African Journal of Biotechnology Vol. 10(16), pp. 3237-3242, 16 April, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1713

Full Length Research Paper

Efficacy of natural zeolite and pigments on yolk color and performance of laying hens

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Accepted 18 February, 2011

An *in vivo* study was conducted to evaluate pigment adsorptive ability of a natural zeolite in laying hens. This experiment was performed with three hundred sixty Hy-line W-36 strain of laying hens at 43 weeks of age. After a two weeks adaptation period, they received six experimental diets with a 3 × 2 factorial arrangement in a completely randomized design. The experimental groups were fed with corn-soy-wheat based diets containing three levels of natural zeolite (0.0, 1.5 and 3.0%) and 2 levels of synthetic pigment (0.0 and 0.04%) for a 6-week period. Each treatment contained 60 birds, which were randomly divided into 4 replicates of 15 birds each. The results showed that egg production, egg weight, shell thickness and the shell percent values did not significantly differ between treatments, but that the yolk color index was significantly reduced and increased by the addition of zeolite and pigment levels to the diet, respectively.

Key words: Natural zeolite, pigment, yolk color, laying hens.

INTRODUCTION

Aflatoxins (AF) are a class of mycotoxins produced mostly by the *Aspergillus flavus* and *Aspergillus parasiticus*. These toxins are seen worldwide, contaminating wheat, corn, soybeans and sorghum, which are normally used for poultry diets (Miller, 1995). Aflatoxins caused a variety of effects in poultry, including a decrease in body weight gain and efficiency of feed utilization. Measures used by the livestock industry to protect animals from the toxic effects of AF include the use of adsorbents (Masimanco et al., 1973; Phillips et al., 1990a, b). At the present time, one of the more promising and practical approaches is the use of adsorbents. The major advantages of these adsorbents include expense, safety and easy administration through addition to animal feeds. However, not all adsorbents are equally effective in protecting poultry against the toxic effects of AF and several adsorbents have been shown to impair nutrient utilization (Chung et al., 1990; Kubena and Harvey, 1993; Scheideler, 1993).

An adsorbent compound obtained from natural zeolite has demonstrated an ability to absorb mycotoxins. The bioavailability of potentially important nutrients such as vitamins or some feed supplements such as pigments or anticoccidials could also be reduced or sequestered by these clays (Ramos et al., 1996; Gray et al., 1998; Miazzo et al., 2000). However, the adsorption ability of these feed additives may vary depending on their geological origin. The zeolite structure is a specific arrangement in which the unit cell contains 24 tetrahedra, 12 AlO_4 and 12 SiO_4. When fully hydrated, there are 27 water molecules and there is also, one monovalent cation for each aluminium (Al) present. Therefore, the non-specific effect of zeolites which have a more rigid structure than other aluminosilicates and their ability to adsorb such nutrients could be much more selective (Miazzo et al., 2000).

Yolk color is strongly associated with food choices. Pigments commonly added to laying diets for marketing reasons. Egg yolk color plays a large role in consumer acceptance of table eggs (Bunell and Bauernfeind, 1962). There are several natural and synthetic sources of pigments used in poultry feed. The principal natural feed ingredient sources of carotenoids used during the past several decades include yellow corn, corn gluten meal

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Abbreviations: AF, Aflatoxins; **FCE,** feed conversion efficiency; **YI,** yolk color index.

Table 1. Chemical composition of zeolite.

Ingredients (%)	Zeolite
SiO_2	77.71
Al_2O_3	12.54
H_2O (Crystal)	-
Fe_2O_3	0.89
Na_2O	3.89
CaO	1.03
MgO	1.03
K_2O	2.75
TiO_2	0.16

and dehydrated alfalfa meal. Among the synthetic pigments, canthaxanthin, apo-ester and others have been developed (Castaneda et al., 2005). Papa et al. (1985) reported that, synthetic sources of pigments have the largest coloring capability of the egg yolk. Therefore, the color of egg yolk provided by carotenoid pigments are added to the diets of laying hens and subsequently, deposited in the egg (Perez-Vendrell et al., 2001). In the present *in vivo* study, the pigment adsorptive ability of natural zeolite in laying hens was assayed.

MATERIALS AND METHODS

A locally prepared natural zeolite was used for the experiment. Feeds were formulated to meet the requirements of laying hens suggested by Hy-Line W-36 Commercial Management Guide (2005). Feed and water were provided *ad-libitum* and a 16:8 L:D (Light:dark) program was followed throughout the experimental period. Laying hens were housed in standard battery cages. The room had cages distributed in two decks. The experimental protocols were reviewed and approved by the Animal Care Committee of the Ferdowsi University of Mashhad, Iran.

