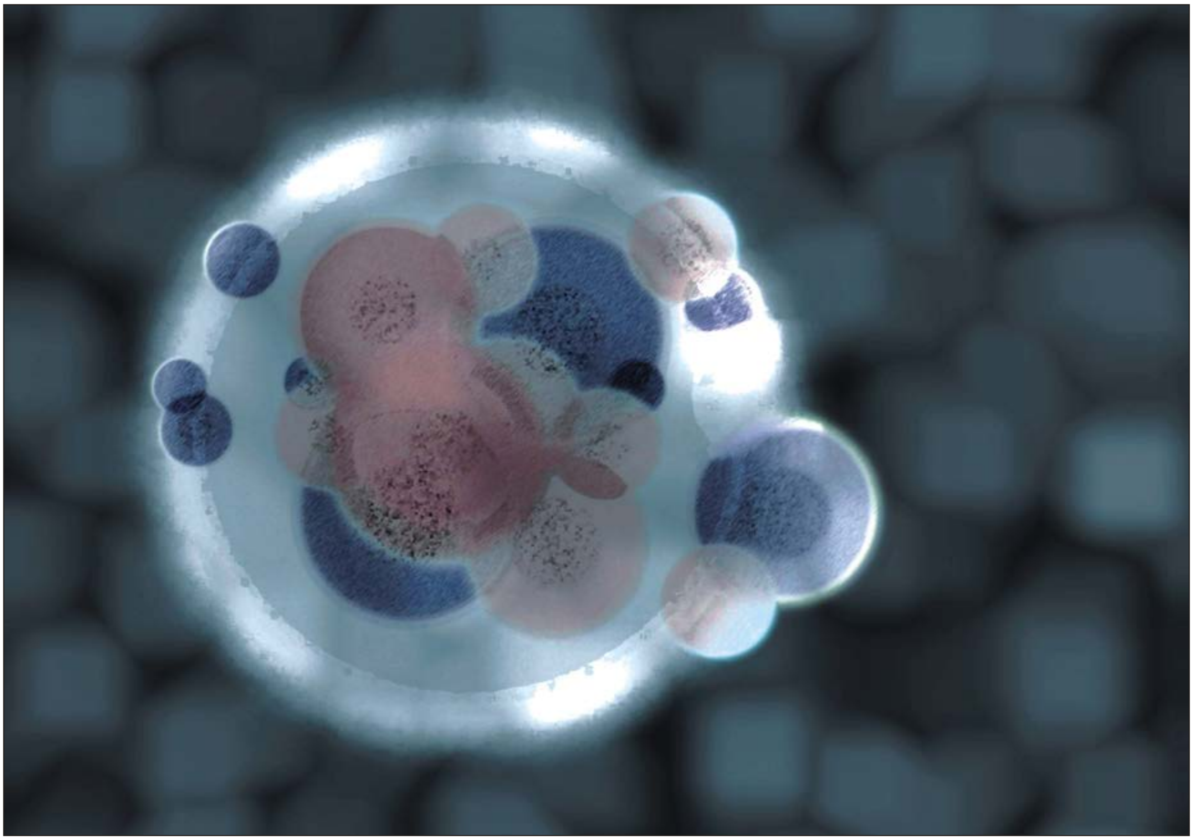

Journal of Biological Sciences

ISSN 1727-3048

Journal of Biological Sciences 8 (2): 328-334, 2008
ISSN 1727-3048

Influence of Dietary Zeolite Supplementation on the Performance and Egg Quality of Laying Hens Fed Varying Levels of Calcium and Nonphytate Phosphorus

H. Nassiri Moghaddam, R. Jahanian, H. Jahanian Najafabadi and M.M. Madaeni
Department of Animal Science, Faculty of Agriculture,
Ferdowsi University of Mashhad, Mashhad 91775-1163, Iran

Abstract: Natural zeolites have been shown to influence calcium and phosphorus utilization in laying hens. A 4×2×2 factorial arrangement of treatments was used to investigate the effects of dietary inclusion of zeolite (0, 1.5, 3 and 4.5% of diet) into the diets with sufficient or deficient Ca (3.26 and 2.45%) and non-phytate P (0.25 and 0.19%) contents on egg performance and egg quality parameters of 28 weeks-old Hy-Line (W-36) Leghorn hens. The trial lasted 98 days (2 weeks adaptation and 12 weeks recording periods), when the hens were 42 weeks-old. Significant dietary effects of feeding zeolite were observed for hen-day egg production, egg mass, egg weight, Haugh score and shell thickness at the initial 6 weeks of recording period, while dietary zeolite supplementation tended to/or had no significant effects on studied parameters at the second 6 weeks of experimental period. Dietary added zeolite caused a significant increases in egg weight ($p<0.05$), egg production and egg mass ($p<0.01$). Hen-day egg production and egg mass at the second 6 weeks of recording period were significantly improved ($p<0.01$) by feeding low non-phytate P (NPP) diets. There was a significant interaction between NPP level and added zeolite for egg production (36 to 42 weeks of age), egg weight and eggshell thickness (30 to 36 weeks of age) and egg mass. Dietary Ca level interacted with P level for shell thickness, eggshell percentage and shell ash, so that the given parameters were affected by Ca:NPP ratio other than dietary Ca and NPP contents. In general, the present findings indicate that dietary inclusion of zeolite up to 1.5% has a potential to improve laying performance, particularly eggshell quality.

