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The Effect of Natural Zeolite as Feed Additive on Performance and Egg Quality in Old Laying Hens

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A R T I C L E I N F O

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A B S T R A C T

This experiment was aimed to use natural zeolite as a feed additive to diet in old laying hens. 36 laying hens of ISA White strain were used, at age of 72 week. The hens were divided into three treatments and each treatment consisted of six replicates with tow hens each. A standard basic ration was used consisting of maize and soybean meal, and the zeolite was added according to the design of the experiment as follows, without zeolite (control T1), + 0.5% zeolite (T2) and + 1% zeolite (T3). The experiment lasted for 6 weeks. The collected data of performance and egg quality parameters were evaluated of 3-week basis period (1st period 72 -75-week, 2nd period 75-78 week) and the entire period (72-78 week). Results indicated that there was no significant effect of adding natural zeolite on the studied performance parameters feed intake (g/hen/d) and feed conversion ratio (g/g) or on the egg production (%), egg weight (g/hen) and egg mass (g/hen/d). However, it must be noted that the hens fed with zeolite at level of +0.5 and + 1% showed a higher egg productivity comparing to hens without zeolite. Regarding the egg quality parameters, no significant effect was observed between the treatments. However, there were only significantly improvement in eggshell ratio (%) and eggshell density (mg/cm^2) when the data of 72-78 week were pooled and analysed. In this study, the zeolite supplementation showed no effects on blood plasma composition. In conclusion, the adding of natural zeolite at inclusion rate of 1% had positive effect on egg production and improved the shell egg quality of old laying hens.

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Introduction

The consumption of the poultry product especially eggs has consistently increased worldwide. Nowadays, optimizing the egg production requires having efficient hens breed and as the same time to apply an adequate management, good health practices and feeding programs. The supply of adequate diets with feed additives may improve nutrient utilization. One of these additives is the natural zeolite, which can use in farm animal nutrition due to its unique antibacterial properties, safety and efficacy (Mumpton and Fishman, 1977; Olver, 1989). The most common natural zeolite is clinoptilolite (Armbruster, 2001). Zeolite has specific physical structure, which provides it with special properties, and so it is able to adsorb gases and toxins, mycotoxins, ammonia, water, and eliminate heavy metals and radioactive elements, which might be harmful to animals (Mumpton, 1999; Zhou, 2008). The absorptive characteristics of zeolite are due to their high cation-exchange capacity, which affects tissue uptake and utilization of $NH4^+$, $Cu2^+$, $Pb2^+$, $Cd2^+$, Cs^+, and other cations in animals (Mumpton and Fishman, 1977; Oğuz, 2011). Additionally, zeolite has the ability to selectively exchange its own cations for ions from the environment. Therefore, zeolite appears to be stable in gastrointestinal tract of animals and as unique selective adsorbers. Many experiments showed that the dietary inclusion of zeolites improved the performance and egg quality by layer hens in early or late egg production periods. Olver (1983) showed that an addition of 5% zeolite to layer feed with different protein content (13 and 16%) led to significantly improving the feed conversion ratio but there was no effect on feed intake. Results of earlier experiments used different levels of zeolite in layer diet indicated no significant effect on feed intake (g/bird/day) or feed conversion ratio (g/g) (Öztürk et al., 1998; Berto et al., 2013). Also, Efthimiou et al. (2011) reported that no significant different in feed intake between hens getting 3% zeolite in their feed comparing to control hens without zeolite. Several experiments were conducted to study the effects of using natural zeolite on the egg production and egg quality in different production periods, and the results varied between an improvement in egg production and no effect of adding zeolite on these parameters. Studies from Merabishvili et al. (1980) and Olver (1989) found that adding of zeolite to layer diet improved the egg production. Olver (1983) found that adding the zeolite by 5% to layer diets contains different levels of protein significantly improved egg productivity (%) compared to hens that did not get zeolite. In a study of Roland et al. (1990) it was found that adding zeolite by 1.5% to the diets of laying hens led to a significant increase ($P \leq 0.05$) in egg production when the phosphorous level in diet was below the hens requirements. In addition, Strakova et al. (2008) found that adding 1% of zeolite increased egg production by 1.7% compared to control hens without zeolite. Other study indicated that adding 1% of the zeolite improved egg production by 1% compared to the control group during the period of 22-68 weeks of hens' ages (Herzig et al., 2008). In contrary, many scientific studies reported that the addition of zeolite in different percentage to the diet and at different production period of layer hens did not affect the egg production (%) (Öztürk et al., 1998; Berto et al., 2013Samara, 2003; Kaya et al., 2013). Skeletal abnormalities, bone deformities and egg breakage and fracture are major problems in laying hens which influenced by number of factors like nutrition, genetics, environment and age and causing a significant loss in egg production and quality (Roberts, 2004). Bain (1997) reported that damaged and cracked eggshell occurs in 6 up to 8% of laid eggs specially in the last third of the egg production period. Nys (1999) mentioned that application of synthetic zeolites improve the eggshell quality since they create a complex with Ca. Previous results of several studies reported that feeding laying hens with natural zeolite improves eggshell quality (Olver, 1989; Roland et al., 1985; Keshavarz and McCormick, 1991). These studies suggested dietary zeolite may also have an effect on egg production and egg quality in old laying hens. Therefore, this study was planned to specially focus on the effect of natural zeolite as additive on egg production performance and egg quality of old laying hens for 6 weeks feeding period.

