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Influences of Different Sources of Natural Pigments on the Color and Quality of Eggs from Hens Fed a Wheat-Based Diet

Research Article

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ABSTRACT

This experiment was conducted to evaluate effects of different sources of natural pigments on yolk color and laying hen performance. A completely randomized design with six treatments and six replicates was used. The six experimental treatments were: a control diet containing yellow corn grain, a reference diet containing wheat and barley grain instead of corn; a red pepper diet composed of reference diet and 2% red pepper; a dried carrot diet containing the reference diet and 5% dried carrot meal; a dried tomato pulp diet composed of the reference diet and 5% dried tomato pulp and an alfalfa meal diet containing the reference diet and 5% alfalfa meal. Results indicated that egg weight, daily egg mass, feed conversion ratio and egg yolk color were not affected by treatments. In conclusion, if the goal of producers is an acceptable level of egg yolk color and performance is not under consideration, then the use of a 5% alfalfa meal in a wheat-barley based diet is suggested.

KEY WORDS carotenoid, egg quality, layer, pigment, yolk color.

INTRODUCTION

The degree of yolk color is an important criterion in table eggs for consumption as well as manufacturing of egg-containing food products (De-Groote, 1970). Therefore, there has been interest in evaluating the yolk-pigmenting properties of different feed sources. The color of egg yolks is produced by oxycarotenoids, as xanthophyll pigments, derived from the feed ingredients (Zahroojian *et al*. 2011). Sources of xanthophylls can be natural or synthetic. The supplementation of the laying hen's diet with synthetic pigments is more expensive than natural coloring sources, since the former must be imported (Hoppe, 1998). The principal natural feed sources of xanthophylls used during the past several decades include yellow corn and alfalfa meal. Corn is one of the essential feedstuffs of the hen's diet, and it contains lutein pigment, which affects the egg yolk color. Although corn is used at high levels in the diet of laying hens, it does not enhance the red pigmentation of the yolk properly (Gurbuz *et al*. 2003). In countries like Iran, where the production of yellow corn is limited, wheat and barley are the only grains usually considered by poultry producers in diet formulations. Wheat-barley based diets usually contain insufficient color pigments to enhance egg yolk pigmentation (Gurbuz *et al*. 2003). Therefore, when wheat and barley are used as the main sources of energy, additional pigmenting additives should be added. Recently, poultry rations have been in a continuing state of change with many new ingredients and special oxycarotenoid concentrates being developed. These products include red pepper, dried tomato pulp, and dried carrot meal. There is limited information available regarding the effectiveness of different natural pigmenting sources (red pepper, dried tomato pulp, alfalfa meal and dried carrot meal) in wheat-barley based diets of layers. Thus, the objective of the present study was to evaluate the effects of different natural pigment sources in diets on yolk color and laying hen performance.

MATERIALS AND METHODS

This study used a completely randomized design with 540 Hy-Line (w-36) hens at 40 weeks of age. They were selected based on egg production and body weight from 7000 birds, and were allocated to 6 treatments with 6 replicates, each of 15 hens.

The experiment continued for a period of 12 weeks (40 to 52 weeks of age). In the pre-experimental period (36 to 40 wk), egg production, egg weight, and initial body weight were recorded for hens fed on a typical corn-soybean meal diet. The hens were selected based on the pre-experimental egg production and body weight so that the average performance of the hens in each treatment was similar at the start of the experiment. Each cage was provided with an individual feeder and two automatic drinkers. The cages were located in a temperature-controlled room, and the photoperiod during the experiment was fixed at 16 hrs per day.

Red pepper, alfalfa meal, dried tomato pulp and dried carrot meal used in the experiment were analyzed for dry matter (DM), metabolizable energy (ME), crude protein (CP), crude fat, crude fiber and ash. The analyzed composition of the natural pigment sources is presented in Table 1. The DM contents of experimental natural pigment sources were determined by drying at 102 ˚C for 16 h in a forced air oven.

