

Influence of Different Sources of Natural Pigmenting on Egg Quality and Performance of Laying Hens

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ABSTRACT

This experiment was conducted to evaluate effects of different sources of natural pigments on egg quality characteristics and laying hens performance. A completely randomized design, with six treatments and six replicate was used. Experimental treatments were control diet containing yellow corn grains, reference diet containing wheat and barley grain instead of corn, red pepper composed of reference diet and 2% red pepper, dried carrot meal containing reference diet and 5% dried carrot meal, dried tomato pulp composed of reference diet and 5% dried tomato pulp and alfalfa meal containing reference diet and 5% alfalfa meal. Results indicated that egg production and egg quality characteristics (except egg yolk color) were not affected by treatments. In conclusion, in case producers are not interested in an optimum grade of egg yolk color and performance is not under consideration, using of 5% alfalfa meal in wheat-barley based diets is suggested.

Keywords: Egg quality; pigment; layer; carotenoid; yolk color.

INTRODUCTION

The degree of yolk color is an important criterion in table eggs for consumption as well as manufacturing of egg-containing market food products (De-Groote, 1970). Therefore, there has been interest in evaluation of yolk pigmenting properties resulted from different feed sources. The color of egg yolks is produced by oxycarotenoids, as xanthophylls pigments, derived from the feed ingredients (Zahroojian *et al.*, 2011). Sources of xanthophylls can be natural or synthetic. Since some of the synthetic color additives have cancerous effects in consumers (Oktay and Olgun, 1972), the natural color additives are preferred for improving egg yolk

pigmentation. Furthermore, the supplementation of the laying hens' diet with synthetic pigments is more expensive than natural coloring sources since the former should be imported (Hoppe, 1998). The principal natural feed ingredient sources of xanthophylls used during the past several decades include yellow corn and alfalfa meal. Corn is one of the essential feedstuff of the hens diet and it contains lutein pigment which affects the egg yolk color. Although corn is used at high levels (40%) in the diet of the 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 the diet formulation. Wheat-barley based diet usually contains insufficient color pigments to enhance egg yolk pigmentation (Gurbuz *et al.* 2003). Therefore, when wheat and barley are used as main source of energy, additional pigmenting additives should be added into the diet of laying hens. Recently, poultry rations

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are in a continuing state of change with many new ingredients and special oxycarotenoids concentrates being developed. These products include red pepper, dried tomato pulp, and dried carrot meal. There is limited information available for the impact of different natural pigmenting sources (red pepper, dried tomato pulp, alfalfa meal and dried carrot meal) in wheat-barley based diet of layers. Thus, the objective of the present study was to evaluate the effects of different natural pigmenting sources in diet on egg quality and laying hens performance.

MATERIALS AND METHODS

This study carried out in completely randomized design with 540 layer hens Hy-line W-36 strain at 40 weeks of age, selected based on egg production and

body weight from 7000 birds, these allocated in six treatments with six replicate that each replicate had 15 hens. The experiment was developed for a period of 12 weeks (40 to 52 week of age). In the pre-experimental period (36 to 40 week), 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 would be similar at the start of the experiment. Each cage was provided with an individual feeder and two automatic pipette drinkers. The cages were located in a temperature-controlled room, and the photoperiod during the experiment was fixed at 16 hours. The layout of the experiment is shown in Table 1.

Table 1. Layout of experiment

Treatments	Composition	No. of replicate in each treatment	No. of bird in each replication	Total No. of birds
Control diet	containing yellow corn grain as natural pigmenting source	6	15	90
Reference diet	Containing 35% wheat and 28.53% barley grain instead of corn	6	15	90
Red paper	reference diet supplemented with 2% red pepper	6	15	90
Alfalfa meal	reference diet supplemented with 5% alfalfa meal	6	15	90
Dried carrot meal	reference diet supplemented with 5% dried carrot meal	6	15	90
Dried tomato meal	reference diet supplemented with 5% dried tomato pulp	6	15	90
Grand total		36	90	540

Diets were formulated using UFFDA feed formulation package according to the nutrient requirements of white laying hens (NRC, 1994) and balanced to be iso-nitrogenous and iso-caloric, and meet all other nutrient

requirements of the birds. Different sources of natural pigment were analyzed in order to better interpretation of results. The composition and calculated analyses of experimental diets is outlined in Table 2.

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 analyses						
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: 9000 IU vitamin A, 1.78 mg vitamin B₁, 6.6 mg vitamin B₂, 30 mg niacin, 10 mg pantothenic acid, 3 mg vitamin B₆, 0.15 mg biotin, 1500 mg choline, 0.015 mg vitamin B₁₂, 2000 IU vitamin D, 18 IU vitamin E, 2 mg vitamin K₃.

² Mineral premix supplied per kg of diet: 10 mg Cu, 0.99 mg I, 50 mg Fe, 100 mg Mn, 0.08 mg Se, 100 mg Zn.

³ Enzyme mixtures contain a commercial cellulase and xylanase enzymes.