Materials and Methods

Animals, Housing and Diet

The experiment was carried out in the experimental unit at the Faculty of Agriculture and Veterinary Medicine-Thamar University, Yemen. The animal protocols were approved by the department of animal production of the Faculty of Agriculture and Veterinary Medicine, Thamar-University.

A total of 36 laying hens (ISA White) of 71 weeks old were obtained from a local commercial farm. After 7 days of acclimation period hens were weight and divided into 3 treatments with 6 pens (replicates) of 2 hens each (12 hens/treatment). The live body weight and egg production were similar by all hens at the start of the experiment. The hens were reared in floor pens with identical size (1 m^2) and each pen was provided with wood shaving letter, drinker, feeder, and nest. The environmental temperature was between 24-26°C and the applied light program was 16 h/day (12 natural and 4 artificial) until the end of experiment. The used diet was of corn–soya bean meal basis and was formulated according to the NRC (1994) to meets the nutritional requirements for laying hens in this production period. The diet was offered in mash form. Table (1) shows the ingredients and chemical composition of basal diet used in this experiment.

Table 1. Composition and nutritive value of experimental diets

Ingredients	%
Corn	65
Soya bean meal	19
Wheat bran	1.5
Layer concentrate*	5
Limestone (ground)	9.5
Total	%100
Composition (calculated)	
Dry matter (%)	89.9
Metabolizable energy (Kcal/kg)	2740
% Crude Protein	16.6
% Calcium	3.9
Available Phosphorus %	0.5
Methionine %	0.46
Lysine %	0.86

*Layer concentrate content Vitamin and mineral premix, limestone, protein 28% and 2740 Kcal

The natural zeolite (NZ) was added to the basal diet and the dietary treatments were as following: control without NZ (T1), basal diet with 0.5% NZ (T2) and basal diet with 1% NZ (T3). A locally prepared natural zeolite was used for the experiment and was obtained from a commercial firm and its chemical analyses showed in table (2). All dietary treatments were isocaloric and isonitrogenous. Water was offered ad libitum during the completely experimental period. This experiment lasted for 6 weeks (from 72-78 weeks of hen's age).

Table 2: Composition of natural zeolite used in this experiment

Minerals	g/kg	Minerals	g/kg
SiO_2	622-7.5	L.O.I	78-136
Al_2O_2	110-121	BaO	< 0.8
CaO	9-3.6	P_2O_5	0.1- 0.4
K_2O	27-52	ZnO	0.08-0.25
Fe_2O_3	12-34	SrO	0.14-0.47
MgO	3-6	PbO	< 0.2
Na_2O	1.2-7.8	ZrO_2	0.05-1
TiO_2	1.5-3.4	SO_3	0.1-0.4
MnO	0.2-1.6	Cl	< 0.3

Studied Parameters

To measure the performance and egg quality parameters, the main period was subdivided to 2 periods (72-75 weeks 1st period, 75-78 weeks 2nd period) and the entire (total) period 72-78 weeks (6 weeks).