Key words: Zeolite, egg production, eggshell quality, Ca and P level, laying hen

INTRODUCTION

For many years nutritionists have recognized that certain non-nutritive materials affect the nutritive efficiency of diets and that some nutrient-containing materials possess dietary effects beyond that which might be expected from their known nutrient composition. During recent years, aluminosilicates in the form of zeolites have been used in poultry ration to evaluate their utility as feed additives (Mumpton and Fishman, 1977).

Zeolites are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations, having infinite, three-dimensional structures. They have the ability to lose and gain water reversibly and to exchange constituent ionic cations without major changes of structure (Mumpton and Fishman, 1977; Street, 1994). They can selectively adsorb gas and steam molecules, or based on ions selectivity they can exchange own cations for other ones (Boranic, 2000; Melenova *et al*., 2003). Zeolites are also used as effective adsorbents of toxin agents, particularly aflatoxins from the feeds (Parlat *et al*., 1999; Phillips, 1999; Rizzi *et al*., 2003). They effectively minimize adverse effects of aflatoxins on feed intake, performance and feed conversion efficiency (Parlat *et al*., 1999; Oguz and Kurtoglu, 2000) and reduce mycotoxin concentration in the livers of affected animals (Rizzi *et al*., 2003). Additionally, natural or synthetic zeolites can adsorb excess ammonium levels and subsequently help to reduce the problem related to ammonia generation (Roland *et al*., 1985). They have been, therefore, used in the treatment of animal wastes to reduce odor and create a healthy environment for confined animals (Mumpton and Fishman, 1977).

Statistically significant improvement of feed conversion after clinoptilolite (CPL) feeding (2% of diet) to piglets in the period from weaning to slaughter was observed (Papaioannou *et al*., 2004). Clinoptilolite in the diet for layer hens (50 g kg^{-1}) increased the numbers of laid eggs, stability of eggshell and efficiency of feed utilization; however, neither the onset of the egg lay cycle, nor the egg weight were affected (Olver, 1997). Rabon *et al*. (1995) recorded significant increased in

Corresponding Author: H. Nassiri Moghaddam, Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad 91775-1163, Iran
Tel: (+98) 511 8795618 Fax: (+98) 511 8787430

serum aluminum and zinc concentration in laying hens following zeolite supplementation, which can be associated with improved quality of the eggshell and bone development. Various researchers have hypothesized that the beneficial effects of zeolite on shell quality may be related to its high affinity for calcium and its high ion-exchange capacity (Roland *et al.*, 1985; Roland, 1988). It has been well documented that high levels of dietary Non-Phytate P (NPP) decrease eggshell quality (Arscott *et al.*, 1962; Elliot and Edwards, 1991). Another hypothesis for the effects of zeolites on shell quality, therefore, is that the aluminum included in zeolite may complex with P and may reduce the availability of P (Roland, 1990; Elliot and Edwards, 1991). This may result in slight improvements in shell quality. Feeding low levels of dietary P is thought to reduce serum P and facilitates the utilization of skeletal calcium in shell formation during overnight (Roland, 1990; Roland *et al.*, 1991). If the mechanism of zeolite is reducing the availability of P, the effect of zeolite on eggshell quality depends on dietary P level. Thus, the lower P intake by laying hens result in the less beneficial effect of zeolite on shell quality and the greater possibility that zeolite may have adverse effects on egg production (Roland, 1990). There are conflicting reports for the effects of zeolite supplementation of low Ca and/or P diets on egg yield and egg quality. The goal of presented study, therefore, was to investigate the effects of zeolite on laying performance and egg quality of White Leghorn hens fed diets with different Ca and P contents.