All treatments (except 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. Studied productive traits were 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 the egg laid by birds of different experimental groups. Considering five eggs from each replication were collected randomly during the 4th, 7th and 12th week of the experimental period. Egg width and length were measured by using egg weighing scale and slide calipers according to protocol reported by Ogawa Seiki Company; 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 as 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 unit was 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, according to report by Holfmann-La Roche, 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). 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) was 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).

Red pepper, alfalfa meal, dried tomato pulp and dried carrot meal used in the experiment was analyzed for dry matter (DM), metabolizable energy (ME), crude protein (CP), crude fat, crude fiber and ash. Analyzed composition of natural pigmenting sources is presented in Table 3. The DM contents of experimental natural pigmenting sources were determined by drying at 102°C for 16 h in a forced air oven. The ME contents of experimental natural pigmenting sources were determined indirectly using the formula suggested by Wiseman (1987). The crude protein, crude fat, crude fiber and ash contents of experimental natural pigmenting sources were measured according to methods 976.06, 920.39, 987.10 and 942.05 of Association of Official Analytical Chemists (AOAC, 1990), respectively. Calcium and phosphorus contents of natural pigment sources were determined by the methods of Page *et al.* 1982.

Data were analyzed as a complete randomized design. Statistical analysis of data was carried out using SAS statistical package program (SAS, 2002) and means were tested with Duncan's multiple range tests at $p < 0.05$.

Table 3. Analyzed composition of natural pigmenting sources

treatment	DM ¹ (%)	ME ² (kcal/kg)	CP (%)	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

¹DM (%): Dry matter, ²ME (kcal/kg): Metabolizable energy, estimated in according with Wiseman (1987).

RESULTS

The data on mean production performance (egg production, egg weight, egg mass and feed conversion ratio) of laying hens during 40 to 52 weeks of age are presented in Table 4. As presented in Table 4, there were no significant differences ($P > 0.05$) between the dietary treatments on egg production. A significant ($P < 0.05$) increase in egg weight was observed with red pepper compared to the control diet treatment. The highest egg weight was obtained with the diet contained red pepper. Also, a significant ($P < 0.05$) increase

in egg mass was observed with reference diet, 2% red pepper and 5% dried tomato pulp compared to the control diet treatment. The highest and lowest egg mass were observed on reference diet and control treatment, respectively. Additionally, feed conversion ratio of reference diet, red pepper and dried tomato pulp were better than other treatments ($P < 0.05$). The best feed conversion ratio was observed on reference diet. Overall, performance reference diet, 2% red pepper and 5% dried tomato pulp were better than other treatments.

Table 4. Effects of dietary treatments on performance of layer during 40 to 52 weeks of age

treatments	Egg production (%)	Egg weight (g)	Egg mass (g/day/bird)	Feed conversion ratio
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

^{a-c} within a column of similar superscript means no differ significantly.

The results of egg quality characteristics are shown in Table 5. As seen in Table 5, there were no significant differences ($P > 0.05$) in egg thickness, egg shell weight, shape index, yolk index, specific gravity, Haugh unit and cholesterol content in yolk among treatments. Egg yolk

colors of all treatments differed significantly ($P < 0.05$) from each other. The highest and lowest score of yolk color was obtained with the diet contained red pepper (14.33) and with reference diet (1.58), respectively.

Table 5. Effects of dietary treatments on egg quality characteristics of layer during 40 to 52 weeks of age

variables	Experimental diets						SEM
	Control diet	Reference diet	Red pepper	Dried carrot meal	Alfalfa meal	Dried tomato pulp	
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

^{a-c} Means within a column no similar superscript differ significantly ($p \leq 0.05$).

DISCUSSION

The results reported in the present study had no significant effect ($P > 0.05$) of different pigmenting sources on egg production of layer chickens. These results can be attributed to same contents of energy and crude protein in dietary treatments. Similar observation reported that pigment supplementation had not been associated with changes in egg production (Angeles and Scheideler, 1998; Garcia *et al.*, 2002; Soto-Salanova, 2003). These non-significant differences agreed well with the result of the 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 result of egg production suggested that the use of 2% red pepper, 5% dried carrot pulp, 5% alfalfa meal and 5% dried tomato pulp in the diet of laying hens had no significant effect on egg production. The egg weight in control diet was significantly lower ($P < 0.05$) in comparison with 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 significant ($P < 0.05$) between treatments. A significant ($P < 0.05$) increase in egg mass was observed with reference diet, 2% red pepper and 5% dried tomato pulp compared to the control diet treatment. The highest and lowest egg mass were observed on reference diet and control treatment, respectively. It appears that higher egg mass output in reference diet, red pepper and dried tomato pulp was probably reflection of higher egg production and egg weight of this groups. Feed conversion ratio of reference diet, red pepper and dried tomato pulp were better than other treatments ($P < 0.05$). The lowest and highest feed conversion ratio was observed on reference diet and 5% alfalfa meal. The increase in feed conversion ratio in alfalfa meal treatment may be attributed to a number of 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 unit 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 unit. In current study, egg shell thickness was not affected by dietary treatments. This result could be due to the nearly similar calcium, phosphorus and vitamin D₃ contents in all treatments diets. In agreement with our results, Jafari *et al.* (2006) reported no significant differences in egg shell thickness and Haugh unit of laying hens fed on diets containing up to 50, 100 and 150 kg/ton 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 improved the shell thickness in layer diets.

