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Interactive Influence of Formycine® Gold and Pigment on Egg Quality and Performance of Laying Hens

Research Article

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ABSTRACT

Sodium bentonite can bind to dietary pigment and decreases yolk color scale. Sodium bentonite is one of the main parts of Formycine® Gold, the effect of different levels of Formycine® Gold and dietary pigment on egg quality, egg yolk color and performance criteria of laying hens from 26 to 34 week of age were studied. One hundred ninety two Hy-Line hens were divided into 24 groups of 8 birds each and randomly assigned to 6 dietary treatments of 4 replicates each. The study was conducted in a completely randomized design with a 2 × 3 factorial arrangement of treatments based on 2 levels of pigment (0 and 0.4 g/kg) and 3 levels of Formycine® Gold (0, 1 and 2 g/kg). Feed intake, body weight, feed to egg ratio and egg productions were recorded at the end of the weeks 28, 30, 32 and 34. Egg quality characteristics including egg weight, specific gravity, yolk color scale, egg shell percentage and thickness were evaluated every other week. Formycine® Gold and dietary pigment had no effect on performance for entire of the experiment. Egg shell weight and thickness increased by 0.4 g/kg dietary pigment at 29-30 weeks of age. Hen-day egg production was not influenced by dietary treatments. Egg specific gravity was similar in hens fed different levels of Formycine® Gold and pigment, except for 29-30 weeks of age. Regardless of Formycine® Gold levels, diet supplemented with pigment considerably increased yolk color scale. It seems that, by decreasing the amount of sodium bentonite in Formycine® Gold, it can be used without any adverse effect on egg quality, egg pigmentation and performance of laying hens.

KEY WORDS Formycine® Gold, laying hen, pigment, sodium bentonite.

INTRODUCTION

Egg yolk and skin color have been important scales in consumer acceptance of poultry products. This has leaded to 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). In addition, carotenoids have been promoted for their antioxidant potential (Bendich, 1989) as well as their usefulness for pigmenting food products. As epidemic diseases of animals have threaten the safety of what goes on our food plates, the topic of biosecurity is receiving a lot of mainstream media attention. Two of the most important sanitary problems related with livestock biosecurity are *Salmonella* and aflatoxin contamination (Yu *et al.* 2004; Devegowda and Murthy, 2005). *Salmonella* is widespread and can be found on a large number of farms and impacts many species of animals, including humans, other mammals and birds (Lu *et al.* 2003). The quality of ingredients used for food production by poultry feed mill factory is important because it can affect flock quality and the wholesomeness of a flock's meat and eggs. Aflatoxins are recognized as a class of mycotoxins produced by fungal species of genus *Aspergillus* (*A. flavus* and *A. parasiticus*) that routinely contaminate feed ingredients of poultry diets (Wilson and Payne, 1994). Aflatoxins cause a variety of losses in poultry including poor growth and low efficiency of feed conversion, increased high mortality (Smith and Hamilton, 1970; Leeson *et al.* 1995), liver pathology, immune suppression (Santin, 2000) and changes in relative organ weights (Edds and Bortell, 1983; Kubena *et al.* 1990; Kubena *et al.* 1993), kidney and spleen lesions (Glahn *et al.* 1991) and increased susceptibility to some environmental and infectious agents (Ibrahim *et al.* 2000; Oguz *et al.* 2003). Formycine® Gold premix is a broad spectrum disinfectant feed additive that includes ammonia, formaldehyde, propionic acid and sodium bentonite. Its efficiently prevents harmful feed contamination, and develops a continuous effect in the animal gut, avoiding the growth of bacteria, fungal and viral contamination.

Sodium bentonite is a part of Formycine® Gold that has a high capacity for swelling and absorbing mycotoxins, fecal excreta moisture and ammonia, but it may exhibit less adsorbent-selective additive, antagonistic or synergistic interaction with other compounds (Ramos *et al.* 1996). Vital nutrients (vitamins) or feed additives (pigments or anticoccidials) could be decreased or sequestered by sodium bentonite (Ramos *et al.* 1996; Gray *et al.* 1998; Hashemipour *et al.* 2010; Miazzo *et al.* 2000). Therefore, the aim of this study was to investigate the pigment adsorption ability of Formycine® Gold and its influence on egg quality and performance of laying hens.