Performance Parameter

The feed was restricted with 120 g/hen and day and the rest of feed was weighed of weekly basis to calculate the exact feed consumption (g/d/hen). Eggs from each replicate were collected, counted, and weighed daily for the calculation of egg production (EP) ratio (%) and egg weight and egg mass (EM) using following equations:

$$\mathrm{EP}\ (\%) = \frac{\text{the number of collected eggs}}{\text{number of hens} \times \text{days}} \times 100$$

$$\mathrm{EM}(\mathrm{g/d/hen}) = \mathrm{EP}\ (\%) \times \text{mean of egg weight (g)}$$

The feed conversion ratio (FCR) was calculated using the following equation:

$$\mathrm{FCR}\ (\mathrm{g/g}) = \frac{\text{Feed intake (g)}}{\text{Egg mass (g)}}$$

Egg Quality Parameters

At the end of 1st period (end of 75th weeks) and the end of the experiment period (78th weeks), two eggs from each replicate (12 eggs per treatment) were collected for determination of egg qualities parameters. The following parameters were determined: egg weight, egg form index, shell weight ratio, the eggshell density and egg white and yolk ratio. Egg form index was determinate during the measurement of the length divided by width of the egg according to (Reddy et al.,1979; Anderson et al., 2004) with a manual caliper. To determine, the egg white, yolk and eggshell, the eggs were broken, and the yolk were separated handily and weighed. Individual shell (with membrane) was cleaned from adhering albumen and drying at room temperature overnight. The albumen (egg white) was determined by difference. The ratio of albumen (AR), yolk (YR) and shell (SR) were determined using the following equations:

YR (%) = yolk weight (g)/ egg weight (g)×100

AR (%) = albumen weight (g)/ egg weight (g)×100

SR (%) = shell weight (g)/ egg weight (g) ×100

The eggshell density (shell weight per unit surface area mg/cm2) was calculated from eggshell weight (mg) per unit egg surface area (cm2). Egg surface area (S) was calculated from the egg weight (EW) according to formula:

S= 3.978 × EW0.7056 (26)

Hemato-Biochemical Parameters

At the end of the experiment blood samples were collected from wing vein from 3 hens per treatment into anticoagulant EDTA treated for determination of red blood cells (RBC's), hemoglobin (Hb), packed cell volume (PCV). Serum was collected by centrifugation for 15 minutes at 3000 rpm and it stored at –20℃ until determination of blood metabolites (total protein, albumen, glucose, cholesterol, and aspartic transaminase) by using commercial kits. Blood minerals (calcium and phosphorus) were determined using commercial kits.

Statistical Analysis

The statistical analysis was performed using software package program (SPSS 14 for windows 2018). Results are presented as mean and standard error of mean (SEM). Significant differences among treatment means were investigated using Duncan's new multiple range test. Effects were considered significant at P< 0.05.

Results and Discussion

Productive Performance Parameters

Results regarding feed intake, feed conversion ratio (FCR) of old laying hens supplemented with natural zeolite are shown in table 3. There are no significant differences were observed among all tested treatments in individually period (72-75 and 76-78 week) or in total period (72-78 week). These results agreed with previous studies, which indicated that the use of zeolite at different level as additive to layer hens diet had insignificant effect on the feed intake and feed conversion ratio (Öztürk et al., 1998; Gezen et al., 2009; Kaya et al., 2013; Kermanshahi et al., 2011; Moghaddam et al., 2008). Additionally, results found that dietary zeolite did not affect the feed intake (Roland et al., 1980). In contrast, showed Olver (1989) that zeolite supplementation increased the daily feed intake. In addition, it was found that the feed intake by hens fed with 1% clinoptilolite was 4.1 g lower per one egg produced comparing to the control hens (Strakova et al., 2008), while Evans (1980) reported that the feed intake increased with zeolite increasing in the diet to ovoid a dilution effect. On the other hands, Emam et al. (2019) indicated that addition of 1% and 2% zeolite to layer diet did not influence the feed intake but improved significantly the feed conversion ratio, which is partially in agreement with our results relate to feed intake. According to table 3, dietary zeolites had no effect on egg production, egg weight and egg mass. There was only an insignificant (P>0.05) increasing in percentage of egg production by hens getting NZ in their diet as compared to the hens in control treatment (without NZ), this improvement reach in mean 3.8% and 4.9% in the total period (6 weeks) by hens fed 0.5% and 1% zeolite respectively. These results are consistent with previous studies (Merabishvili et al., 1980; Strakova et al., 2008; Herzig et al., 2008; Fremen 2003) who found that zeolites have a positive effect on egg production reached an amount between 1-3%. These results are in similarity with the findings of previously studies, which indicated no significant effect on egg production, egg weight, and egg mass of laying hens fed varying level of natural zeolite (Gezen et al 2009; Berto et al., 2013; Kermanshahi et al., 2011). On the other hand, studies showed that 2% (Machacek et al., 2010; Fendri et al., 2012) of zeolite significantly affected the mean egg weight and an increasing (p≤0.5) in egg mass by hens getting zeolite in diet (Emam et al., 2019). The increasing of egg production (%) in this study, it might be due to the beneficial effects of the zeolite which including, adsorption of toxins that are damaging and even fatal to the animals live, the improved utilization of feed nutrients, positive effects on the intestinal microflora and the mechanism of digestion, protection of animals against harmful mycotoxins and elimination of heavy metals and radioactive elements effects (Mumpton and Fishman, 1977; Zhou, 2008; Papaioannou et al., 2005).