MATERIALS AND METHODS

General schedule: This experiment was conducted to study the effects of natural zeolite on laying hen performance. The study here was carried out in the experimental farm of Ferdowsi University of Mashhad (Mashhad, Iran), from middle weeks of May to middle of August. The birds were randomly housed in layer cages at a density of six birds per pen (461 cm^2 per bird). An experimental unit consisted of one pen supplied with individual feed pan and nipple drinker. A total of three hundred eighty four Single Comb White Leghorn (Hy-Line W-36) laying hens at 28 weeks of age, were placed in 64 cage units (six birds per cage) and randomly assigned to one of the sixteen experimental diets (4 pen replicates for each treatment group). The diets were formulated to be isocaloric (2.9 kcal ME g^{-1}) and isonitrogenous (15% CP) and to meet or exceed National Research Council (1994) nutrient requirements for laying hens (Table 1). The experimental diets consisted of a 4×2×2 factorial arrangement with four levels of zeolite supplementation (0, 1.5, 3 and 4.5% of diet), two levels of Ca [3.25 and 25% lower than National Research Council (1994)

Table 1: Composition of the experimental diets and their calculated analysis

	0% zeolite				1.5% zeolite				3% zeolite				4.5% zeolite			
Ingredients	NRC	P[4]	Ca[4]	Ca/P[4]	NRC	P	Ca	Ca/P	NRC	P	Ca	Ca/P	NRC	P	Ca	Ca/P
Corn	65.68	65.95	67.68	67.63	62.72	62.99	66.25	66.20	62.08	62.35	66.28	66.55	59.96	59.99	63.31	63.59
Soybean meal	20.53	20.48	19.48	19.39	21.09	21.04	20.22	20.13	21.92	21.87	21.13	21.07	21.40	21.66	21.69	21.64
Wheat bran	2.00	2.00	3.88	4.17	2.00	2.00	2.57	2.85	-	-	-	-	-	-	-	-
Sunflower oil	2.20	2.12	1.50	1.50	3.09	3.01	2.00	2.00	3.39	3.31	2.12	2.04	4.00	4.00	3.02	2.93
Limestone	5.92	6.11	3.80	3.99	5.92	6.11	3.79	3.98	5.91	6.10	3.78	3.98	5.91	6.10	3.78	3.97
Oyster shell	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	0.76	0.44	0.76	0.42	0.77	0.44	0.77	0.43	0.78	0.45	0.78	0.45	0.80	0.47	0.78	0.45
Common salt	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34
Mineral premix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.08	0.07	0.07	0.07	0.08	0.08	0.07	0.08	0.08	0.08	0.07	0.07	0.09	0.09	0.08	0.08
L-Lysine HCl	-	-	-	-	-	-	-	-	-	-	-	-	0.50	0.35	-	-
Zeolite[3]	-	-	-	-	1.50	1.50	1.50	1.50	3.00	3.00	3.00	3.00	4.50	4.50	4.50	4.50
Nutrient composition																
ME (kcal kg^{-1})	2900	2900	2900	2900	2900	2900	2900	2900	2900	2900	2900	2900	2900	2900	2900	2900
Crude protein (%)	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Met+Cys (%)	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58
Lysine (%)	0.74	0.74	0.73	0.73	0.74	0.74	0.73	0.73	0.75	0.75	0.74	0.74	1.13	1.02	0.75	0.75
Threonine (%)	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.56	0.56	0.56	0.56	0.54	0.55	0.56	0.56
Tryptophan (%)	0.20	0.20	0.19	0.19	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.19	0.20	0.20	0.20
Arginine (%)	0.91	0.91	0.91	0.91	0.92	0.92	0.91	0.91	0.92	0.92	0.92	0.91	0.90	0.91	0.92	0.92
Calcium (%)	3.26	3.26	2.45	2.45	3.26	3.26	2.45	2.45	3.26	3.26	2.45	2.45	3.26	3.26	2.45	2.45
Available P (%)	0.25	0.19	0.25	0.19	0.25	0.19	0.25	0.19	0.25	0.19	0.25	0.19	0.25	0.19	0.25	0.19
Sodium (%)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15

[1] Poultry mineral premix provided per kilogram of diet: Mn, 88 mg; Cu, 6.6 mg; Fe, 8.5 mg; Zn, 88 mg; Se, 0.3 mg.
[2] Poultry vitamin premix provided per kilogram of diet: vitamin A (as retinyl acetate), 6600 IU; cholecalciferol, 1000 IU; vitamin E (as α-tocopherol), 10 IU; vitamin K, 2.0 mg; riboflavin, 2.2 mg; Ca pantothenate, 6.4 mg; niacin, 20.6 mg; choline chloride, 110 mg; vitamin B_{12}, 21.2 μg; ethoxyquine, 1.1 mg.
[3] Supplied by Afrand Toska Company, Iran.
[4] P: The diets low in P content; Ca: the diets low in Ca content; Ca/P: the diets deficient in both Ca and P contents