MATERIALS AND METHODS

One hundred ninety two, 25-wk-old, Hy-Line (w-36) laying hens were housed in double-deck cage batteries with a stocking density of 440 cm^2/bird at 25 ˚C.

The egg production and body weight of birds was approximately uniform at the beginning of the study. The birds were randomly assigned to 1 of 6 dietary treatments, with each treatment replicated 4 times among the batteries with 8 birds for each replicate.

The study was conducted in a complete randomized design with a 2 × 3 factorial arrangement of treatments based on 2 levels of pigment (0 and 0.4 g/kg) as described below and 3 levels of Formycine® Gold (0, 1 and 2 g/kg) (Pol. Industrial Francolí, 30, 43080 Tarragona, Spain). Experimental diets were formulated to meet the recommendations of Hy-Line International (2007). The birds were fed with a basal diet for a week. The pigment consisted of 20 g 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 remained fixed for the whole experimental period.

All dietary treatments were isocaloric and isonitrogenous. Composition of the experimental diets is shown in Table 1. The study was conducted over 8 weeks so that the experiment was terminated at 34 weeks of age.

Feed and water were provided *ad libitum*. Birds were provided with lighting program of 16L: 8D and continuous ventilation.

Performance and egg quality characteristics

Hen-day egg production was measured daily throughout the experimental period and the eggs were weighed weekly. Body weight of hens were measured at the beginning (26 wk), middle (30 wk) and end (34 wk) of the experiment. Feed consumption for each replicate was measured weekly and then feed conversion efficiency was calculated as the gram of feed consumption per gram of egg weight. At the end of each week, egg production was calculated. Egg weight was measured from 8 randomly collected eggs per replicate in each week. The data of performance were reported biweekly at 28, 30, 32 and 34 weeks of age.

Five eggs from each replicate were randomly collected at the end of each week, kept at 4 ˚C and then analyzed for the following egg quality: egg weight, specific gravity, shell thickness, shell percentage and yolk color scale. Specific gravity was evaluated by the flotation method (Holder and Bradford, 1979) and was calculated as:

Specific gravity= A / (A-B)

Where:
A: egg weight in air.
B:egg weight in distilled water.

Eggs were broken separately on a flat surface to measure yolk color scale and subsequently shell weight and shell thickness. The shell (including the shell membranes) was washed in warm water and allowed to dry at room temperature overnight; eggshell thickness was determined using a micrometer. Yolk color scale was measured using the Roche Yolk Color Fan (Vuilleumier, 1969). The number of cracked and unclean eggs was recorded daily.

Statistical analyses

Data were analyzed using GLM procedure of SAS version 9.1 (SAS, 2004). The model included the fixed effects of Formycine® Gold, pigment and the interaction between Formycine® Gold and pigment. Significant differences among the means were declared at $P < 0.05$. Means showing significant differences in ANOVA were compared using Tukey studentized range (HSD) test. In order to obtain normal distribution, all data were normalized using JMP 7 (SAS Institute) software before analysis.

Table 1 Composition of the experimental diets

Pigment[1] (g/kg)	0			0. 4		
Formycine® Gold (g/kg)	0	1	2	0	1	2
Ingredients (%)						
Corn	56.43	56.43	56.43	56.43	56.43	56.43
Soybean meal	27.3	27.3	27.3	27.3	27.3	27.3
Wheat bran[1]	1	1	1	0	0	0
Bone meal	2.32	2.32	2.32	2.32	2.32	2.32
Limestone	7.68	7.68	7.68	7.68	7.68	7.68
Vit. and min. premix[3]	0.5	0.5	0.5	0.5	0.5	0.5
Salt	0.43	0.43	0.43	0.43	0.43	0.43
Tallow	3.96	3.96	3.96	3.96	3.96	3.96
DL-Met	0.18	0.18	0.18	0.18	0.18	0.18
Pigment[2]	0.0	0.0	0.0	1	1	1
Formycine® Gold	0.0	0.1	0.2	0.0	0.1	0.2
Sand	0.2	0.1	0.0	0.2	0.1	0.0
Calculated analysis						
ME (MJ/kg)	11.78	11.78	11.78	11.78	11.78	11.78
CP, %	15.25	15.25	15.25	15.25	15.25	15.25
CF, %	2.95	4.09	5.23	2.95	4.09	5.23
Ca, %	4.25	4.25	4.25	4.25	4.25	4.25
Ava. P, %	0.42	0.42	0.42	0.42	0.42	0.42
Linoleic acid, %	2.66	2.92	3.18	2.65	2.91	3.17
Na, %	0.17	0.17	0.17	0.17	0.17	0.17
Lys, %	0.78	0.78	0.78	0.78	0.78	0.78
Met, %	0.41	0.4	0.39	0.41	0.40	0.39
Met + Cys, %	0.67	0.67	0.67	0.67	0.67	0.67

