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The Effect of Zeolite and Organic Acid Mixture Supplementation in the Layer Diet on Performance, Egg Quality Traits and Some Blood Parameters

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Abstract: This study was conducted to investigate the effect of zeolite, organic acid mixture and the combination of zeolite and organic acid mixture supplementation in a layer diet on performance, egg quality traits and some blood parameters. The experiment was carried out with a total of 96 Lohman White commercial hens 44 weeks of age in 6 replicate cages with each cage containing 4 hens who were allocated randomly to one of four dietary treatments. The hens were fed a basal diet (C), a basal diet plus 20 g kg^{-1} zeolite (Z), a basal diet plus 2 g kg^{-1} organic acid mixture (consisting of 70% propionic acid, 5% citric acid and 25% soft acid) (OA) and a basal diet plus 20 g kg^{-1} Zeolite and 2 g kg^{-1} Organic Acid mixture (ZOA). Feed intake, egg production, egg weight, feed conversion ratio and body weight did not statistically differ amoung the groups ($p>0.05$). Supplementing the basal diet with OA and ZOA significantly increased eggshell strength and weight. Eggshell strength was also higher on average in hens on the ZOA diets than those receiving OA. All of the additive treatments significantly increased eggshell thickness compared with that of the control group. Dietary treatment reduced serum albumin, glucose, ALP, AST and Ca contents compared with the control group ($p<0.05$). But treatment did not affect serum cholesterol, total protein and p levels. It can be concluded that the addition of organic acid mixture and zeolite combinations to layer diets can be beneficial in improving egg quality traits, especially shell strength and thickness.

Key words: Laying hens, zeolite, organic acid, performance, egg quality traits, blood parameters

INTRODUCTION

Zeolites are a family of aluminosilicates with an unusual crystalline structure enclosing pores occupied by cation and water molecules (Olver, 1983). Each zeolite species has its own unique crystal structure and hence its own set of chemical and physical properties (Eleroglu *et al*., 2011). They have the ability to lose and gain water reversibly and to exchange constituent ionic cations without major structural changes (Fendri *et al*., 2012). The exploitation of these properties underlies the use of zeolites in a wide range of industrial and agricultural applications particularly animal nutrition since, the mid-1960s (Papaioannou *et al*., 2005). Zeolites are also used as effective adsorbents of toxin agents particularly aflatoxins from the feeds (Rizzi *et al*., 2003; Moghaddam *et al*., 2008). Parlat *et al*. (1999) showed that zeolite supplementation in Japanese quail diets effectively minimizes the adverse effects of aflatoxins on feed intake, performance and feed conversion ratio. In the other study, the inclusion of clinoptilolite in laying hen's diets at a level of 50 g kg^{-1} increased the number of eggs laid and the feed conversion ratio, however the egg weight was not affected (Olver, 1997). Rabon *et al*. (1995) recorded a significant increase in serum aluminum and zinc levels in laying hens following the addition of zeolite to their diets which can be associated with improved egg quality parameters. Some researchers have hypothesized that the benificial 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). The effects of dietary zeolites in poultry have been investigated extensively and a growth-promoting effect evident in mineral utilization and metabolism has been reported. The beneficial effects may be related to the Al, Si, Zn, Na or K concentrations of zeolite because these minerals have been shown to influence mineral metabolism and electrolyte balance, leading to increased bone formation (Roland *et al*., 1993; Utlu *et al*., 2007).

Organic acids have been used for decades in feed preservation. However, regarding poultry production, only limited studies have been conducted to explore the effects of organic acid supplementation on the performance and egg quality parameters of laying hens.

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Soltan (2008) reported that the inclusion of organic acids at different levels in diets improved eggshell thickness and yolk index while significantly reducing the albumen index. Yesilbag and Colpan (2006) found that the supplementation of organic acid mixture in laying hen's diets did not affect shell-breaking strength. Adding organic acids to feed can lower the gastric pH and low gastric pH accelerates the conversion of pepsinogen to pepsin which improves the absorption rate of proteins, amino acids and minerals (Park *et al*., 2009). Dietary acidification increased gastric proteolysis and protein and amino acid digestibility in pigs (Giesting and Easter, 1985). The acidic anion has been shown to complex with Ca, P, Mg and Zn which results in the improved digestibility of these minerals (Li *et al*., 1998; Edwards and Baker, 1999). Pileggi *et al*. (1956) reported that citric acid had a specific effect on phytate phosphorus. Studies by Boling *et al*. (1998, 2000) showed that dietary organic acid effectively improved phytate phosphorus utilization in chicks. Abdel-Fattah *et al*. (2008) reported that broiler chicks fed a diet supplemented with organic acids containing acetic acid, citric acid or lactic acid at 1.5 and 3.0% levels had significantly higher Ca and P blood concentrations.