The ME contents of the sources were calculated by using the formula suggested by Wiseman (1987). The crude protein, crude fat, crude fiber and ash contents of the sources were measured according to methods 976.06, 920.39, 987.10 and 942.05 of AOAC (1990), respectively. Calcium and phosphorus contents were determined by the methods of Page *et al*. (1982).

Diets were formulated using an UFFDA feed formulation package according to the nutrient requirements of white laying hens (NRC, 1994). They were balanced to be iso-nitrogenous and iso-caloric, and to meet all other nutrient requirements of the birds. Red pepper, alfalfa meal, dried tomato pulp and dried carrot meal were analyzed in order to permit appropriate feed formulation. The composition and calculated analyses of experimental diets is outlined in Table 2.

All treatments (except the control diet) were supplemented with a commercial cellulase and xylanase enzyme mixture. Diets were made in mash form and provided daily according to expected intake, and water was available *ad libitum* throughout the experiment.

Egg production (%), egg weight (g), egg mass (g/day/bird) and feed conversion ratio were recorded daily (Jafari *et al*. 2006). Egg quality characteristics were measured for five eggs from each replicate that were collected randomly during the 4th, 7th and 12th week of the experimental period. Egg width and length were measured by using slide calipers (Ogawa Seiki Co. Ltd; Tokyo, Japan). The egg was then carefully broken on a glass plate to measure egg quality characteristics. Egg shape index was calculated according to Hasin *et al*. (2006) for each egg from its average length and width. The egg shape index= (average width of egg/average length of egg) × 100. The albumen index was calculated by dividing the average height of thick albumen by its width according to Hasin *et al*. (2006). For each egg, the shell thickness was determined at three different points in the middle part of the egg using a Mitutoyo caliper 7313 micrometer with 0.01 mm precision. Haugh units were calculated from the weight and height of albumen of egg using the formula suggested by Haugh (1937).

$$HU= 100 \log (H+7.57-1.7 W^{0.37}$$

Where:
HU: haugh unit.
H: height of thick albumen.
W: egg weight (g).

The yolk color score was determined by comparing with the roche yolk color (RYC) fan (F. Hoffmann-La-Roche Ltd., Basal, Switzerland). The RYC fan is a standardized tool which shows the range of yolk colors from 1 (very light yellow) to 15 (very dark yellow) as produced under natural feeding conditions (Vulleumier, 1969). The yolk index was calculated as the ratio of average yolk height to average yolk width following removal of the yolk from the albumen. Also, egg shell weight and specific gravity (Densitometer, Mettler-Toledo, Iso-14001, Switzerland) were measured according to Holder and Bradford (1979). Egg yolk cholesterol content was measured in accordance with standards of the Association of Official Analytical Chemists (AOAC, 1990). Data were analyzed as a completely randomized design. Statistical analysis of data was carried out using the SAS statistical program (SAS, 2002), and differences between means were tested with Duncan's multiple range tests at $P < 0.05$.

RESULTS AND DISCUSSION

The data on mean production performance (egg production, egg weight, egg mass and feed conversion ratio) of the laying hens from 40 to 52 weeks of age are presented in Table 3.

Table 1 Analyzed composition of natural pigment sources

Pigmented product	DM (%)	ME (kcal/kg)	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Ash (%)	Calcium (%)	Phosphorus (%)
Red pepper	88.21	1450	14.04	9.01	23.20	10.53	0.60	0.37
Dried tomato pulp	94.46	1760	20.40	8.80	33.67	4.50	0.52	0.47
Alfalfa meal	92.22	1673	19.84	3.42	21.08	10.60	1.47	0.23
Dried carrot meal	93.86	1721	4.03	0.52	11.63	5.30	0.66	0.50

DM: dry matter and ME: metabolizable energy.
DM and ME estimated to according to Wiseman (1987).