[1, 2] Pigment provided by 20 g lucanthin and 20 g xanthin per 100 kg diet mixed as premix with wheat bran.
[3] Supplied per kg 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 and Fe: 75 mg.

RESULTS AND DISCUSSION

Effect of dietary pigment and Formycine® Gold on performance parameters including body weight, feed consumption, egg production and feed to egg ratio are shown in Table 2. The mentioned parameters were not significantly affected by laying hens containing dietary Formycine® Gold and pigment for the whole of the study. No interaction effect was also observed for Formycine® Gold and pigment.

In contrast to our results, Arab-Abousadi *et al.* (2007) showed that broilers fed diet containing Formycine® Gold had better daily feed intake, weight gain and feed conversion ratio when compared to control group. The use of sodium bentonite and pigment did not show considerable effect on laying hen feed intake, feed conversion efficiency and egg production in the study of Hashemipour *et al.* (2010). The effects of Formycine® Gold and pigment on egg weight, specific gravity, cracked and unclean eggs are presented in Table 3. Regardless of pigment levels, the egg weight at 31-32 weeks increased exponentially under Formycine® Gold treatment. Hens fed diet supplemented with 1 g kg^{-1} Formycine® Gold produced higher eggs at 31-32 weeks of age than those fed other levels (P<0.05).

Cracked and unclean eggs were not affected by Formycine® Gold or pigment levels. Egg shell weight and egg shell thickness were not affected by dietary treatments (Table 4) except at 29-30 weeks of age for dietary pigment supplementation. Hens received 0, 1 and 2 g kg^{-1} Formycine® Gold laid statistically similar egg shell parameters in all weeks (P>0.05). Egg specific gravity of hens fed diet containing 0.4 g kg^{-1} significantly improved at 29-30 weeks of age (P<0.05). Egg of pigment, weight, specific gravity, cracked and unclean eggs for Formycine® Gold or pigment were not statistically significant (P>0.05).

The results of this study for egg specific gravity, egg weight and cracked eggs are in agreement with those of Hashemipour *et al.* (2010) who reported that levels of pigment and sodium bentonite had no influence on egg weight and cracked eggs percentage, although the specific gravity increased by dietary pigment supplementation in the laying hens. Egg yolk color scale was increased by pigment supplementation in layer diets (Table 5). Hens fed diet containing 0.4 g kg^{-1} pigment produced eggs with higher yolk scales than those fed the control group (P<0.05). Formycine® Gold had no significant effect on egg yolk color scale (P>0.05). However, interaction between Formycine® Gold and pigment was significant (P<0.05).

Table 2 Effect of dietary Pigment and Formycine® Gold on performance of laying hens