There is no recorded data on the use of zeolite and organic acid combination in laying hens diets. The use of zeolite in combination with organic acids may have synergetic effects on egg quality parameters. The primary aim of the present study was to determine the effect of zeolite, organic acid mixture and their combination on performance, egg quality traits and some blood parameters in laying hens.

MATERIALS AND METHODS

Animals, diet and management: Ninety six, 44 weeks old Lohmann layers were blocked according to the location of their cages (50×46×46 cm) and then randomly assigned to receive one of four dietary treatments for 12 weeks. Each dietary treatment was replicated in six cages with each cage containing 4 hens. The experimental diets included a standard commercial layer diet (control), a diet including 20 g kg^{-1} Zeolite (Z), a diet including 2 g kg^{-1} organic acid mixture (consisting of 70% propionic acid, 5% citric acid and 25% soft acid) (OA) and a diet including 20 g kg^{-1} zeolite plus 2 g kg^{-1} OA (ZOA) for 12 weeks. The ingredients and chemical composition of the basal diets are given in Table 1. The experimental diets were formulated to meet NRC recommendations and analyzed using AOAC (1990) Methods.

Sample collection and analytical procedure: Feed intake and egg production were recorded daily egg weight was measured bi-weekly and body weights were measured at the beginning and end of the experiment. Feed Conversion Ratio (FCR) was recorded as kilograms of feed consumed per kilograms of eggs produced. Before determining egg weight, 12 eggs from each experimental group were stored for 24 h at room temperature. About 12 eggs were randomly selected from each experimental group every month to evaluate egg quality parameters, including shape index, shell strength, shell thickness, albumen index, yolk index, yolk color (Yolk Colour Fan, the CIE Standard Colorimetric System, F. Hoffman-La Roche Ltd. Basel, Switzerland) and haugh units. Eggs were assessed according to the method utilized by Kaya and Macit (2012).

Table 1: Ingredients and nutrient composition of experimental diet

Experimental diet	Percentage
Ingredients	
Corn	53.73
Soybean (46% CP)	18.72
Bonkalite	7.50
Fish meal	2.16
Sunflowerseed meal (36% CP)	5.35
Vegetable fat	0.50
Dicalcium phosphate[1]	2.04
Marble	9.80
Vitamin-mineral premix[2]	0.30
Salt	0.23
Methionine[3]	0.40
Lysine[4]	0.25
Chemical composition (on a DM basis)	
Dry matter (%)	88.17
Crude protein (%)	16.56
Crude fiber (%)	4.18
Ether extract (%)	2.74
Ash (%)	13.60
ME (kcal kg^{-1})	2650.00

[1]Each kg contained 24% Ca and 17.5% P; [2]The premix provided per 1 kg of diet; vitamin A: 15.000 IU; cholecalciferol: 1500 ICU; DL-α-tocopheryl acetate: 30 IU; menadione: 5.0 mg; thiamine: 3.0 mg; riboflavin, 6.0 mg; niacin: 20.0 mg; panthotenic acid: 8.0 mg; pyridoxine: 5.0 mg; folic acid: 1.0 mg; vitamin B12: 15 μg; Mn: 80.0 mg; Zn: 60.0 mg; Fe: 30.0 mg; Cu: 5.0 mg; I: 2.0 mg and Se: 0.15 mg; [3]DL-methionine; [4]L-lysine hydrochloride

At the end of the experiment, blood samples taken from the subcutaneous vena ulnaris were collected from 2 hens from each cage and stored in additive-free vacutainers. Serum was obtained following centrifugation at 3,000 g for 10 min at 20°C and kept at -20°C until laboratory analyses were performed. Serum parameters-albumin, cholesterol, total protein, glucose, ALP, AST, ALT, Ca and P were measured using commercial kits (DDS® Spectrophotometric kits, Diasis Diagnostic Systems Co., Istanbul, Turkey) with a Mindray Perfect Plus 400 autoanalyzer.

Statistical analysis: Data from the present experiment were statistically analyzed with ANOVA using the GLM procedure of SPSS (1996). The effects of the dietary treatments on response variables were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

The performance parameters of hens fed a diet supplemented with Zeolite (Z) and Organic Acid mixture (OA) or a combination of Z and OA (ZOA) between 44 and 56 weeks of age are shown in Table 2. Feed additives had no impact on feed consumption, egg production, egg weight, feed conversion ratio, damage egg ratio and body weight.