Table 3. The effect of NZ on performance (feed consumption, FCR, egg production, egg weight and egg mass)

Period	* Treatment				
	0 NZ	0.5% NZ	NZ1 %	SEM	P-Value
Feed intake (g/bird/d)					
week72 – 75	113	114	115	1.18	0.88
weeks75 – 78	115	116	117	0.77	0.33
(72–78)Total	114	115	117	0.82	0.57
FCR (g/g)					
week72 – 75	2.12	1.96	2.13	0.04	0.10
week75 – 78	1.96	2.01	1.99	0.03	0.82
(72–78)Total	2.04	1.98	2.05	0.03	0.49
Egg production(%)					
weeks72 – 75	89.3	94.9	93.7	1.61	0.35
weeks75 – 78	92.9	94.4	97.6	1.49	0.44
(72–78)Total	91.1	94.6	95.6	1.33	0.36
Egg weight (g/bird)					
weeks72 – 75	62.7	61.7	60.8	0.69	0.53
weeks75 – 78	63.5	62.2	60.5	0.71	0.24
(72–78)Total	63.1	61.9	60.6	0.68	0.35
Egg mass (g/bird/d)					
weeks72 – 75	56.1	58.6	56.9	1.21	0.71
weeks75 – 78	59.1	58.8	59.3	1.01	0.98
(72–78) Total	57.6	58.7	58.1	0.96	0.91

* N= 6 SEM = standard error of mean

Egg Quality Parameters

Data on the eggshell ratio, eggshell density (mg/cm^2), egg form index, ratio of egg white (albumen) and yolk are shown in table 4. There is no significant difference between all dietary treatments regarding all above-mentioned tested parameters when data analyzed individually in the 1st and 2nd collecting experimental periods or in entire experimental periods (72-78 weeks). As shown in table 4, there are no differences between all dietary treatments in relation to parameters of egg form index, egg white ratio (albumen) and egg yolk ratio, which were in similarity to the results of earlier studies (Öztürk et al., 1998; Berto et al., 2013; Rizzi et al., 2003), but in contradictory to the results of (Fendri et al., 2012) and (Emam et al., 2019). Additionally, the supplementation of natural zeolite (0.5% or 1%) did not affect the eggshell quality parameters (eggshell ratio and eggshell density) in the 1st and 2nd experimental periods. However, there were only significant differences when the data of 72-78 weeks were pooled regarding the quality parameters eggshell ratio (%) and eggshell density (mg/cm^2) in this experiment. According to pooled data egg shell ratio and egg shell density were increased significantly ($p<0.05$) only by 1% natural zeolite versus other treatments (0.5% NZ and 0 NZ), which indicate an obviously improvement in egg shell quality by addition of 1% NZ to hens layer diets in this experiment. These results are in agreement with previously scientific findings, which indicated that an addition of 1-4% zeolite to the diet of laying hens with different age led to an improvement in the egg shell quality, including an increase in egg shell weight and ratio, egg shell density, egg shell thickness, stronger egg shell and higher egg shell ash (Gezen et al., 2009; Samara, 2003; Moghaddam et al., 2008; Emam et al., 2019; Fendri et al., 2012). Roland et al. (1985) hypothesized that the beneficial effect of NZ on eggshell quality is related to its high affinity to Ca and its high ion-exchange capability. Additionally, it is suggested that improvement of eggshell quality may associated with zeolite content of elements Si, Al and Na which affect positively the metabolism of Ca (Watkins and Southern, 1991).