	Pigment (g kg^{-1})			Formycine® Gold (g kg^{-1})				P-value Source of variation		
	0	0.4	±SEM	0	1	2	±SEM	Pig.	Form.	Pig. × Form.
Body weight (g)										
26 wk	1349	1350	16.87	1346	1354	1358	20.66	0.826	0.947	0.283
30 wk	1444	1455	14.40	1466	1438	1444	17.64	0.600	0.490	0.314
34 wk	1516	1524	14.28	1522	1514	1525	17.50	0.736	0.900	0.178
Feed intake (g)										
27-28 wk	88.6	87.7	1.42	88.2	88.0	88.1	1.74	0.705	0.995	0.36
29-30 wk	92.6	92.5	0.77	92.8	93.5	91.3	0.94	0.943	0.274	0.11
31-32 wk	94.5	94.6	0.69	95.1	94.6	94.0	0.84	0.997	0.234	0.091
33-34 wk	100.3	100.6	1.03	100.6	100.4	100.5	1.26	0.843	0.991	0.184
27-34 wk	94.0	93.8	0.77	94.1	94.1	93.5	0.94	0.913	0.842	0.102
Egg production (%)										
27-28 wk	87.9	84.0	1.90	87.0	86.3	84.5	2.33	0.176	0.726	0.100
29-30 wk	88.1	87.7	1.90	88.1	88.3	87.4	2.38	0.564	0.807	0.06
31-32 wk	85.6	85.4	1.76	86.8	85.3	84.5	2.16	0.946	0.757	0.235
33-34 wk	87.0	85.3	1.55	85.9	86.3	86.3	1.89	0.922	0.988	0.29
27-34 wk	86.5	85.6	1.58	85.9	86.5	85.6	1.94	0.707	0.946	0.146
Feed to egg ratio										
27-28 wk	1.77	1.83	0.027	1.80	1.78	1.82	0.033	0.216	0.393	0.051
29-30 wk	1.80	1.82	0.470	1.83	1.82	1.79	0.058	0.609	0.656	0.072
31-32 wk	1.87	1.84	0.028	1.87	1.81	1.89	0.034	0.490	0.318	0.215
33-34 wk	1.90	1.97	0.040	1.95	1.94	1.93	0.050	0.400	0.928	0.084
27-34 wk	1.84	1.87	0.030	1.86	1.84	1.86	0.037	0.873	0.703	0.090

Pig: pigment; Form: Formycine® Gold and Pig × Form: interaction of pigment and Formycine® Gold.
SEM: standard error of the means.

Table 3 Effect of dietary Pigment and Formycine® Gold on egg quality of laying hens

	Pigment (g kg^{-1})			Formycine® Gold (g kg^{-1})				P-value Source of variation		
	0	0.4	±SEM	0	1	2	±SEM	Pig[1].	Form.	Pig. × Form.
Egg weight (g)										
27-28 wk	56.9	57.2	0.46	57.1	57.3	57.8	0.562	0.609	0.793	0.759
29-30 wk	58.5	58.3	0.55	58.2	58.3	58.7	0.673	0.88	0.853	0.386
31-32 wk	59.2	60.3	0.45	59^{b}	61.3^{a}	59^{b}	0.559	0.083	0.006	0.405
33-34 wk	60.5	60.3	0.48	60.4	60.2	60.5	0.592	0.742	0.916	0.422
Cracked	0.378	0.46	0.101	0.255	0.411	0.59	0.124	0.575	0.19	0.679
Unclean egg	1.84	2.51	0.324	2.14	2.18	2.2	0.397	0.164	0.993	0.732
Specific gravity										
27-28	1.080	1.080	< 0.001	1.081	1.080	1.080	< 0.001	0.936	0.677	0.265
29-30	1.077^{a}	1.080^{b}	0.001	1.080	1.077	1.080	0.001	0.004	0.326	0.968
31-32	1.082	1.081	0.001	1.082	1.079	1.082	< 0.001	0.197	0.047	0.099
33-34	1.078	1.077	0.001	1.078	1.077	1.077	0.001	0.283	0.821	0.023

Pig: pigment; Form: Formycine® Gold and Pig × Form: interaction of pigment and Formycine® Gold.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Supplementing of laying hen diet with pigments could lead to changes in egg yolk color, however, Hashemipour *et al*. (2010) and Kermanshahi *et al*. (2011) found simultaneous use of aluminosilicates (sodium bentonite or zeolite) and pigment prevent yolk pigmentation. The reason of this effect might can prevent be high capacity of aluminosilicates that able to absorb dietary pigment and subsequently reduce yolk color scale. Formycine® Gold is composed of formaldehyde, propionic acid and sodium bentonite. Since, the added value of net sodium bentonite of Formycine® Gold in this study is lower than that of sodium bentonite content used in previous mentioned studies; it seems Formycine® Gold itself had no strong effect on egg yolk color scale. However, when specific gravity increased at 29-30 weeks of age by dietary pigment supplementation, at the same time shell thickness was also increased (Tables 3 and 4).

It is generally accepted that there is strong correlation between egg thickness and egg specific gravity (Hashemipour *et al*. 2010; Kermanshahi *et al*. 2011).