Three hundred and sixty laying hens of the Hy-Line W-36 commercial strain at approximately 67% production and uniform body weight were used at 43 week of age. After a two-week adaptation period, from 45 week of age, the experiment was started. In a 3 × 2 factorial arrangement with a completely randomized design experiment, 60 birds from each treatment were randomly divided into 4 replicates (15 birds/3-cages unit per replicate). The experimental diets contained 3 levels of zeolite (0.0, 1.5 and 3.0%) and 2 levels of pigment (0.0 and 0.04%) and were fed to the laying hens for 6 weeks. A cage dimension was 45 × 45 cm, equaling 2025 cm^2 of floor space. With 5 hens per cage, each bird had approximately 405 cm^2 of floor space. The pigment consisted of 20 grams of yellow lucantin pigment and 20 grams of red xanthin pigment mixed with an appropriate amount of wheat bran to make a uniform premix (0.4 g/kg of complete feed). This pigment concentration was considered for the whole experimental period. The chemical composition of zeolite is shown in Table 1. All dietary treatments were isocaloric and isonitrogenous. The ingredients and calculated chemical composition of the experimental diets is shown in Table 2.

The feed intake for each replicate was determined from the difference of feed offered and feed weighed back during each 4-week periods. Feed conversion efficiency (FCE) was calculated as the unit of feed intake per unit of egg weight laid (g/g). Egg production and egg weight were determined on 4 eggs randomly collected at the end of each week. The number of cracked and shell less eggs were also recorded. Thereafter, the collected eggs were broken to determine shell weight, shell thickness using micrometer and yolk color index (YI) using Roche color fan.

Data were analyzed by the General Linear Models procedure of SAS (SAS Institute, 1995) as a 3 × 2 factorial arrangement. Means for treatments showing significant differences in the analysis of variance were compared using Tukey's HSD test. Statistical significance was accepted at $P < 0.05$. Yolk color and shell weight data were analyzed using the repeated measures techniques of the SAS in PROC MIXED procedure. Contrast between treatment means were used to establish significance of the difference between 0.0, 1.5 and 3.0% zeolite. Levels of 0.0 and 1.5 versus 3.0% of zeolite were contrasted. In order to obtain normal distribution, all data were normalized using JMP 7 (SAS institute) software before analysis.

RESULTS AND DISCUSSION

The performance and some of the egg quality data of the birds fed different levels of zeolite and pigment are presented in Table 3. The data indicated that, none of the parameters were affected by feeding zeolite and pigment. The results for shell weight and YI are shown in Table 4. The YI was significantly affected by zeolite and pigment levels. Hens fed zeolite tended ($P = 0.062$) to lay eggs with a lighter shell weight (%) during the entire period compared with the control group. This effect was significant in 49 to 50 weeks of age, in a quadratic manner. A linear decrease in YI between 47 and 48 weeks of age was obtained as zeolite was increased. The addition of pigment significantly increased the YI in all recorded periods. The zeolite × pigment interaction effect was significant during weeks 47 to 48, 49 to 50 and the whole period, as shown in Table 4. The time significantly influenced percent of shell weight and yolk color when time was modeled as a classification variable (Table 5).

The use of clay supplements in animal and poultry feed is not new. Recent studies involving the use of clays as dietary supplements have given results which suggest that some clay products might have direct beneficial effects upon animal performance. Zeolites are used as effective adsorbents of toxic agents, particularly aflatoxins from the feeds (Parlat et al., 1999; Phillips, 1999; Ortatatli and Oguz, 2001; Rizzi et al., 2003). However, they indicated that zeolites may have other effects when included in animal diets (Desheng et al., 2005).

The addition of aluminosilicate to chick diets at a level of 1% did not negatively affect chick performance, organ weights or serum parameters or cause any pathological changes. This result indicates that, aluminosilicates did not negatively affect dietary nutrients at this dietary inclusion level (Ledoux et al., 1995). Feed intake and feed conversion ratio were not influenced by supplementing zeolite to diets; it concur with studies of Salari et al. (2006), Özturk et al. (1998) and Miazzo et al. (2005). Olver (1989) reported no significant dietary effects between treatments which were observed with respect to body weight, age at first egg, egg weight, haugh units or feed intake per hen. However, significant dietary effects in favor of clinoptilolite feeding were noticed with the

Table 2. Ingredients and nutrient composition of experimental diets.