Elliot and Edwards stated that there was no performance improvement associated with the supplementation of natural zeolite into laying hens diets. The results of the present study regarding the effects of zeolite on performance were in agreement with the findings of Kermanshahi who concluded that zeolite did not have any effect at the 0, 1.5 and 3.0% level on egg production, cracked and shell-less eggs, egg weight, feed intake and the feed conversion rate of laying hens during 45-50 weeks of age. Similarly, it was reported that a diet supplemented with 0, 20, 40, 60 and 80 g kg^{-1} natural zeolite (Ozturk *et al*., 1998) had no positive or negative effect on the performance of laying hens in terms of egg production, egg weight, body weight, body weight gain, feed intake and Feed Conversion Ratio (FCR). Samara also showed that there were no significant effects on the egg production, egg weight, feed consumption and feed conversion ratio of laying hens fed 0 and 10 g kg^{-1} of natural zeolite. In contrast to the results, others have observed that the addition of zeolite to a layer diet has positive effects on egg production (Olver, 1983, 1997) and the egg weight of 30-36 weeks old laying hens (Moghaddam *et al*., 2008) and that the addition of natural zeolite into the broiler diet improved feed conversion ratio.

The performance parameters in this study were not affected ($p>0.05$) by the supplementation of OA into laying hens diets (Table 2). A similar observation was reported by Yesilbag and Colpan (2006) who determined that the addition of an organic acid combination (formic and propionic acids and their ammonium salts) into laying hens diets had no significant effect on egg weight, feed consumption, feed conversion ratio and body weight, though egg production increased due to the addition of OA. The results of this study with regard to organic acid were not in agreement with those of Soltan (2008). They concluded that organic acid supplementation has positive effects on egg production and feed conversation in laying hens of an older age.

The effects of the dietary treatments on the egg quality traits are presented in Table 3. Shape index, yolk color, yolk index, albumen index and haugh score were not affected by dietary treatment, though eggshell strength, eggshell thickness and eggshell weight were.

In contrast, Kermanshahi *et al.* (2011) observed that yolk color index decreased significantly but shell thickness was not affected by the addition of increased zeolite into the layer diet. In this study, supplementing the basal diet with OA or ZOA significantly increased eggshell strength and egghell weight compared to the control and zeolite groups. Data from the results of the study with regard to eggshell strength were on average higher for the ZOA diets than those supplemented with OA (Table 3). The use of zeolite in combination with

Table 2: Effects of zeolite and organic acids mixture supplementation on laying performance parameters in hens

Performance parameters								
						Body weight (g)		
Groups	Feed consumption (g/day)	Egg production (%)	Cracked egg yield (%)	Egg weight (g)	FCR[1]	Initial	Final	Gain
Control	153.56	86.06	63.19	2.86	0.21	1588.08	1600.08	12.00
Z	140.87	86.12	62.67	2.63	0.48	1632.50	1697.50	65.00
OA	143.17	85.93	64.14	2.61	0.19	1596.17	1666.25	70.08
ZOA	148.95	84.84	62.49	2.83	0.01	1584.67	1601.17	16.50
SEM	5.82	2.34	1.03	0.10	0.14	33.67	42.44	39.44
P	0.18	0.96	0.63	0.09	0.37	0.74	0.30	0.61

[1]FCR = Feed Conversion Ratio (kg feed consumed per kg egg produced)

Table 3: Effects of zeolite and organic acids mixture supplementation on egg quality parameters in hens

Egg quality parameters								
Groups	Shape index (%)	Shell strength (kg/cm^2)	Shell thickness ($mm\times10^{-2}$)	Shell weight (g)	Yolk color	Yolk index (%)	Albumen index (%)	Haugh unit
Control	77.46	2.290^{b}	0.360^{b}	8.040^{b}	12.33	43.32	8.96	84.50
Z	76.50	2.280^{b}	0.370^{ab}	8.310^{b}	12.36	44.70	9.23	84.64
OA	77.88	2.880^{ab}	0.390^{a}	9.040^{a}	12.42	43.54	8.63	83.28
ZOA	77.13	3.260^{a}	0.390^{a}	8.640^{ab}	12.58	42.63	8.62	83.78
SEM	0.63	0.230	0.007	0.220	0.17	0.80	0.35	1.62
P	0.47	0.008	0.040	0.018	0.70	0.34	0.55	0.93

[a, b]Means within columns with different superscripts differ at $p<0.05$

Table 4: Effects of zeolite and organic acids mixture supplementation on metabolic profile of hen's serum

Response variables[1]									
Groups	Alb[1]	TP[1]	Glu[1]	Chol[1]	ALP[1]	AST[1]	ALT[1]	Ca[1]	P[1]
Control	3.05[a]	4.50	257.00[a]	146.00	282.50[a]	237.00[a]	68.50	24.87[a]	6.43
Z	1.90[b]	3.01	172.50[b]	102.50	139.50[b]	161.50[b]	39.00	16.02[b]	4.42
OA	1.74[b]	3.60	224.00[ab]	113.00	168.50[b]	151.50[b]	45.60	17.09[b]	4.08
Z+OA	1.83[b]	3.48	189.00[b]	134.50	126.50[b]	175.00[b]	44.00	14.83[b]	4.79
SEM	0.26	0.42	19.47	13.57	33.14	18.84	13.64	2.00	0.74
P	0.01	0.14	0.04	0.15	0.02	0.03	0.10	0.02	0.17