recommendations for Ca i.e., 2.45%] and two levels of dietary NPP [0.25 and 25% lower than National Research Council (1994) recommendations for NPP i.e., 0.19%]. The diets were presented in mash form and feed and water were provided for ad libitum consumption throughout the trial. A photoperiod of 14 h day^{-1} was given. Prior to main recording period, the birds given experimental diets for 2 consecutive weeks as an adaptation period and feed intake records were maintained during this period. The main trial period was subdivided into six periods by biweekly breakouts. Egg production was recorded daily for each cage unit and an average egg production rate (hen-day percentage) was calculated for every period. Because the experiment was began in the middle weeks of spring and continued until summer and the high levels of unsaturated fat sources were included into some of experimental diets; the diets, therefore, were prepared weekly and feed consumption was also measured weekly and used to calculate average daily feed intake and feed efficiency (g feed to g egg). All eggs laid during the last 3 days of every week were weighed to give an average egg weight. Twenty four eggs from each treatment (6 eggs per replicate) laid at the last 2 days of every 14 days period were sampled randomly to measure eggshell breaking strength, shell thickness, egg shell percentage, shell ash and Haugh score. Shell thickness was measured in 5 places (air cell, equator and sharp end) by means of a micrometer screw gauge. The shell membranes were not removed. Haugh score was calculated with the HU formula (Eisen *et al*., 1962), based on the height of albumen determined by a micrometer and egg weight. Shell ash percentage was determined following 8 h drying period in oven, thereafter 8 h ashing by muffle furnace (AOAC, 1995).

Statistical analysis: The experiment had a complete randomized design with 4×2×2 (zeolite by Ca by NPP) factorial arrangement of treatments. All data were subjected to analysis of variance using the General Linear Models (GLM) procedures of SAS (SAS, 1999). Data were expressed in means of two separate periods (30 to 36 weeks and 36 to 42 weeks of age) to represent the periodical changes of egg production rate and egg quality measurements. Significant differences among treatment means were measured by Duncan's multiple range test (Duncan, 1955) at $p<0.05$.

RESULTS

The various egg production characteristics are shown in Table 2. Hen-day egg production was not affected by dietary treatments during the initial 6 experimental weeks, but in the second 6 weeks zeolite supplementation did improve egg production significantly ($p<0.001$). Egg mass was also increased by dietary added zeolite in the same period. Increased egg mass was not unexpected because added zeolite improved egg production and egg weight concomitantly, the two parameters which make egg mass. Similarly, when the diets deficient in NPP content were fed egg production

Table 2: Effects of zeolite supplementation of diets with various Ca and NPP contents on laying hen performance

Zeolite (%)	Ca (%)	NPP (%)	Egg production (%) 30-36 weeks	Egg production (%) 36-42 weeks	Feed intake (g day^{-1} per hen) 30-36 weeks	Feed intake (g day^{-1} per hen) 36-42 weeks	Feed efficiency (feed/egg) 30-36 weeks	Feed efficiency (feed/egg) 36-42 weeks	Egg mass (g day^{-1} per hen) 30-36 weeks	Egg mass (g day^{-1} per hen) 36-42weeks
0.0	3.26	0.25	86.66	80.34	107.21	102.72	2.21	2.25	48.85	45.50
0.0	3.26	0.19	90.07	86.67	104.16	96.37	2.02	1.97	51.55	48.87
0.0	2.45	0.25	88.72	80.00	101.15	94.32	2.01	2.05	50.33	45.99
0.0	2.45	0.19	90.07	83.86	105.71	103.29	2.06	2.13	51.27	48.31
1.5	3.26	0.25	88.78	87.67	108.17	103.05	2.11	2.03	51.36	50.76
1.5	3.26	0.19	85.90	87.50	103.72	99.05	2.10	1.94	49.34	50.96
1.5	2.45	0.25	89.10	87.67	103.67	98.64	2.05	1.96	50.71	50.29
1.5	2.45	0.19	86.86	88.33	104.73	102.97	2.16	2.05	48.58	50.17
3.0	3.26	0.25	87.41	82.83	102.35	94.46	2.06	2.02	49.78	46.81
3.0	3.26	0.19	89.10	86.00	104.98	97.51	2.04	1.91	51.55	50.99
3.0	2.45	0.25	86.27	84.42	106.84	106.25	2.20	2.20	48.63	48.33
3.0	2.45	0.19	91.67	89.67	106.07	103.30	2.00	1.99	53.05	52.02
4.5	3.26	0.25	83.98	85.33	105.16	100.98	2.17	2.04	48.42	49.50
4.5	3.26	0.19	89.43	86.67	102.58	98.53	2.02	1.98	50.81	49.83
4.5	2.45	0.25	88.14	87.67	106.39	101.85	2.06	1.98	51.58	51.41
4.5	2.45	0.19	86.86	86.33	108.52	101.53	2.14	2.00	50.66	50.70
SEM			1.908	1.433	3.729	4.967	0.069	0.093	1.212	0.979
			Probability							
Zeolite			NS	***	NS	NS	NS	NS	NS	***
Calcium			NS	NS	NS	NS	NS	NS	NS	NS
Phosphorus			NS	**	NS	NS	NS	NS	NS	**
Zeolite×Calcium			NS	NS	NS	NS	NS	NS	NS	NS
Zeolite×Phosphorus			NS	*	NS	NS	NS	NS	*	**
Calcium×Phosphorus			NS	NS	NS	NS	NS	NS	NS	NS
Zeolite×Calcium×Phosphorus			NS	NS	NS	NS	NS	NS	NS	NS