Table 2 Composition and calculated analyses of experimental diets

	Dietary treatments					
	Control diet	Reference diet	Red pepper	Dried tomato pulp	Alfalfa meal	Dried carrot meal
Ingredients, %						
Yellow corn	59.27	0.00	0.00	0.00	0.00	0.00
Soybean meal	22.38	18.06	17.50	15.50	15.46	15.71
Barley grain	0.00	28.53	27.19	26.10	26.21	25.93
Wheat grain	0.00	35.00	35.00	35.00	35.00	35.00
Soybean oil	5.67	7.10	7.10	7.10	7.10	7.10
Dicalcium phosphate	1.65	1.10	1.10	1.10	1.10	1.10
DL-methionine	0.19	0.17	0.17	0.18	0.16	0.17
Lysine	0.00	0.06	0.04	0.12	0.09	0.09
Oyster shell	9.92	9.10	9.00	9.00	8.98	9.01
Sodium chloride	0.42	0.38	0.35	0.35	0.35	0.34
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Enzyme mixture[3]	0.00	0.05	0.05	0.05	0.05	0.05
Red pepper	0.00	0.00	2.00	0.00	0.00	0.00
Dried tomato pulp	0.00	0.00	0.00	5.00	0.00	0.00
Alfalfa meal	0.00	0.00	0.00	0.00	5.00	0.00
Dried carrot meal	0.00	0.00	0.00	0.00	0.00	5.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated content:						
AME, kcal/kg	2895	2898	2896	2896	2897	2898
Crude protein, %	14.89	15.06	15.10	14.92	14.97	15.01
Calcium, %	3.84	3.85	3.83	3.84	3.84	3.85
Available P, %	0.31	0.32	0.32	0.31	0.30	0.30
Methionine, %	0.35	0.36	0.36	0.35	0.35	0.36
Met + Cys, %	0.58	0.60	0.59	0.58	0.60	0.59
Lysine, %	0.71	0.73	0.73	0.72	0.72	0.73

[1] Vitamin premix supplied per kg of diet: vitamin A: 9000 IU; vitamin B_{12}: 0.015 mg; vitamin D: 2000 IU; vitamin E: 18 IU; vitamin K_3: 2 mg; vitamin B_1: 1.78 mg; vitamin B_2: 6.6 mg; vitamin B_6: 3 mg; Niacin: 30 mg; Pantothenic acid: 10 mg; Biotin: 0.15 mg and Choline: 1500 mg.
[2] Mineral premix supplied per kg of diet: Cu: 10 mg; I: 0.99 mg; Fe: 50 mg; Mn: 100 mg; Se: 0.08 mg and Zn: 100 mg.
[3] Enzyme mixture contained a commercial cellulase and xylanase enzymes.

Table 3 Effects of dietary treatments on performance of laying hens from 40 to 52 weeks of age

Treatments	Egg production (%)	Egg weight (g)	Egg mass (g/day/bird)	Feed conversion ratio (g feed/g egg mass)
Control diet	75.84	64.77[b]	49.20[c]	1.962[ab]
Reference diet	79.28	66.98[ab]	53.04[a]	1.854[c]
Red pepper	77.65	68.00[a]	52.78[ab]	1.890[bc]
Dried carrot meal	77.20	66.04[ab]	50.99[abc]	1.936[abc]
Alfalfa meal	75.75	66.41[ab]	50.24[bc]	1.992[a]
Dried tomato pulp	79.89	65.64[ab]	52.44[ab]	1.896[bc]
SEM	0.669	0.382	0.441	0.015

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.

There were no significant differences in egg production (P>0.05) between the dietary treatments. A significant (P<0.05) increase in egg weight was observed from hens receiving the red pepper diet compared to the control diet.

Also, a significant (P<0.05) increase in egg mass was observed for hens on the reference diet, the 2% red pepper diet and the 5% dried tomato pulp diet compared to the control diet.

The highest and lowest egg mass were observed for the reference diet and control diet treatments, respectively. The best feed conversion ratio was observed on the reference diet. Overall, the reference diet, the 2% red pepper diet and the 5% dried tomato pulp diet gave better performance than the other treatments.