Hematological and Biochemical Parameters

The results of the effect of dietary zeolite supplementation on tested hematological and biochemical parameters in blood were shown in Table 5. Hematological parameters have been considered as an important indicator for the healthy birds. In this study, there were insignificant difference among all experimental treatments in total protein, hemoglobin, packed cell volume (PCV) and Red blood cells (RBC). These findings are agreed partially with older results (Emam et al., 2019; Machacek et al., 2010) regarding the hematological traits RBC count, PCV and hemoglobin. On the other hand, these data did not agree with previous results of Strakova et al. (2008) who found significant difference in total RBC count and hemoglobin levels, which were higher in the experimental group treated by adding natural Zeolite compared to the control group. Biochemical parameters were not influenced by zeolite addition. As shown in Table 5 the values for these measured parameters such as total protein, albumin, glucose, cholesterol, AST, calcium and phosphors ranged within reference ranges, which agreed with (Strakova et al., 2008; Papaioannou et al., 2002) who observed no changes in serum concentrations of minerals (specially Ca and P) in sows at the long-term administration of zeolite. Based on these findings, it is worthy to mention that the zeolite supplementation at levels of 0.5% and 1% in diets of aged layer hens did not have adverse effects on blood plasma composition.

Table 4: The effect of NZ on egg quality (egg form index, eggshell, shell density, egg white and egg yolk)

Period	Treatment*				
	0 NZ	0.5% NZ	1 %NZ	SEM	P- Value
Egg form index					
75–72 week	74.9	74.4	74.1	0.47	0.91
78–75 week	74.6	75.1	75.0	0.51	0.88
Total (78–72)	74.7	74.6	74.9	0.40	0.96
Egg shell (%)					
72 - 75week	8.9	9.0	9.6	0.18	0.78
75 - 78 week	9.0	9.1	9.4	0.12	0.45
Total(72-78)[1]	9.0[a]	9.1[a]	9.6[b]	0.11	0.032
Egg shell density (mg/cm^2)					
72 – 75week	76.4	76.0	81.5	1.45	0.23
75 – 78 week	76.6	76.9	78.9	0.91	0.55
Total(72–78)[1]	76.3[a]	76.7[a]	81.3[b]	0.82	0.033
Egg white (Albumen) (%)					
72 – 75week	62.8	61.3	62.9	0.54	0.42
75 – 78 week	63.2	63.2	63.1	0.41	0.99
Total(72–78)	63.0	62.7	63.0	0.39	0.97
Egg yolk (%)					
72 – 75week	26.9	27.3	26.8	1.21	0.71
75 – 78 week	26.3	26.4	26.3	1.01	0.98
Total(72–78)	26.7	26.9	26.6	0.96	0.91

[*] N=12 [1]N= 11 SEM= standard error of mean, [a,b] letters in the same row are significantly different by $p \leq 0.05$.

Table 5. Effect of zeolite supplementation on blood biochemical parameters of laying hens

Parameter	Treatment*				
	0 NZ	0.5% NZ	1 %NZ	SEM	P-Value
Hemoglobin (g/l)	11.3	11.5	11.3	0.22	0.92
PCV (%)	32.6	33.3	33.3	0.82	0.95
RBC ($\times 10^6$/mm^3)	2.40	2.53	2.57	0.08	0.72
Total protein (g/dl)	4.6	5.2	5.0	0.18	0.38
Albumin (g/d)	2.2	2.3	2.1	0.58	0.08
Glucose (g/dl)	269	253	275	9.5	0.20
Cholesterol (g/dl)	160	204	127	16.1	0.13
AST (i.u/l)	158	155	159	6.5	0.98
Ca (mg/dl)	17.2	17.2	17.0	0.05	0.23
P (mg/dl)	6.2	5.5	5.4	0.38	0.70

Conclusion

From this study, it could be concluded that natural zeolite can be used as additives in layer hens diet and the application of 1% natural zeolite with basal diet had positive effect on the egg production (%) and improved the egg shell quality of old laying hens without any negative effects

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