Ingredients (%) / Pigment levels (g/kg)	0.0, 0.4	0.0, 0.4	0.0, 0.4
Corn	40.00	40.00	40.00
Wheat	23.86	20.36	16.86
Soybean meal	17.87	18.75	19.63
Barley	4.00	4.00	4.00
Wheat bran	1.50	1.50	1.50
Tallow	1.87	3.00	4.12
Oyster shell	8.84	8.83	8.82
Dicalcium phosphate	1.11	1.12	1.13
Salt	0.28	0.28	0.29
Vitamin and mineral premix[2]	0.50	0.50	0.50
Lys HCl	0.06	0.04	0.03
DL-Met	0.11	0.12	0.12
Zeolite	0.00	1.50	3.00
Calculated analysis			
ME, Kcal/kg	2700	2700	2700
CP,	15.00	15.00	15.00
CF,	3.26	3.22	3.17
Ca,	3.69	3.69	3.69
Ava. P,	0.33	0.33	0.33
Na,	0.17	0.17	0.17
Lys,	0.73	0.73	0.73
Met+Cys,	0.60	0.60	0.60
Arg,	0.85	0.86	0.87
Trp,	0.19	0.20	0.20

[1]Pigment provided by 20 gram lucanthin and 20 gram xanthin per 100 kg diet mixed as premix with wheat bran (0.4 g/kg of completed feed). [2]Supplied per kilogram of diet: vitamin A, 10000 IU; vitamin D_3, 9790 IU; vitamin E, 121 IU; vitamin K_2, 2 mg; vitamin B_{12}, 0.02 mg; thiamin, 4 mg; riboflavin, 4.4 mg; niacin, 22 mg; pyridoxine, 4 mg; biotin, 0.03 mg; folic acid, 1 mg; Ca-pantotenate, 40 mg; choline chloride, 840 mg; ethoxyquin, 0.125 mg; Zn, 65 mg; Mn, 75 mg; Cu, 6 mg; Se, 0.2 mg; I, 1 mg; Fe, 75 mg.

Table 3. Effect of zeolite and pigment on egg production, cracked and shell less egg, egg weight, shell thickness, feed intake and feed conversion efficiency of laying hens during 45 to 50 weeks of age.

Treatment	EP[1] (%)	Cracked (%)	Shell less (%)	EW[2] (g)	ST[3] (mm)	Feed intake (g/hen/day)	FCE[4] (g:g)
Zeolite (%)							
0.0	60.57	3.45	0.22	61.16	0.39	120.48	3.27
1.5	65.84	2.59	0.82	62.26	0.39	123.55	3.02
3.0	63.54	2.03	0.16	61.47	0.38	124.44	3.18
± SEM	1.114	0.97	0.213	0.610	0.003	2.253	0.076
Pigment (g/kg)							
0.0	63.43	2.79	0.56	61.05	0.38	123.16	3.18
0.4	65.64	2.59	0.24	62.20	0.39	122.48	3.01
±SEM	1.132	0.792	0.174	0.498	0.003	1.840	0.086
***P*- values**							
Zeolite	0.316	0.785	0.121	0.436	0.483	0.446	0.256
Pigment	0.179	0.702	0.137	0.122	0.532	0.797	0.421
zeolite × pigment	0.469	0.193	0.685	0.803	0.804	0.542	0.514

Means in each column with no superscripts are not statistically significant ($P > 0.05$). [1]Egg production; [2]egg weight; [3]shell thickness; [4]feed conversion efficiency.

Table 4. Effect of zeolite and pigment on shell weight and yolk color index of layers during 45 to 50 weeks of age.

Age (week)		Shell weight (g)				Shell weight[1] (%)				Yolk color index[2]			
		45 to 46	47 to 48	49 to 50	45 to 50	45 to 46	47 to 48	49 to 50	45 to 50	45 to 46	47 to 48	49 to 50	45 to 50
Zeolite (%)													
0.0		5.12	5.41	5.49	5.34	8.53	8.91	8.96[a]	8.78	6.27	5.75[a]	6.57	6.20
1.5		5.19	5.37	5.42	5.33	8.52	8.68	8.51[b]	8.58	6.17	5.36[ab]	5.60	5.71
3.0		5.24	5.39	5.45	5.34	8.39	8.78	8.69[ab]	8.64	6.35	5.31[b]	5.61	5.76
±SEM		0.047	0.072	0.092	0.043	0.113	0.090	0.093	0.055	0.091	0.112	0.074	0.049
Pigment (g/kg)													
0.0		5.14	5.31	5.41	5.29	8.57	8.81	8.68	8.69	4.05[b]	3.38[b]	3.55[b]	3.66[b]
0.4		5.22	5.44	5.50	5.38	8.38	8.80	8.78	8.65	8.48[a]	7.56[a]	8.30[a]	8.11[a]
±SEM		0.038	0.059	0.075	0.048	0.092	0.074	0.076	0.045	0.074	0.083	0.060	0.040
Zeolite	**Pigment**												
0.0	0.0	5.07	5.36	5.48	5.30	8.56	8.85	8.97	8.79	3.90	3.45	3.40	3.58
0.0	0.4	5.18	5.48	5.51	5.38	8.45	9.01	8.85	8.78	8.65	8.05	9.75	8.81
1.5	0.0	5.16	5.27	5.31	5.23	8.60	8.69	8.30	8.53	4.10	3.52	3.55	3.72
1.5	0.4	5.26	5.48	5.54	5.42	8.46	8.73	8.74	8.63	8.25	7.20	7.65	7.70
3.0	0.0	5.25	5.37	5.45	5.33	8.58	8.90	8.76	8.74	4.15	3.17	3.70	3.67
3.0	0.4	5.23	5.38	5.45	5.35	8.23	8.67	8.73	8.55	8.55	7.45	7.52	7.84
±SEM		0.067	0.103	0.13	0.061	0.160	0.128	0.132	0.078	0.135	0.159	0.121	0.069
Source of variation			***P*-values**										
Zeolite		0.203	0.814	0.861	0.966	0.985	0.244	0.025	0.062	0.409	0.014	0.454	0.490
Pigment		0.130	0.137	0.405	0.069	0.251	0.908	0.335	0.628	0.0001	0.0001	0.0001	0.0001
zeolite × pigment		0.694	0.791	0.647	0.371	0.851	0.322	0.114	0.213	0.132	0.016	0.001	0.001
Zeolite (Lin)[3]		0.121	0.528	0.747	0.959	0.897	0.259	0.249	0.090	0.502	0.004	0.682	0.284
Zeolite (Quad)[3]		0.614	0.964	0.664	0.799	0.915	0.209	0.012	0.048	0.251	0.718	0.240	0.612
contrast													
0.0, 1.5 versus 3.0%		0.210	0.595	0.953	0.864	0.953	0.725	0.727	0.570	0.297	0.066	0.556	0.421

