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Interactive Effect of Sodium Bentonite with Pigments on Performance and Egg Quality of Laying Hens

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Abstract: In this experiment sodium bentonite was evaluated for its effect on egg yolk pigmentation and performance when synthetic pigment was added to the diet of laying hens. About 256 Hy-Line W-36 laying hens 35 weeks old were allocated to 8 experimental diets with 4 replicates each. Four levels of sodium bentonite (0.0, 1.0, 2.0 and 3.0%) and 2 levels of synthetic pigment (0.0 and 0.04%) were used for the 12 week experiment with a 4×2 factorial arrangement and a completely randomized design. Pigment was supplied by 20 g of lucantin and 20 g of xanthin that were diluted with 9960 g wheat bran. About 1 week before the experiment, the birds fed dietary treatments for adaptation. The measured records were feed intake, feed conversion ratio, egg production, egg weight, specific gravity, shell percent, shell thickness and yolk color index. The addition of sodium bentonite decreased ($p<0.05$) the specific gravity and yolk color index compared to the control diet. The addition of pigment significantly increased specific gravity and as expected, increased the yolk color index compared to the control diet.

Key words: Sodium bentonite, pigment, yolk color, laying hens, weight, Iran

INTRODUCTION

Aflatoxins (AF) are secondary fungal metabolites mainly produced by *Aspergillus* sp. (Miller, 1995). They can grow on different food, feed and waste materials and under a wide variety of environmental conditions in terms of moisture, pH and temperature which enables them to have a widespread distribution. There are >20 aflatoxin derivatives of which aflatoxin B1 (AFB1) is the most toxic (Hussein and Brasel, 2001).

In poultry, their effects are associated with liver damage, immunosuppression and poor performance (Kermanshahi *et al*., 2007; Leeson *et al*., 1995; Abo-Norag *et al*., 1995). Practical and effective methods for detoxifying AF-contaminated feedstuffs have been suggested. Recently, nutritionally inert absorbents have been used in the diet to detoxify AF-contaminated feedstuffs. Some absorbents may sequester mycotoxins and decrease mycotoxins absorption from the gastrointestinal tract, avoiding their toxic effects in livestock (Ramos *et al*., 1996).

In vitro and *in vivo* studies have shown that bentonites (aluminosilicates) have the ability to absorb mycotoxins (Ramos and Hernandez, 1997), fecal moisture and ammonia (Hale, 2005). Efficacy of anticoccidials that have a high molecular weight could be decreased by these feed additives (Gray *et al*., 1998). Egg yolk and skin color play important roles in consumer acceptance of poultry products. This has encouraged the addition of carotenoid pigments to layer and meat-type poultry diets which are subsequently deposited in the skin, fat and egg (Perez-Vendrell *et al*., 2001).

Aluminosilicates have a high capacity for swelling and absorbing mycotoxins, fecal moisture and ammonia but might exhibit less adsorbent-selective additive, antagonistic or synergistic interactions with other compounds (Ramos *et al*., 1996). 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. Therefore, the objective of the present study was to evaluate the pigment adsorption ability of locally available sodium bentonite and its effects on performance and egg quality in laying hens.

MATERIALS AND METHODS

The experiment was conducted with locally available sodium bentonite. Experimental diets were formulated to meet the recommendations of laying hens as described by the Hy-Line International (2005). Feed and water were provided *ad libitum* and a 16L:8D prog was followed throughout the experimental period. Laying hens were

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housed in cages in controlled room conditions with temperature maintained at about 25°C and there was no control on relative humidity. The cages were arranged on 2 decks. The experimental protocols were reviewed and approved by the Animal Care Committee of the Ferdowsi University of Mashhad, Iran.

Birds, feeding and management: About 256, 35 weeks old laying hens of the Hy-Line W-36 commercial strain at approximately 71% production and uniform body weight were used in this study. After 1 week adaptation from week 36 laying hens were allocated to 8 experimental diets with 4 replicates each (8 birds/2-cage unit per replicate), thus forming a 4×2 factorial arrangement with a completely randomized design. Cage dimensions were 40×40 cm, equaling 1600 cm^2 of floor space. With 4 hens per cage, each bird had approximately 400 cm^2 of floor space. Four levels of sodium bentonite (0.0, 1.0, 2.0 and 3.0%) and 2 levels of pigment (0.0 and 0.04%) were fed during the 12 week experiment.