[1]Alb: Albumin (mg/dL); TP: Total Protein (g/dL); Glu: Glucose (mg/dL); Chol: Cholesterol (mg/dL); ALP: Alkaline Phosphatase (unit/L); AST: Aspartate Aminotransferase (unit/L); ALT: Alanine Aminotransferase (unit/L); Ca: Calcium; P: Phosphorus; [a,b]Means within columns with different superscripts differ at $p<0.05$

organic acids may have synergetic effects on eggshell strength by increasing mineral utilization. All of the additive treatments significantly increased eggshell thickness compared with that of the control group. Moghaddam *et al.* (2008) reported that eggshell strength was not affected by dietary zeolite supplementation, because of the positive effect of zeolite on shell thickness. In contrast to the present study, Fendri *et al.* (2012) found that the addition of zeolite in the diet of laying hens increased shell strength. Results from this study with regard to zeolite's impact on egg quality are in agreement with the findings of Ozturk *et al.* (1998) who concluded that adding zeolite into the layer diet had no positive or negative effect on shape index, yolk color, yolk index, albumen index, haugh score, eggshell strength and eggshell weight.

Similar to the results of the present study, Yesilbag and Colpan (2006) reported that adding OA to layer diet at different levels did not affect shell thickness, shell strength, yolk index, albumen index and haugh unit. Park *et al.* (2009) found that eggshell strength and thickness were not effected by the supplementation of OA at 0.3 and 0.4% levels in the layer diet which differed from the findings of this study. Results in the current study regarding OA are in agreement with findings obtained by Swiatkiewicz who reported that the addition of 5 g kg^{-1} of OA (a blend of formic, propionic and acetic acid) improved eggshell strength at 46, 58 and 70 weeks of age in laying hens. In addition, they stated that OAs have positive effects on egg shell traits, possibly by increasing calcium utilization (normal-3.70% Ca, reduced-3.25% Ca) and phosphorous (normal-0.65% P, reduced 0.60% P) to rations.

The data regarding the serum parameters of laying hens including albumin, total protein, glucose, cholesterol, Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), calcium and phosphorus are summarized in Table 4. The inclusion of Z, OA and ZOA in the basal diet caused a decrease in albumin, glucose, ALP, AST and calcium.

Utlu *et al.* (2007) reported that zeolite supplementation did not affect serum Ca but p concentrations decreased significantly in the serum of laying hens compared to the control group. There have been several reports in the literature indicating a response to zeolites in the blood serum of hens fed broiler diets (Kececi *et al.*, 1998; Oguz *et al.*, 2000; Eleroglu *et al.*, 2011) though the addition of zeolite into diets did not significantly alter the serum biochemical parameters, total protein, glucose, cholesterol, Ca and P reported in these studies. But Park *et al.* (2002) indicated that the blood cholesterol concentration of hens on a broiler diet was significantly lower in those receiving 3.0% natural zeolite treatments than those in the control group. Oguz *et al.* (2000) have also reported that the dietary clinoptilolite supplementation (1.5%) significantly reduced serum AST activity and increased ALT activity in broiler chickens. The expected effects of zeolites might exhibit variation due to factors such as the Al and p content of the zeolite and the level of Ca and p in the layer diets. Eleroglu *et al.* (2011) suggested that the structure of the mineral, the geographical source of the involved zeolite or its unique crystal structure, size, shape of cavities, porosity and metal oxide content as well as environmental conditions and animal species could be responsible for these inconsistent findings.

Similar to the results of the present experiment with regard to OA, a study conducted by Yesilbag and Colpan (2006) also reported no changes in serum parameters such as cholesterol and ALT by different concentrations of acetic acids (Attia *et al.*, 2013). In contrast to the results of the present study, however Soltan (2008) reported elevation in the serum protein and albumin of laying lens fed organic acid.

In this study, the effects of supplementations of zeolite, organic acid mixture and a combination of zeolite and organic acid mixture on performance, egg quality traits and some blood parameters were investigated and their effectiveness was compared. Feed additives had no effect on performance. However, these supplementations, especially the combination of zeolite and organic acid

mixture had a consistent impact in improving eggshell quality and some blood parameters containing ALP, AST and Ca.

CONCLUSION

It can be said that the addition of organic acid mixture and zeolite combinations to a layer diet can be beneficial in improving egg quality traits, especially shell stiffness and thickness.

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