Egg yolk colors of all experimental diets differed significantly ($P < 0.05$) from each other. The highest and lowest score of yolk color was obtained with the diet contained red pepper (14.33) and 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 color additive with reference diet at 2%, reddish or red egg yolk colors with Roche Yolk Color (RYC) score higher than 14 was obtained which are preferred in cake industry (Fletcher and Halloran, 1981; Papa *et al.* 1985). RYC value of reference diet containing 28.53% barley and 35% wheat and without color additive was found quite low (1.58). All natural pigmenting sources added were able to provide yolk color scores higher than that provided by the control group. Skider *et al.* (1998) found 1.87 yolk color score from 62% wheat based diet for 3 weeks. Wheat-based

diet usually fails to produce eggs with standard yolk color (color 1 to 2) on RYC fan score (Saha *et al.*, 1999). Although the wheat is main crop produced in Iran, it cannot be used as main diet of the laying hens due to its adverse effect on egg yolk color. However, addition of 5% dried tomato pulp and 5% dried carrot meal into that diet resulted in a low RYC value (3 and 3.92, respectively). Skider *et al.* (1998) reported a yolk color value of 3.12 during third week of 4% dried carrot meal diet supplementation. This value was very close of the value obtained in our experiment. The addition of 5% alfalfa meal into reference diet resulted in an optimum RYC value (6.08), which is so close to the optimum RYC value preferred by Iranian consumers. As expected, the diet containing corn resulted in 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. Saxena *et al.* (1982) reported that feeding hens diets containing 400 g/kg of yellow corn increased markedly the egg yolk score of produced eggs from 2 to 8 in period of 3-7 days.

The results indicated that the addition of red pepper and alfalfa meal to reference diet instead of carrot pulp and tomato pulp can be used to increase dietary xanthophylls levels and potentially enhance resulting 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. 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). Besides birds health condition, the breed and age of hens and some environmental factors may affect yolk

color (El Baushly and Raterink, 1989).

CONCLUSIONS

In conclusion, results of the present study showed that substitution of corn with wheat and barley had effect on egg yolk color of laying hens. Therefore, layer diets based on wheat-barley require pigment supplementation to achieve required egg yolk color. The results of the present study showed that using alfalfa meal, reference diet, red pepper, dried carrot meal and dried tomato pulp in wheat-barley based diets could be a suitable for improvement of egg yolk color. Egg yolk color could reach an optimum grade by supplementation of 5% alfalfa meal on wheat-barley based diets, however, the addition of 5% alfalfa meal to wheat-barley based diets resulted in highest feed conversion ratio. On other hand, RYC value of the diet containing 5% dried carrot meal and 5% dried tomato pulp were found quite low (3.92 and 3, respectively). Egg yolk color is an important factor for egg marketing in several countries. Thus, if the

goals of producers were not egg yolk quality and an optimum grade in egg yolk color and performance but egg yolk color, the using of 5% alfalfa meal is suggested. Additionally, our results revealed that red pepper could be used as natural color additive to enhance yolk pigmentation (in favor of red color) and red pepper at rates of 2% of feed can support consistently RYC value of 14.33, which is preferred in cake industry. Future studies are suggested to find out the proper levels of alfalfa meal, red pepper, dried carrot meal and dried tomato pulp in wheat-barley based diets of laying hens for optimum performance and yolk color that are desirable for consumers.

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تأثير مصادر مختلفة من الأصباغ الطبيعية على نوعية البيضة وأداء دجاج البيض

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ملخص

أجريت هذه التجربة لتقييم تأثير مصادر مختلفة من الأصباغ الطبيعية على خصائص نوعية البيضة وأداء دجاج البيض، حيث استخدم التصميم العشوائي الكامل بست معاملات وستة مكررات، واشتملت المعاملات على المعاملة الضابطة المحتوية على حبوب الذرة الصفراء، ومعاملة مرجعية محتوية على حبوب القمح والشعير كبديل للذرة، واحتوت المعاملة الثالثة على الغذاء في المعاملة المرجعية والفلفل الأحمر بواقع 2%، والمعاملة الرابعة على الغذاء في المعاملة المرجعية و5% جزر مجفف، والمعاملة الخامسة على الغذاء في المعاملة المرجعية و5% لب البندورة المجفف، والمعاملة السادسة على الغذاء في المعاملة المرجعية و5% عليقة البرسيم الحجازي، وقد أوضحت النتائج أن إنتاج البيض وخصائص نوعية البيضة (فيما عدا لون المح) لم تتأثر بالمعاملة، وكخلاصة فإنه إذا كان هدف المنتجين هو الحصول على الدرجة المثلى من لون المح وكان الأداء ليس مقصودا، فإنه ينصح باستخدام 5% عليقة البرسيم الحجازي مع عليقة حبوب القمح والشعير كعلف بديل.

الكلمات الدالة: نوعية البيضة، صبغة، دجاج البيض، كاروتيني، لون المح.

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