http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (7): 1333-1337, 2006
ISSN 1028-8880

Effects of Addition of Marigold Flower, Safflower Petals, Red Pepper on Egg-yolk Color and Egg Production in Laying Hens

E. Rowghani, A. Maddahian and M. Arab Abousadi
Department of Animal Science, College of Agriculture,
Shiraz University, Shiraz, Iran

Abstract: The egg-yolk pigmentation properties of Marigold Flower (MF), Safflower Petals (SP), Red Pepper Meal (RPM) and Commercial Pigment (CP) were evaluated when used at different levels with a practical basal laying hens diet. A total of 240 laying hens were randomly divided into 48 groups, 5 birds per group cage. One of 12 experimental diets was fed to four cages (four replicates per each treatment). The experimental period lasted four weeks and during this period the birds had free access to feed and water. Test diets were produced by adding various levels of additives to basal diet. The egg production was recorded daily and egg-yolk color was examined using the Roche Color Fan (RYCF). Twelve yolks per diet were evaluated every three days during the experimental period. The results showed that, egg and yolk weight, daily feed intake, feed conversion ratio and egg production were not affected significantly by the dietary treatments. The egg-yolk color changed significantly ($p<0.01$) due to added pigments. The yolk color was improved within 10-13 days after feeding diets. The highest color pigmentation was obtained with the diet contained 3% RPM (12.55) whereas, the lowest color pigmentation obtained with the basal diet (5.54). MF was significantly ($p<0.01$) more effective in producing higher RYCF values than SP at all levels. Increasing the level of RPM, resulted in an increase reddish color pigmentation of yolk. The addition of 0.5% RPM and 0.6% CP resulted in optimum color pigmentation (9.67 and 9.57, respectively). The results indicated that RPM can be used as a potential natural color pigment in the diet of laying hens instead of CP and additionally the use of the level of RPM depends on the yolk color desired by the specific market.

Key words: Red pepper, marigold flower, safflower petals and egg-yolk color

INTRODUCTION

The degree of yolk color is an important criterion for table eggs for consumption as well as for manufactures of egg-containing market food products (De Groote, 1964; Saunders, 1964). For this reason, there has been interest in evaluating the yolk pigmenting properties of different feed sources. The egg-yolk color is the result of the deposition and coloring capacity of xanthophylls in the yolk. In order to improve yolk color, both natural and artificial color additives are used in the hen's diet (Couch and Farr, 1971; Guenthner *et al*., 1973; Fletcher and Halloran, 1981, 1983; Henken, 1992; Vdedibie and Opera, 1998; Taweesak, 2000; Bocanegra *et al*., 2004). As natural color pigment sources, ground-clover, marigold and red chili are widely used in poultry diets to enhance yolk color. Corn, marigold and ground-clover contain lutein, which leads to yellow color, whilst ground red pepper contains capsanthin and capsorubin leading to red color in yolk (Karunajeewa, 1980; Fletcher and Halloran, 1981; NRC, 1993). Hens are not able to synthesize the color pigments, but they have the ability to transport about 20-60% of pigments into yolk from ingested feed (Bartov and Bornstein, 1980). Many factors, such as dietary ingredients and nature of added pigments can affect yolk pigmentation (Hilton *et al*., 1974; Belyavin, 1981). Besides, birds health condition, the genus and age of hens and other environmental conditions may affect yolk coloring (El Baushly and Raterink, 1989). Based on the demand of artificial-free food by consumers, feed used for human consumption should be as natural as possible. The present study was conducted to investigate the effect of addition of marigold flower, safflower petals and red pepper which are available in sufficient amount and are cheap in Iran, as alternative feed additives to improve yolk pigmentation when added to the diet of laying hens at different levels.