[a, b, c, d] Means in each column with different superscript are significantly different ($P < 0.05$).[1]Shell weight to egg weight ratio.[2] yolk color was determined with a Roche yolk color fan. [3]linear (Lin) or quadratic (Quad) response estimated using orthogonal polynomial contrasts.

Table 5. Means for yolk color and shell weight modeling time as a classification variable[1].

Parameter	Period			±SEM	P-value
	45 to 46	47 to 48	49 to 50		
Yolk color	6.27	5.49	5.89	0.058	0.0003
Shell weight (g)	5.39	5.37	5.45	0.125	0.892
Shell weight (%)	8.47	8.87	8.73	0.061	0.0187

[1]A factorial experiment including 3 levels of zeolite (0.0, 1.5 and 3.0%) and 2 levels of pigment (0.0 and 0.04%).

number of eggs laid per hen, shell thickness and efficiency of feed utilization. Also, significant differences between strains were observed regarding all measurements taken except feed intake.

It has been reported that, feeding laying hens with zeolite improves eggshell quality (Roland et al., 1985; Rabon et al., 1991). There was an interactive effect of zeolite and pigment on the yolk index (YI) in two recorded periods (47 to 48 and 49 to 50 weeks of age). The lack of consistency on YI could be related to its level in the diet and its structure. The eggs of hens treated with clinoptilolite were lighter in color than those of the control group ($P < 0.05$), and the tendency to yellowness in eggs, was increased by clinoptilolite, probably through the affinity of red pigments for adsorbents and a consequent prevalence of yellow tonality. Zeolites are crystalline hydrated aluminosilicates containing cations of alkaline metals (Dvorak, 1989). Venglovsky et al. (1990) investigated the effect of the adsorption capacity of natural zeolite and bentonite on fecal coliform microorganisms in pigs. They found that slurry treated with zeolite did not contain any fecal coliforms after 14 days of contact, whereas, in those treated with bentonite; coliforms were not seen after 7 days of contact. In contrast with our results on the interaction effect of zeolite, Rizzi et al. (2003) stated that, hen dosed with clinoptilolite laid eggs lighter in color than those of the control group ($P < 0.05$) and the yolk color changed from denser yellow to lighter yellow by clinoptilolite inclusion, probably through the affinity of pigments for adsorbents and a consequent prevalence of yellow tonality. In our study, pigment as expected significantly increased the YI compared with the control diet. Oguz et al. (2000) showed a lower concentration of zeolite in the diet which was more effective for all measured parameters than a higher concentration (15 versus 25 g/kg). The expected effects of zeolite might exhibit variation due to such factors as concentration, the aluminum and phosphorus content of the aluminosilicate, the level of calcium and phosphorous in the diet and age of laying hens.

Conclusions

Under the conditions of this study, it was concluded that dietary zeolite decreased the pigmentation of egg yolks but had no effect on egg production and other performance criteria in laying hens. It was clear that lower yolk pigmentation was observed with the higher usage of zeolite in the laying hen diet. Based on the differences in clays in terms of their adsorbent capacities, it was suggested that, when a higher yolk color index is required then, smaller amount of zeolite must be considered for use in laying hen diets. More research is also needed to clarify whether the available natural zeolites in market have different adsorptive ability in laying hens.

ACKNOWLEDGEMENT

This study was supported by the Excellence Center for Animal Sciences and Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran.

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