The pigment consisted of 20 g of yellow lucantin pigment and 20 g of red xanthin pigment mixed with an appropriate amount of wheat bran to make a uniform premix (0.4 g kg^{-1} of complete feed). This pigment concentration was considered for the whole experimental period. The composition of sodium bentonite is shown in Table 1. All dietary treatments were isocaloric and isonitrogenous. The compositions of the experimental diets are shown in Table 2.

Data collection: The feed intake for each replicate was determined weekly and then the Feed Conversion Efficiency (FCE) was calculated as the g of feed intake per g of egg weight. At the end of each week, egg production was calculated. Egg weights were determined from 4 eggs per replicate that were randomly collected each week. The number of cracked and shell-less eggs was recorded daily. Specific Gravity (SG) was determined by the flotation method (Holder and Bradford, 1979). Thereafter, these eggs were broken one by one on a flat surface to determine the shell weight, shell thickness and Yolk color Index (YI) using the Roche color fan (DSM nutritional products Co.). The shell of the broken eggs was washed under gently flowing tap water to release albumen residues and was then air-dried and weighed. The shell thickness at the equator of each eggshell was measured using a micrometer. Specific gravity was measured based on the method described by Holder and Bradford (1979). SG = A/(A-B); A: egg weight in air and B: egg weight in distilled water.

Statistical analysis: Data were analyzed by ANOVA, using the GLM procedure of SAS (1995) appropriate for a completely randomized design with 4 levels of sodium bentonite and 2 levels of pigment in a factorial

Table 1: Chemical composition of sodium bentonite and zeolite

Ingredients (%)	Sodium bentonite (Experiment 1)
SiO_2	67.00
Al_2O_3	16.30
H_2O (Crystal)	6.90
Fe_2O_3	3.30
Na_2O	2.60
CaO	1.80
MgO	1.50
K_2O	0.48
TiO_2	0.12

Table 2: Ingredients and nutrient composition of experimental diets

	Pigment[1] levels (g kg^{-1})			
Ingredients (%)	0.0, 0.4	0.0, 0.4	0.0, 0.4	0.0,0.4
Corn	43.82	41.80	39.71	37.64
Wheat	25.00	25.00	25.00	25.00
Soybean meal	18.23	18.64	19.06	19.48
Wheat bran	1.00	1.00	1.00	1.00
Tallow	1.00	1.73	2.50	3.27
Limestone	8.50	8.46	8.41	8.36
Dicalcium phosphate	1.38	1.38	1.38	1.39
Salt	0.35	0.28	0.22	0.15
Vitamin and mineral premix[2]	0.50	0.50	0.50	0.50
Lys HCl	0.07	0.06	0.06	0.05
DL-Met	0.15	0.15	0.16	0.16
Sodium bentonite	0.00	1.00	2.00	3.00
Calculated analysis				
ME (Kcal kg^{-1})	2750.00	2750.00	2750.00	2750.00
CP(%)	15.00	15.00	15.00	15.00
CF (%)	3.16	3.14	3.12	3.11
Ca (%)	3.64	3.64	3.64	3.64
Ava. P (%)	0.37	0.37	0.37	0.37
Na (%)	0.17	0.17	0.17	0.17
Lys (%)	0.76	0.76	0.76	0.76
Met+Cys (%)	0.65	0.65	0.65	0.65
Arg (%)	0.86	0.86	0.86	0.87
Trp (%)	0.20	0.20	0.20	0.20

Pigment provided by 20 g lucanthin and 20 g xanthin per 100 kg diet mixed as premix with wheat bran (0.4 g kg^{-1} 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 ($ZnSO_4$), 65 mg; Mn ($MnSO_4{\bullet}H_2O$), 75 mg; Cu ($CuSO_4{\bullet}5H_2O$), 6 mg; Se (Na_2SeO_3), 0.2 mg; I (KI), 1 mg; Fe ($FeSO_4{\bullet}H_2O$), 75 mg

arrangement. Tukey's HSD was used to compare significantly different treatment means ($p<0.05$). Specific gravity, yolk color and shell weight data collected at different months after feeding 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, 1.0, 2.0 and 3.0% SB. The following contrasts were tested: Levels of 0.0, 1.0 and 2.0 vs. 3.0% of SB. In order to obtain normal distribution, all data were normalized using JMP 7 (SAS institute) software before analysis.