NS: Not Significant; *$p<0.05$; **$p<0.01$; ***$p<0.001$

Table 3: Effects of zeolite supplementation of diets with various Ca and NPP contents on egg parameters

			Egg weight (g)		Haugh unit		Breaking strength (kg cm^{-2})	
Zeolite (%)	Ca (%)	NPP (%)	30-36 weeks	36-42 weeks	30-36 weeks	36-42 weeks	30-36 weeks	36-42 weeks
0.0	3.26	0.25	56.34	56.63	81.54	74.46	3.93	3.33
0.0	3.26	0.19	57.26	56.38	81.29	71.30	3.95	3.61
0.0	2.45	0.25	56.72	57.42	75.92	73.69	3.75	3.38
0.0	2.45	0.19	56.93	57.61	76.61	77.90	3.50	3.06
1.5	3.26	0.25	57.85	57.90	73.14	78.10	3.73	3.64
1.5	3.26	0.19	57.43	58.24	72.21	70.13	4.24	3.84
1.5	2.45	0.25	56.89	57.36	73.10	72.60	3.86	3.75
1.5	2.45	0.19	55.92	56.79	78.14	74.77	3.56	3.45
3.0	3.26	0.25	56.95	56.53	73.36	73.82	3.54	3.45
3.0	3.26	0.19	57.86	59.28	73.79	73.58	3.60	3.86
3.0	2.45	0.25	56.38	57.24	73.29	73.73	3.91	3.50
3.0	2.45	0.19	57.87	58.07	75.76	74.43	3.51	3.79
4.5	3.26	0.25	57.65	58.03	75.00	72.92	4.03	3.93
4.5	3.26	0.19	56.81	57.51	75.89	74.79	3.46	3.30
4.5	2.45	0.25	58.54	58.64	77.69	78.38	3.77	3.72
4.5	2.45	0.19	58.31	58.73	76.25	69.37	4.01	3.53
SEM			0.463	0.632	2.089	3.113	0.241	0.219
			Probability					
Zeolite			*	0.066	**	NS	NS	NS
Calcium			NS	NS	NS	NS	NS	NS
Phosphorus			NS	NS	NS	NS	NS	NS
Zeolite×Calcium			**	0.086	*	NS	NS	NS
Zeolite×Phosphorus			*	0.089	NS	NS	NS	NS
Calcium×Phosphorus			NS	NS	NS	NS	NS	NS
Zeolite×Calcium×Phosphorus			NS	NS	NS	NS	NS	NS

NS: Not Significant; *p<0.05; **p<0.01

Table 4: Effects of zeolite supplementation of diets with various Ca and NPP contents on eggshell quality measurements

			Shell thickness (mm)		Eggshell percentage (%)		Eggshell ash (%)	
Zeolite (%)	Ca (%)	NPP (%)	30-36 weeks	36-42 weeks	30-36 weeks	36-42 weeks	30-36 weeks	36-42 weeks
0.0	3.26	0.25	0.370	0.382	9.10	9.27	92.77	93.33
0.0	3.26	0.19	0.366	0.367	9.02	8.96	91.83	92.11
0.0	2.45	0.25	0.351	0.363	9.14	8.82	88.06	91.15
0.0	2.45	0.19	0.368	0.371	9.45	9.12	91.41	93.24
1.5	3.26	0.25	0.385	0.390	9.65	9.10	94.12	93.99
1.5	3.26	0.19	0.398	0.359	9.49	8.68	94.80	90.07
1.5	2.45	0.25	0.368	0.373	9.37	8.99	92.32	91.06
1.5	2.45	0.19	0.376	0.372	9.74	9.38	94.29	93.47
3.0	3.26	0.25	0.391	0.385	9.50	9.34	93.72	93.66
3.0	3.26	0.19	0.370	0.392	9.03	9.16	92.86	94.26
3.0	2.45	0.25	0.362	0.370	9.10	9.20	90.89	90.79
3.0	2.45	0.19	0.381	0.383	9.43	9.43	95.66	96.07
4.5	3.26	0.25	0.376	0.394	9.50	9.55	94.47	94.51
4.5	3.26	0.19	0.358	0.348	9.34	8.93	89.91	89.71
4.5	2.45	0.25	0.391	0.378	8.87	8.76	92.14	90.30
4.5	2.45	0.19	0.365	0.377	9.55	9.08	93.46	93.89
SEM			0.008	0.010	0.233	0.239	1.573	1.618
			Probability					
Zeolite			*	NS	NS	NS	0.072	NS
Calcium			0.107	NS	NS	NS	NS	NS
Phosphorus			NS	0.105	NS	NS	NS	NS
Zeolite×Calcium			0.063	NS	NS	NS	NS	NS
Zeolite×Phosphorus			*	0.109	NS	NS	NS	NS
Calcium×Phosphorus			NS	*	**	**	**	**
Zeolite×Calcium×Phosphorus			NS	NS	NS	NS	NS	NS

