other diets ($P < .05$). On the other hand birds receiving 126 g/kg CP diet supplemented with phytase had significantly lower lymphocyte counts but significantly higher heterophil counts and H/L ratio compared with other diets ($P < .05$). Our results do not agree with those of Rama Rao et al. [38], who observed no significant differences among the male broiler parent chicks fed on the various levels of dietary protein. The differences in their results and ours may be due to the fact that their chicks were given higher levels of dietary protein (18 to 23% CP) than our hens in the present study. We were unable to find other research reports on the effects of dietary protein or phytase on differential counts of white blood cells in laying hens.

Table 11. Effects of dietary crude protein level (150, 138, and 126 g/kg) and phytase supplementation (0 and 300 FTU/kg) on white blood cell counts and heterophil to lymphocyte (H/L) ratio of laying hens (wk 7 of the experiment, wk 63 of age)[1]

Treatments		Heterophils	Lymphocyte	Monocyte	Eosinophil	Basophil	H/L ratio
Protein (P)							
150		26.83±8.82	70.25±9.67	0.92±1.24	0.83±1.40	1.17±0.83	0.40±0.18
138		29.83±7.91	67.67±8.89	1.08±1.38	0.25±0.62	1.17±1.53	0.46±0.17
126		40.08±7.37	57.00±7.90	1.17±1.11	0.83±1.40	0.92±1.00	0.73±0.24
Phytase (E)							
0		31.50±8.03	65.44±9.80	1.28±1.41	0.56±1.10	1.22±1.31	0.51±0.20
300		33.00±11.33	64.50±11.19	0.83±0.98	0.72±1.32	0.94±0.94	0.56±0.29
SEM		1.62	1.73	0.20	0.20	0.19	0.04
CV		20.32	11.98	119.83	193.47	110.30	33.47
Sources of variation				P values			
Protein (P)		.001	.001	.89	.42	.84	.001
Phyatse (E)		.50	.72	.30	.69	.49	.44
P × E		.001	.00	.59	.62	.78	.01
P	E						
1	0	32.67±6.50^{b}	64.50±8.55b^{c}				0.52±0.17b^{c}
1	1	21.00±6.87^{c}	76.00±7.29^{a}				0.28±0.11^{d}
2	0	24.33±5.99^{c}	72.83±8.42^{ab}				0.35±0.13^{dc}
2	1	35.33±5.39^{ab}	62.50±6.22^{c}				0.58±0.13^{b}
3	0	37.50±5.89^{ab}	59.00±8.01^{c}				0.57±0.19^{ab}
3	1	42.67±8.29^{a}	55.00±7.87^{c}				0.81±0.28^{a}
CV		20.32	11.98				33.47
P value		.001	.001				.001

[1] Means (±SD), [ab] Means within column (main effects) with different superscripts are significantly different (P < .05), Duncan's least significance multiple-range test were applied to compare means.

3.4 Serum Parameters

Based on the results presented in Table 12, decreasing dietary CP level decreased significantly serum levels of total cholesterol ($P < .05$). Birds receiving diets containing phytase also produced significantly lower serum total cholesterol when compared with other treatments ($P < .05$). These findings were partly similar to those reported by Ghiyasi et al. [39], who reported that decreasing levels of dietary protein (17 to 15%) decreased serum total cholesterol concentrations in broiler chickens. On the other hand, they found no effect of dietary CP levels in the serum levels of triglycerides, LDL-cholesterol and HDL-cholesterol, whereas in the present study birds given low protein diets had markedly lower serum triglycerides and LDL-cholesterol concentrations but significantly higher serum concentrations of HDL-cholesterol as compared to high CP diets. An inference could, thus, be drawn from the results of the present study that dietary protein level can be reduced from 150 to 138 g/kg in layer diets with desirable effects on blood lipid profile.

Table 12. Effects of dietary crude protein level (150, 138, and 126 g/kg) and phytase supplementation (0 and 300 FTU/kg) on serum biochemical parameters (mg/dL) of laying hens (wk 7 of the experiment, wk 63 of age)[1]

Treatment	Total	Triglycerides	HDL-	LDL-
Protein (P)				
150	182.27±79.28[a]	2199.55±1198.17[a]	54.09±17.20[a]	97.18±23.75[a]
138	140.36±36.70[b]	1313.64±814.49[b]	44.82±10.38[b]	81.27±18.01[b]
126	138.58±22.84[b]	956.67±374.107[b]	48.58±1.10[ab]	79.83±15.55[b]
Phytase (E)				
0	170.06±70.52[a]	1705.63±1294.05	51.81±14.71	91.19±23.24
300	138.39±27.26[b]	1268.61±546.56	46.78±9.79	81.22±16.60
SEM	9.24	168.39	2.130	3.48
CV	44.79	67.77	37.45	36.81
Sources of variation	P values			
Protein (P)	.03	.001	.12	.04
Phyatse (E)	.04	.09	.16	.08
P × E	.05	.13	.05	.06

[1]Means (±SD), [ab]Means within column (main effects) with different superscripts are significantly different (P < .05), Duncan's least significance multiple-range test were applied to compare means.

4. CONCLUSION

In the present study, decreasing dietary protein levels decreased EP, EM and FI and increased FCR whereas phytase supplementation have no beneficial effect on productive performance of laying hens and egg quality traits. However, feeding low protein diets decreased serum levels of total cholesterol, triglycerides and LDL-cholesterol but decreased serum levels of HDL-cholesterol when compared to the control group. In addition, dietary supplementation with phytase significantly decreased serum total cholesterol level.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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