RESULTS AND DISCUSSION

Effect of sodium bentonite and pigment on egg production, cracked and shell less egg, egg weight, shell thickness, shell weight, feed intake and feed conversion

Table 3: Effect of sodium bentonite and pigment on egg production, cracked and shell less egg, egg weight, shell thickness, shell weight, feed intake and feed conversion efficiency of laying hens during 36-47 weeks of age

Treatments	EP[2] (%)	Cracked (%)	Shell less (%)	EW[3] (g)	ST[4] (mm)	SW[5] (g)	SW[6] (%)	Feed intake (g/hen/day)	FCE[7] (g:g)
SB[1] (%)									
0.0	69.79	2.040	0.220	66.37	0.401	5.590	8.450	118.690	2.570
1.0	65.40	2.190	0.820	66.32	0.403	5.570	8.410	120.380	2.780
2.0	69.21	1.840	0.170	67.05	0.406	5.710	8.560	123.490	2.670
3.0	67.50	2.010	0.500	67.83	0.402	5.580	8.230	123.660	2.710
±SEM	1.432	0.366	0.277	1.042	0.003	0.056	0.170	1.889	0.069
Pigment (g kg^{-1})									
0.0	66.71	1.970	0.450	66.90	0.401	5.570	8.350	119.670	2.690
0.4	69.24	2.070	0.400	66.88	0.405	5.660	8.480	123.440	2.670
±SEM	1.012	0.258	0.196	0.737	0.002	0.040	0.120	1.336	0.048
Source of variation	----------------	----------------	----------------	----------------	p-values	----------------	----------------	----------------	----------------
SB	0.156	0.928	0.157	0.640	0.689	0.252	0.796	0.202	0.233
Pigment	0.090	0.783	0.270	0.837	0.282	0.126	0.467	0.058	0.741
SB x pigment	0.219	0.950	0.609	0.966	0.582	0.341	0.962	0.309	0.808

Means in each column with no superscripts are not statistically significant ($p>0.05$), [1]Sodium bentonite; [2]Egg production; [3]Egg weight; [4]Shell thickness; [5]Shell weight; [6]shell weight to egg weight ratio; [7]Feed conversion efficiency

Table 4: Effect of sodium bentonite and pigment on specific gravity and yolk color index of laying hens during 36-47 weeks of age