NS: Not Significant; *p<0.05; **p<0.01

rate and egg mass was increased during 36 to 42 weeks of age. Hen-day egg production (36 to 42 weeks of age) and egg mass (in both periods) were influenced by zeolite × NPP interaction. Feeding low NPP diets had a considerable effect on egg production when the diets were devoid of zeolite or contained 3% zeolite, resulted the quadratic zeolite effect to be significant (data not shown). The same view was seen with regard to egg mass. Neither feed intake, nor feed conversion efficiency was affected (p>0.05) by dietary treatments (Table 2).

No significant differences (p>0.05) were observed between the experimental diets in the light of eggshell breaking strength (in both recording periods) and Haugh unit (at 36 to 42 weeks of age); however, there were significant differences among the dietary treatments with regard to Haugh unit (at 30 to 36 weeks of age) and egg weight (Table 3). Zeolite supplementation improved egg weight in the first trial recording period (30 to 36 weeks of age) and tended ($p = 0.066$) to increase it in the second stage. Haugh unit (30 to 36 weeks of age) was reduced by dietary zeolite supplementation. Dietary added zeolite caused a significant increase ($p<0.05$) in shell thickness in the first period (Table 4). Except shell thickness which affected by dietary supplemental zeolite, none of eggshell measurements were significantly altered by main effects of dietary treatments (Table 4). Shell thickness (36 to 42 weeks of age), eggshell percentage and shell ash, however, were affected by Ca×NPP interaction.

DISCUSSION

In the study presented herein, egg production rate and in turn egg mass, were increased ($p<0.001$) by zeolite including into the diets in the period from 36 to 42 weeks of age. The best egg production rate and also egg mass assigned ($p<0.001$) to the birds fed on diets containing 1.5% zeolite supplementation followed by hen given 4.5% zeolite-supplemented diets. Similarly, in his study by three strains of laying hens, Olver (1997) found that hens on the diet containing clinoptilolite (zeolite) laid more eggs per hen than those fed no clinoptilolite. According to Mumpton and Fishman (1977) zeolites can be used to extract ammonia (NH_4^+) by ion exchange. Zeolite could, therefore, help to remove stress-forming ammonia from the hen's intestine thereby allowing increased egg production. The increase in egg production rate and egg mass by dietary including of 4.5% zeolite in the present study appears to be related to higher fat content of given diets other than direct action of zeolite. This effect is called extracaloric effect of fat whereby resulting improved performance and feed efficiency beyond that expected from the dietary nutrient composition (National Research Council, 1994).

The hens fed on low NPP diets increased ($p<0.01$) egg production and egg mass. It has been well documented that high levels of dietary phosphorus decrease eggshell quality (Arscott *et al*., 1962; Elliot and Edwards, 1991). Some researchers hypothesized that the aluminum content of zeolite may complex with P and may reduce the availability of P (Roland, 1990; Elliot and Edwards, 1991). This may result in slight improvements in shell quality. Furthermore, feeding low levels of dietary P is thought to reduce serum P and facilitates the utilization of skeletal calcium in shell formation during the night (Roland, 1990; Roland *et al*., 1991) and in turn improves laying performance. Dietary added zeolite interacted with NPP with regard to egg production (36 to 42 weeks of age) and egg mass, in that zeolite supplementation improved egg production and egg mass when the diets were sufficient in phosphorus content. In agreement with our findings, Fethiere *et al*. (1990) claimed that a decrease in feed intake and thus a decrease in P intake in the presence of sodium aluminosilicates, is directly related to poor hen performance, particularly when low levels of P are fed.