Table 4 Effect of dietary Pigment and Formycine® Gold on egg shell quality of laying hens

	Pigment (g kg^{-1})			Formycine® Gold (g kg^{-1})				P-value Source of variation		
	0	0.4	±SEM	0	1	2	±SEM	Pig[1].	Form.	Pig*Form
Shell weight (g)										
27-28 wk	5.24	5.22	0.062	5.3	5.2	5.18	0.076	0.799	0.525	0.176
29-30 wk	5.49	5.69	0.071	5.62	5.47	5.68	0.086	0.054	0.233	0.384
31-32 wk	5.26	5.24	0.058	5.24	5.26	0.26	0.071	0.807	0.952	0.834
33-34 wk	5.47	5.49	0.055	5.5	5.48	5.47	0.067	0.760	0.818	0.674
Shell weight (%)										
27-28 wk	9.22	9.13	0.092	9.28	9.09	9.14	0.113	0.492	0.462	0.249
29-30 wk	9.39	9.75	0.093	9.65	9.38	9.68	0.113	0.005	0.257	0.964
31-32 wk	8.89	8.7	0.085	8.88	8.59	8.92	0.099	0.091	0.043	0.285
33-34 wk	9.06	9.13	0.089	9.11	9.11	9.05	0.109	0.623	0.897	0.414
Shell thickness (mm)										
27-28 wk	0.411	0.407	0.004	0.414	0.41	0.404	0.006	0.506	0.484	0.115
29-30 wk	0.425[b]	0.435[a]	0.004	0.434	0.421	0.435	0.005	0.014	0.166	0.556
31-32 wk	0.404	0.398	0.003	0.403	0.397	0.402	0.004	0.314	0.663	0.436
33-34 wk	0.414	0.413	0.004	0.414	0.413	0.413	0.005	0.946	0.985	0.449

Pig: pigment; Form: Formycine® Gold and Pig × Form: interaction of pigment and Formycine® Gold.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effect of dietary Pigment and Formycine® Gold on yolk colour scale[1] of laying hens

		Age (Week) 28	30	32	34
Pigment (g kg^{-1})					
0		8.18[b]	7.91[b]	7.35[b]	7.41[b]
0.4		12.39[a]	11.91[a]	10.56[a]	11.35[a]
±SEM		0.139	0.075	0.113	0.108
Formycine® Gold (g kg^{-1})					
0		10.44	9.9	8.9	9.31
1		10.03	9.8	9.94	9.62
2		10.4	10	9.03	9.22
±SEM		0.17	0.092	0.139	0.132
Source of variation		P-value			
Pigment		< 0.001	< 0.001	< 0.001	< 0.001
Formycine® Gold		0.179	0.06	0.8	0.08
Pigment × Formycine® Gold		0.049	0.017	0.033	0.038
Interactions					
Formycine® Gold (g kg^{-1})	Pigment (g kg^{-1})				
0	0	8.5[b]	8[b]	7.19[b]	7.44[b]
1	0.4	12.18[a]	11.9[a]	10.5[a]	11.44[a]
2	0	8.18[b]	8.25[b]	7.5[b]	7[b]
0	0.4	12.4[a]	11.81[a]	10.6[a]	11.18[a]
1	0	7.9[b]	7.5[b]	7.4[b]	7.8[b]
2	0.4	11.44[a]	10.56[a]	12[a]	12.6[a]
±SEM		0.241	0.13	0.197	0.187

[1] Yolk colour was determined with a Roche yolk colour fan.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Based on anecdotal evidence, there are few studies about interaction effects between aluminosilicates and pigment during the laying period.

Supplementations of clay have generally been used in animal diets for many reasons. They are 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 structure of double hydrogen bonds between aflatoxin B_1 and aluminosilicate (Desheng *et al.* 2005).

Aluminosilicates such as sodium bentonite showed no effect on egg production (Roland, 1990) egg weight (Miles *et al*. 1986) and feed consumption (Miles *et al*. 1986). Dietary pigment supplementation led to increase specific gravity. *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 are used.

CONCLUSION

Under the conditions of this study, it is concluded that dietary Formycine® Gold had no adverse effect on egg yolk pigmentation, egg quality and laying hen performance. Simultaneous use of Formycine® Gold and pigment increased egg yolk color and might be effective on laying hen health status. More research is needed to clarify this.

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