MATERIALS AND METHODS

The experiment was carried out using 25-week old Hyline White layers (n = 240). A basal diet (Table 1) was

Corresponding Author: E. Rowghani, Department of Animal Science, College of Agriculture, Shiraz University, Shiraz, Iran

Table 1: Basal diet composition (%) and calculated nutrient content

Ingredients	(%)
Wheat	43.42
Yellow corn	19.00
Soy bean meal	15.50
Sunflower meal	8.00
Fat	2.00
Calcium carbonate	8.10
Bone meal	2.25
Wheat bran	0.6
Methionine	0.14
Salt	0.2
Vitamin and mineral premix[1]	0.65
Multienzyme [2]	0.14
Nutrient content	
Metabolizable energy kcal kg^{-1}	2750.0
Crude Protein (CP) (%)	16.12
ME/CP	170.59
Linoleic acid (%)	0.54
Lysine (%)	0.73
Methionine+ cysteine (%)	0.65
Calcium (%)	3.57
Available phosphorus (%)	0.44
Sodium (%)	0.12
Xanthophylls mg kg^{-1}	3.8

[1]Vitamin and mineral premix provides per 2.50 kg of product: Vit. A, 7700000 IU; 15000 mg B_1; 4400 mg B_2; 55000 mg B_3; 3000 mg B_6; 8.8 mg B_{12}; 3300000 IU D_3; 6600 IU E; 550 mg K3; 110 mg B_9; 22000 mg B_5; 55 mg H_2; 275 mg colin chloride; 100 mg antioxidant; 66 mg Mn; 33000 mg Fe; 66000 mg Zn; 8800 mg Cu; 300 mg Se and 900 mg I. [2]Multienzyme Source (Gryndazine, Biochem, Co.Tehran, Iran, contained Xylanase and beta-Gluconase

Table 2: Calculated xanthophylls content of experimental diets (mg kg^{-1})

Diets	Corn xanthophylls	MF xanthophylls	RPM xanthophylls	Total xanthophylls
Basal diet (BD)	3.8	-	-	3.8
BD +0.4%MF	3.8	21.60	-	25.40
BD +0.8%MF	3.8	43.20	-	47.00
BD +1.2%MF	3.8	64.80	-	68.80
BD+0.5%RPM	3.8	-	2.00	5.80
BD+1%RPM	3.8	-	4.00	7.80
BD+2%RPM	3.8	-	8.00	11.80
BD+3%RPM	3.8	-	12.00	15.80

MF = Marigold Flower, RPM = Red Pepper Meal

formulated to contain 16.12% crude protein and 2750 kcal kg^{-1} Metabolizable Energy (ME). The basal dietary ingredients and additives were purchased from local market. The test diets were produced by adding various levels of Marigold Flower (MF), Safflower Petals (SP), Red Pepper Meal (RPM) and a Commercial Pigment (CP; at recommended level) to basal diet (contained neither artificial nor natural color additives). Home grown MF, SP and red pepper were grounded before adding to basal diet. Commercial pigment was obtained from Biochem, Co. Tehran, Iran and contained Chinese red pepper and pot marigold pigments in 3 to 1 ratio and was added to basal diet with wheat bran at 1.5 and 98.5%, respectively. The calculated xanthophylls content of the diets (except for SP) is shown in Table 2. Hens were kept in wire cages (five hens per cage). Pre-experiment (14 days) was conducted using basal diet. Twelve different experimental diets were given to 240 hens for 4 weeks. Each diet was given to 4 replicates (5 hens per cages). Hens had free access to feed and water during experimental period and 16 h day^{-1} light was provided. Yolks were classified with the Roche Yolk Color Fan (RYCF) (Vuilleumier, 1969). During 4 weeks of experiment, yolk color was evaluated nine times every 3 days interval in 12 eggs per diet. Egg production was recorded daily and egg weight was determined by collecting the eggs of each cage weekly. Daily feed consumption and conversion ratio were calculated. Data were analyzed as a complete randomized design. Statistical analysis of data was carried out using SAS statistical package program (SAS, 1997) and means were compared with Duncan's multiple range test at $p<0.01$.