Feed intake and feed efficiency ratio were not affected ($p>0.05$) by dietary treatments. In consistent with presented findings, Olver (1989) reported that the clinoptilolite (CPL) supplementation had no effect on the amount of feed consumed. The same author, however, observed that the efficiency of feed utilization by hens fed on CPL-supplemented diets was superior to that of those fed on the control diet in three hen strains studied. Similarly, Kondo and Wagai (1968) found that when the diet of young pigs was supplemented with 50 g CPL kg^{-1} feed efficiency was increased by 35% compared to pigs that received no dietary CPL. It is possible that the adsorption of elements by the CPL could have improved the feed efficiency (Olver, 1989). It appears that the lack of feed efficiency response to added zeolite in the present study may be, in part, related to summative effects of Ca and NPP contents of diets in calculations.

Zeolite inclusion increased egg weight in the first experimental period (30 to 36 weeks of age) and tended ($p = 0.066$) to increase it in the further period. Increasing zeolite level caused a linear response ($p<0.05$) in egg weight, so that the heaviest eggs were assigned to 4.5% zeolite-supplemented diets. Presumably, this observation is due to the presence of higher linoleic acid content in the more zeolite-supplemented diets. It has been well documented that high levels of dietary linoleic acid increase egg size (March and MacMillan, 1990). Haugh score was reduced by dietary added zeolite and the least scores were allocated to the eggs laid by hens fed on 4.5% zeolite-supplemented diets. The exact mechanism for this effect is not clear. To our opinion, however, zeolite supplementation particularly higher zeolite levels, has been reduced P availability for production of phosphoproteins contained in egg albumen, so reduced albumen stability. Eggshell breaking strength was not affected by dietary zeolite supplementation despite of the effect of zeolite on shell thickness. This finding means that shell thickness is not the only requisite for high eggshell strength. The beneficial impact of added zeolite on shell thickness may be related to its high affinity for calcium and its high ion exchange capacity (Roland *et al*., 1985; Roland, 1988). Furthermore, these beneficial effects may also be related to the Al, Si or Na content of zeolites because these elements have been shown to influence Ca metabolism (Roland, 1988; Roland *et al*., 1993).

None of main dietary factors (zeolite supplementation level, dietary Ca or NPP contents) had significant ($p>0.05$) influence on eggshell percentage or shell ash; however, these eggshell measurements were affected by Ca×NPP interaction. Feeding the diets deficient in NPP content reduced eggshell percentage and shell ash when the diets were normal in Ca content, while with low Ca diets decreasing dietary NPP content led to increase in given parameters. It seems that the Ca:NPP ratio is more important in balancing poultry (including laying hens) rations than the amounts of these two nutritionally macroelements. The added zeolite tended ($p = 0.072$) to increase shell ash percentage, probably due to phosphorus-complexing action of aluminum contained in zeolites (Roland, 1990).

While some reports (Olver, 1983, 1989) indicate that zeolites have a positive effect on egg production, some others observed negative effects in terms of egg production (Miles *et al*., 1988; Roland, 1988), egg weight (Miles *et al*., 1988; Fethiere *et al*., 1990) and feed consumption (Miles *et al*., 1988). The expected effects of zeolites may exhibit variations due to such factors as nature, concentration, the aluminum content of the zeolite and the dietary Ca and P levels (Mumpton and Fishman, 1977; Elliot and Edwards, 1991).

In summary, the present results indicate that the dietary supplementation with 1.5% zeolite can improve egg laying performance and egg quality; however, Haugh score as an index for egg albumen quality should be noted when the diets are supplemented with zeolites. It is needed to perform more studies with older hens to confirm if added zeolite has a potential to improve shell quality of eggs laid in older ages.

ACKNOWLEDGMENTS

The authors would like to thank Deputy for Research and Excellent Center of Animal Sciences in Ferdowsi University of Mashhad (Mashhad, Iran) for financial support.

REFERENCES

AOAC, 1995. Official Methods of Analysis. 16th Edn. Association of Official Analytical Chemists, Arlington, VA.

Arscott, G.H., P. Rachapaetayakom and P.E. Bernier, 1962. Influence of ascorbic acid, calcium and phosphorus on specific gravity of eggs. Poult. Sci., 41: 485-488.

Boranic, M., 2000. What a physician should know about zeolites. Lijec. Viesn., 122: 292-298.

Duncan, D.B., 1955. Multiple range and multiple F-tests. Biometrics, 11: 1-42.

Eisen, E.J., B.B. Bohren and H.E. Mckean, 1962. The Haugh unit as a measure of egg albumen quality. Poult. Sci., 41: 1461-1468.

Elliot, M.A. and H.M. Edwards Jr., 1991. Comparison of the effects of synthetic and natural zeolite on laying hen and broiler chicken performance. Poult. Sci., 70: 2115-2130.