		Specific gravity[1]				Yolk color index			
Age (week)									
Treatments		36-39	40-43	44-47	36-47	36-39	40-43	44-47	36-47
SB (%)									
0.0		1.081^{a}	1.063^{a}	1.074	1.073^{a}	10.0000^{a}	8.740	8.570^{a}	9.100^{a}
1.0		1. 077^{a}	1.072^{a}	1.074	1.075^{a}	9.8200^{a}	8.780	8.010^{b}	8.870^{ab}
2.0		1.077^{a}	1.072^{a}	1.064	1.071^{a}	9.7700^{a}	8.820	7.880^{b}	8.820^{b}
3.0		1.042^{b}	1.037^{b}	1.057	1.046^{b}	9.1000^{b}	8.640	7.780^{b}	8.510^{c}
±SEM		0.006	0.007	0.006	0.005	0.1400	0.125	0.085	0.083
Pigment (g kg^{-1})									
0.0		1.063	1.054^{b}	1.061	1.060^{b}	7.3100^{b}	6.170^{b}	5.270^{b}	6.250^{b}
0.4		1.075	1.068^{a}	1.074	1.072^{a}	12.0300^{a}	11.320^{a}	10.850^{a}	11.400^{a}
±SEM		0.004	0.005	0.004	0.003	0.0990	0.088	0.060	0.058
Interactions									
SB (%)	Pigment (g kg^{-1})								
0.0	0.0	1.074	1.055	1.076	1.068	7.4600	6.290	6.010	6.590
0.0	0.4	1.088	1.072	1.073	1.078	12.5400	11.200	11.120	11.620
1.0	0.0	1.077	1.072	1.074	1.074	7.0900	6.040	5.180	6.100
1.0	0.4	1.078	1.073	1.075	1.075	12.5600	11.510	10.840	11.640
2.0	0.0	1.077	1.071	1.056	1.068	7.5200	6.030	4.980	6.180
2.0	0.4	1.076	1.074	1.073	1.074	12.0300	11.610	10.770	11.470
3.0	0.0	1.025	1.021	1.039	1.028	7.2000	6.320	4.890	6.130
3.0	0.4	1.059	1.054	1.075	1.063	11.0100	10.970	10.680	10.890
	±SEM	0.009	0.010	0.008	0.007	0.1990	0.177	0.120	0.117
Source of variation		----------------	----------------	----------------	----------------	p-values	----------------	----------------	----------------
SB		0.024	0.032	0.136	0.029	0.0400	0.9450	0.0040	0.0100
Pigment		0.083	0.015	0.326	0.038	0.0001	0.0001	0.0001	0.0001
SB × pigment		0.493	0.892	0.002	0.678	0.0160	0.0390	0.9890	0.2610
SB (Lin)[3]		0.128	0.229	0.276	0.068	0.0200	0.6870	0.0010	0.0010
SB (Quad)[3]		0.007	0.008	0.851	0.014	0.6810	0.9940	0.0800	0.8530
contrast									
0.0, 1.0, 2.0 vs. 3.0%		0.006	0.009	0.854	0.005	0.0140	0.8470	0.0620	0.0070

[a, b, c, d]Means in each column with different superscript are significantly different ($p<0.05$), [1]SG = A/ (A-B); A: egg weight in air and B: egg weight in distilled water, [2] Yolk color was determined with a Roche yolk color fan, [3]Linear (Lin) or quadratic (Quad) response estimated using orthogonal polynomial contrasts

efficiency of laying hens during 36-47 weeks of age are shown in Table 3. These parameters were not significantly different from 36-47 weeks of age. The feed intake did not increase significantly follow ing bentonite and pigment supplementation.

Although there was no significant difference for Shell Thickness (ST) between 0.0 and 0.04% of pigment in the whole period, a strong trend ($p = 0.055$) was detected.

The sodium bentonite x pigment interaction had no effect on these parameters. The SG and YI of laying hens were assayed from 36-47 weeks of age and in 4 week periods (Table 4). Although there were no significant differences in performance, cracked and shell-less eggs, the Specific Gravity (SG) and Yolk color Index (YI) demonstrated significantly a quadratic and linear response to sodium bentonite treatment, respectively.

Hens that received 1 and 2% sodium bentonite laid eggs with a lower but not significant SG compared to the control treatment. The addition of 3% sodium bentonite into the laying hen diet produced eggs with the lowest SG

Table 5: Means for yolk color, specific gravity and shell weight modeling time as a classification variable

	Weeks				
Variables	36-39	40-43	44-47	±SEM	p-value
Yolk color	9.680	8.740	8.060	0.066	0.0001
Specific gravity	1.069	1.061	1.067	0.003	0.0461

[1]A factorial experiment including 4 levels of sodium bentonite (0.0, 1.0, 2.0 and 3.0%) and 2 levels of pigment (0.0 and 0.04%)

when contrasted with other levels of sodium bentonite ($p<0.05$). The addition of sodium bentonite to the diet had no significant effect on SG between 44 and 47 weeks of age. During this period, pigment supplementation numerically increased the SG but this effect was only significant in the overall period (36-47 weeks). There was no interaction effect of sodium bentonite x pigment on specific gravity. The addition of sodium bentonite to the diet decreased YI in a linear manner during the 4 weeks period and overall except between 40-43 weeks of age. The lowest YI was found in hens fed 3% sodium bentonite. Undoubtedly the addition of pigments into the laying hen diet could lead to more pigments in the yolk. Pigment supplementation improved the YI ($p<0.05$) compared to the control birds. The sodium bentonite x pigment interaction effect was significant during the 4 weeks period and overall period of study (Table 4). Table 5 contains specific gravity and yolk color over time. There was a significant effect attributable to time for specific gravity and yolk color index.