The results of egg quality characteristics (Table 4) showed that there were no significant differences between treatments (P>0.05) in egg thickness, egg shell weight, shape index, yolk index, specific gravity, haugh units and cholesterol content in yolks. Egg yolk colors of all treatments differed significantly (P<0.05) from each other. The highest and lowest scores of yolk color were obtained for eggs from hens receiving the diets containing red pepper (14.33) and the reference diet (1.58), respectively.

The treatments imposed in the present study produced no significant effects (P>0.05) on egg production of layer hens. This result can be attributed to the similar energy and crude protein contents of all dietary treatments. Pigment supplementation has not been associated with changes in production in previous studies (Angeles and Scheideler, 1998; Garcia *et al.* 2002; Soto-Salanova, 2003). This non-significant difference agreed well with the results of previous reports by Dotas *et al.* (1999), Santos-Bocanegra *et al.* (2004) and Skider *et al.* (1998) who worked with tomato pulp, red pepper and carrot meal, respectively. The results regarding egg production suggest that the use of 2% red pepper, 5% dried carrot pulp, 5% alfalfa meal or 5% dried tomato pulp in the diet of laying hens has no detrimental effect on egg production. The egg weight from the control diet was significantly lower (P<0.05) than that from the red pepper treatment.

In agreement with our results, Yannakopoulos *et al.* (1992) found that tomato meal resulted in greater egg weight and suggested that this could be a consequence of its high lysine content. On the other hand, Mitsuhiro *et al.* (1994) did not find any improvement of egg weight when dietary red pepper was used. Egg mass was significantly different (P<0.05) among treatments. A significant (P<0.05) increase in egg mass was observed with the reference diet, 2% red pepper diet, and the 5% dried tomato pulp diet compared to the control diet treatment. The highest and lowest egg mass were from the reference diet and control treatment, respectively. Feed conversion ratios for hens on the reference diet, red pepper diet and dried tomato pulp diet were better than other treatments (P<0.05). The lowest and highest feed conversion ratios were from the reference diet and the 5% alfalfa meal diet. The increase in feed conversion ratio in the alfalfa meal treatment may be attributed to factors such as higher levels of crude fiber, protease inhibitors and anti-nutritional factors (such as tannin) (Ali *et al.* 2003).

There were no significant differences (P>0.05) in egg shell thickness, egg shell weight, shape index, yolk index, specific gravity, haugh units and cholesterol content in yolk among treatments. In a study by Calislar and Uygur (2010), dry tomato pulp had a significant effect on the egg shape index and egg yolk index, whereas, dry tomato pulp had no significant effect on the albumen index and haugh units. In the current study, egg shell thickness was not affected by dietary treatments. This result could be due to the nearly similar calcium, phosphorus and vitamin D_3 contents in all diets. In agreement with our results, Jafari *et al.* (2006) reported no significant differences in egg shell thickness and haugh units of laying hens fed on diets containing up to 50, 100 and 150 kg/t dried tomato pulp compared to hens fed on a control diet. In contrast, Gregoriades *et al.* (1984) found that the inclusion of dried tomato pulp in layer diets improved the shell thickness.

Egg yolk colors of eggs from hens fed all experimental diets differed significantly (P<0.05) from each other. The highest and lowest score of yolk color was obtained with the diet containing red pepper (14.33) and with the reference diet (1.58), respectively. Red pepper contains capsanthin and capsorubin leading to red color in yolk (Fletcher and Halloran, 1981; Karunajeewa, 1980). Thus, when red pepper was used as a color additive to the reference diet at 2%, reddish or red egg yolk colors scored higher than 14, scores that are preferred in the cake industry (Fletcher and Halloran, 1981; Papa *et al.* 1985). The roche yolk color (RYC) value of the reference diet containing 28.53% barley and 35% wheat and no color additive was found quite low (1.58). All natural pigment sources added resulted in yolk color scores higher than that provided by the control group. Skider *et al.* (1998) found a 1.87 yolk color score from a 62% wheat-based diet after 3 weeks. Wheat-based diets usually fail to produce eggs with a standard yolk color on the RYC fan score (Saha *et al.* 1999). Although wheat is the main crop produced in Iran, it cannot be used as the main ingredient of laying hen diets due to its adverse effect on egg yolk color. However, the addition of 5% dried tomato pulp or 5% dried carrot meal into that diet resulted in RYC values of 3 and 3.92, respectively, which are low, but higher than for the control group. Skider *et al.* (1998) reported a yolk color value of 3.12 during the 3rd week from 4% dried carrot meal supplementation. This value was very close to the value obtained in our experiment. The addition of 5% alfalfa meal into the reference diet resulted in a RYC value of 6.08, which is close to the optimum RYC value preferred by Iranian consumers. As expected, the diet containing corn resulted in a darker egg yolk color (RYC=5.75) than the diet containing wheat and barley (RYC=1.58). Yellow corn is among the conventional sources of carotenoids and rich in zeaxanthin.