Fethiere, R., R.D. Miles and R.H. Harms, 1990. Influence of synthetic sodium aluminosilicate on laying hens fed different phosphorus levels. Poult. Sci., 69: 2195-2198.

Kondo, N. and B. Wagai, 1968. Experimental use of clinoptilolite-tuff as dietary supplements for pigs. Yotonkai, May, 1-4.

March, B.E. and C. MacMillan, 1990. Linoleic acid as a modulator of egg size. Poult. Sci., 69: 634-639.

Melenova, L., K. Ciahotny, H. Jirglova, H. Kusa and P. Ruzek, 2003. Removal of ammonia from the waste gas by means of adsorption on zeolites and their subsequent use in agriculture (in Czech). Chem. Listy, 97: 562-568.

Miles, R.D., R.H. Harms and S.M. Laurent, 1988. Influence of sodium zeolite A (Ethacal[R]) on laying hen performance. Nutr. Rep. Int., 34: 1097-1103.

Mumpton, F.A. and P.H. Fishman, 1977. The application of natural zeolites on animal science and aquaculture. J. Anim. Sci., 45: 1188-1203.

National Research Council, 1994. Nutrient Requirements of Poultry. 9th Rev. Edn. Natl. Acad. Press, Washington, DC.

Oguz, H. and V. Kurtoglu, 2000. Effect of clinoptilolite on performance of broiler chickens during experimental aflatoxicosis. Br. Poult. Sci., 41: 512-517.

Olver, M.D., 1983. The effect of feeding clinoptilolite (zeolite) to laying hens. S. Afr. J. Anim. Sci., 13: 107-110.

Olver, M.D., 1989. Effect of feeding clinoptilolite (zeolite) to three strains of laying hens. Br. Poult. Sci., 36: 115-121.

Olver, M.D., 1997. Effect of feeding clinoptilolite (zeolite) on the performance of three strains of laying hens. Br. Poult. Sci., 38: 220-222.

Papaioannou, D.S., C.S. Kyriakis, C. Alexopoulos, E.D. Tzika, Z.S. Pilizopoulou and S.C. Kyriakis, 2004. A field study on the effect of dietary use of a clinoptilolite-rich tuff, alone or in combination with certain antimicrobials, on the health status and performance of weaned, growing and finishing pigs. Res. Vet. Sci., 76: 19-29.

Parlat, S.S., A.O. Yildiz and H. Oguz, 1999. Effect of clinoptilolite on performance of Japanese quail (*Coturnix coturnix japonica*) during experimental aflatoxicosis. Br. Poult. Sci., 40: 495-500.

Phillips, T.D., 1999. Dietary clay in the chemoprevention of aflatoxin-induced disease. Toxicol. Sci., 52: 118-126.

Rabon, Jr., H.W., D.A. Roland Sr., M.M. Bryant, R.C. Smith, D.G. Barnes and S.M. Laurent, 1995. Absorption of silicon and aluminum by hens fed sodium zeolite: A with various levels of dietary cholecalciferol. Poult. Sci., 74: 352-359.

Rizzi, L., M. Simioli, P. Roncada and A. Zaghini, 2003. Aflatoxin B_1 and clinoptilolite in feed for laying hens: Effects on egg quality, mycotoxin residues in livers and hepatic mixed-function oxygenase activities. J. Food Prot., 66: 860-865.

Roland, Sr., D.A., S.M. Laurent and H.D. Orloff, 1985. Shell quality as influenced by zeolite with high ion-exchange capability. Poult. Sci., 64: 1177-1187.

Roland, Sr., D.A., 1988. Further studies of effect of phosphorus and aluminosilicate on egg shell quality. Poult. Sci., 67: 577-584.

Roland, Sr., D.A., 1990. The relationship of dietary phosphorus and sodium aluminosilicate to the performance of commercial leghorns. Poult. Sci., 69: 105-112.

Roland, Sr., D.A., D.G. Barnes and S.M. Laurent, 1991. Influence of sodium aluminosilicate, hydroxyl-sodalite, carnegicite, aluminum sulfate and aluminum phosphate on performance of commercial leghorns. Poult. Sci., 70: 805-811.

Roland, Sr., D.A., H.W. Rabon Jr., K.S. Rao, R.C. Smith, J.W. Miller, D.G. Barnes and S.M. Laurent, 1993. Evidence for absorption of silicon and aluminum by hens fed sodium zeolite A. Poult. Sci., 72: 447-455.

SAS., 1999. SAS Statistics User's Guide. Statistical Analytical System. 5th Rev. Edn. Carry, NC, SAS Institute Inc.

Street, C., 1994. Natural zeolites in animal feeds. Feed Compounder, February.