To the knowledge, the interaction effects between aluminosilicates and pigment during the laying period has not been tested yet. Clay supplements have generally been used in animal diets for many reasons mentioned below. This is used as effective adsorbent of toxic agents particularly aflatoxins present in the feedstuffs (Phillips, 1999; Ortatatli and Oguz, 2001; Rizzi *et al*., 2003). The main adsorptive mechanism of aflatoxins by these binders involves the formation of double hydrogen bonds between aflatoxin B1 and aluminosilicate (Desheng *et al*., 2005).

Kurnick and Reid (1960) reported that the addition of aluminosilicates to the diet improved weight gain reduced the speed of feed transit through the gastrointestinal tract and increased feed intake in broiler chickens. Feed consumption was higher ($p>0.05$) when higher levels of sodium bentonite were added to the diets but no reason found for these variations. This might be due to their absorbent-selective character and the binding of selected nutrients, meaning that the birds have to increase their consumption to meet requirements. In broiler studies feed consumption and the feed conversion ratio were not affected by adding sodium bentonite (Salari *et al*., 2006).

In this study, egg production, egg weight, shell thickness and shell weight percent were not influenced by the experimental diets (Table 4 and 5). These findings are consistent with others (Evans and Farrell, 1992; Ozturk *et al*., 1998) who also found that production criteria were not affected by sodium aluminosilicates. Contrary to our results, Quisenberry and Bradley (1964) reported that sodium bentonite improved the laying frequency, shell quality and egg size. Olver (1989) found that heavier eggs were laid by layers fed sodium bentonite compared to the control group.

However, others observed negative effects in terms of egg production (Roland, 1990a; Fethiere *et al*., 1990; Roland *et al*., 1991), egg weight (Miles *et al*., 1986; Fethiere *et al*., 1990) and feed consumption (Miles *et al*., 1986). The data revealed a significant quadratic effect of sodium bentonite on Specific Gravity (SG). The addition of 3% sodium bentonite decreased ($p<0.05$) SG when compared to the control diet. This result is consistent with the findings of Roland (1990b) which showed that sodium aluminosilicates changed the specific gravity of eggs and hypothesized that the mechanism by which sodium alumiosilicates affect specific gravity is independent of phosphorus. The reduction in the SG of the laid eggs in this study might be attributed to the non-specific selective characteristic of these clays. Olver (1989) reported that laying hens fed 2, 4 and 8% sodium bentonite showed significantly improved feed conversion and several other productive parameters. Based on these observations, it was concluded that 2% sodium bentonite was the optimal inclusion level to obtain the best productive benefits in laying hens.

Supplementing diets with pigment led to increased SG and tended to increase shell thickness. Although the pigments used are classified as carotenoids, their vitamin A activity differs from that of beta carotene. *In vitro* sodium bentonite has a strong affinity for pure carotene (Erwin *et al*., 1957). They suggested that sodium bentonite is not specific for carotene and apparently binds other non-carotenoid pigments as well. Briggs and Spivey (1954) reported similar results in chickens fed purified diets. They reported that purified diets containing vitamin A and 3% bentonite may produce vitamin A deficiency symptoms while a practical commercial diet with 5% bentonite had no deleterious effect.

Chung *et al*. (1990) reported that hydrated sodium calcium aluminosilicate at 0.5 and 1% had no effect on manganese, vitamin A or riboflavin utilization in chickens. However, zinc utilization was reduced by adding Na-Ca aluminosilicate to the chicken diet at higher levels. It seems that the basis of the diet and the level of clays are important issues to be considered in laying hen diets when a combination of pigments and natural clays is used. There was an interactive effect of sodium bentonite and pigment on the Yolk Index (YI) in all recorded periods.

CONCLUSION

Under the conditions of this study, it was concluded that dietary sodium bentonite decreased the pigmentation of egg yolks but had no effect on egg production and other performance criteria in laying hens. Different modes of action and interaction effects of bentonite were seen in this study regarding the yolk color index but it was clear that lower yolk pigmentation was observed with the higher usage of sodium bentonite 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 less sodium bentonite must be used. The lack of similar studies related to this topic does not allow the obtained data to be further evaluated and compared.

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