Table 4 Effects of dietary treatments on quality characteristics of eggs from layer hens during 40 to 52 weeks of age

Variables	Experimental diets						
	Control diet	Reference diet	Red pepper	Dried carrot meal	Alfalfa meal	Dried tomato pulp	SEM
Shell thickness (mm)	0.35	0.34	0.35	0.33	0.34	0.36	0.453
Egg shell weight (g)	5.13	5.14	5.17	5.16	5.15	5.15	0.100
Shape index (%)	73.8	74.4	75.7	75.5	76.3	74.6	0.004
Albumen index (%)	10.32	9.89	11.01	10.46	9.97	10.09	0.401
Yolk index (%)	0.40	0.40	0.40	0.39	0.41	0.39	0.002
Yolk color score	5.75[c]	1.58[f]	14.33[a]	3.92[d]	6.08[b]	3.00[e]	1.001
Specific gravity	1.081	1.080	1.081	1.078	1.081	1.081	0.001
Haugh unit	96.16	95.63	98.73	98.19	95.86	94.26	0.850
Cholesterol (mg/g)	6.11	6.75	6.20	6.01	6.32	6.16	0.069

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.

Saxena *et al.* (1982) reported that feeding hens diets containing 400 g/kg of yellow corn increased markedly the egg yolk score of eggs from 2 to 8 in 3-7 days. The results indicated that the addition of red pepper and alfalfa meal to the reference diet instead of carrot pulp and tomato pulp can be used to increase dietary xanthophylls levels and enhance yolk color. Jafari *et al.* (2006) reported that tomato by-products had no effect on the egg yolk color in laying hens. However, Dotas *et al.* (1999) and Yannakopoulos *et al.* (1992) found that yolk color was higher in eggs produced by hens fed on dried tomato pulp diets compared to a control diet. The effectiveness of a particular natural source as a pigment for poultry products depends on the level and availability of the xanthophylls in the source as well as the chemical nature of the particular xanthophylls (Delgado-Vargas, 1997). In addition to the birds' health, the breed and age of hens and other environmental conditions may also affect yolk coloring (El-Baushly and Raterink, 1989).

CONCLUSION

Egg yolk color could reach an acceptable level by supplementing 5% alfalfa meal to wheat-barley based diets, but the addition of 5% alfalfa meal to a wheat-barley based diet resulted in the highest feed conversion ratio. On the other hand, the RYC value of eggs from hens fed diets containing 5% dried carrot meal and 5% dried tomato pulp were found to be quite low (3.92 and 3, respectively). If the goal of producers is an acceptable egg yolk color and performance is not under consideration, then using of 5% alfalfa meal is suggested. If egg yolk color is not important for consumers and egg production is more important, then the supplementation of 5% alfalfa meal to wheat-barley based diets is not suggested. Our results also revealed that red pepper could be used as a natural color additive to enhance yolk pigmentation (in favor of red color) and red pepper at a rate of 2% of the feed can produce a RYC value of 14.3, a color which is preferred in the cake industry. Future studies should be carried out to find out the best levels of alfalfa meal, red pepper, dried carrot meal and dried tomato pulp in wheat-barley based diets of laying hens in order to produce table eggs with yolk colors that are desirable for consumers without altering laying performance.

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