RESULTS AND DISCUSSION

The pigmentation of egg-yolks has long been recognized as an important quality attribute for this food. Marigold flower and red pepper or their extracts or commercial pigments have been used as sources of pigments to supplement laying hen diets. No significant differences observed among diets for egg characteristics such as egg weight, yolk weight, daily feed intake, feed

Table 3: Production performance and Roche Yolk Color Fan (RYCF) values of eggs from hens fed various added levels of Marigold Flower (MF), Safflower Petals (SP), Red Pepper Meal (RPM) and Commercial Pigment (CP) to basal diet[1]

Diets	Egg weight (g)	Yolk weight (g)	Feed intake (g/hen/day)	Feed conversion (g feed/g egg)	Egg production (%)	RYCF
Basal diet (BD)	52.12	12.71	100.71	2.38	81.96	5.54[i**]
BD+0.4%MF	51.14	12.67	102.63	2.27	88.39	8.09[f]
BD+0.8%MF	50.07	12.37	104.60	2.45	85.49	8.92[e]
BD+1.2%MF	50.47	12.23	100.00	2.49	80.00	9.15[e]
BD+0.4%SP	49.95	12.10	100.36	2.24	89.64	5.51[i]
BD+0.8%SP	51.60	12.37	99.11	2.23	85.89	6.02[h]
BD+1.2%SP	49.75	12.44	102.50	2.43	84.82	6.52[g]
BD+0.5%RPM	50.41	12.35	100.54	2.42	82.32	9.67[d]
BD+1.0%RPM	49.80	12.21	99.64	2.47	81.42	10.93[c]
BD+2.0%RPM	50.06	12.42	101.43	2.42	83.75	12.01[b]
BD+3.0%RPM	50.42	12.06	102.10	2.31	87.77	12.55[a]
BD+0.6%CP	50.72	12.19	100.36	2.35	84.64	9.57[d]
SE	0.18	0.05	0.85	0.02	0.87	0.34

[1]Means in the same column with the same letter are not significantly different at ($p<0.01$), SE = Standard Error

conversion ratio and egg production, but the yolk color changed significantly ($p<0.01$) according to the pigments and their added levels to the basal diet (Table 3). In other words, additives did not have any influence on production performance (Mackay *et al*., 1963; Guenthner *et al*., 1973; Fletcher and Halloran, 1981, 1983; Garcia *et al*., 2002; Soto-Salanova, 2003; Bocanegra *et al*., 2004; Fredriksson *et al*., 2006). The non-significant differences for feed conversion between diets indicated that the differences in yolk color between diets was not depend on the differences in feed intake. In the present study, improvement in yolk color was observed between 10-13 days after feeding experimental diets. Similar trends had been reported by Marusich *et al*. (1960); Mackay *et al*. (1963), Gawecki *et al*. (1977) and Taweesak (2000). In other studies, improvements in egg-yolk colors had been observed only after one week of supplementation with synthetic pigments (Halaj *et al*., 1999; Garcia *et al*., 2002). Since an orange yolk color is preferred by consumers (Erkek and Talug, 1990; Kirkpinar and Erkek, 1996), adding 0.5 or 1% RPM to the basal diet (as a source of red pigment) is more desirable. SP added at 3 levels, did not produced RYCF values higher than 6.52 and at the rate of 0.4% support the same RYCF values as basal diet. SP was inferior to other coloring additives in producing desirable yolk color which is due to it's very low content of xanthophylls and gave RYCF value that varies from 5.51 to 6.52. However, for obtaining yolks classified as desirable in RYCF, it is necessary to use SP or SP extract with RPM, MF or CP in appropriate ratios. The addition of MF (as a source of yellow pigment) at 3 different levels (0.4, 0.8 and 1.2%) produced RYCF values higher ($p<0.01$) than RYCF values obtained with feeding SP at similar levels and basal diet and these results were in agreement with the previous study of Taweesak (2000). MF addition at 1.2% produced RYCF values very close to 10.0 (9.15) which showed it's potentiality for producing desirable egg-yolk color. As compared with RPM, the addition of the highest level of MF (1.2%) was less effective ($p<0.01$) in enhancing egg-yolk color than the lowest level (0.5%) of RPM, which is in agreement with previous findings (Mackay *et al*., 1963; Guenthner *et al*., 1973). The lowest egg-yolk color value (5.54) and the highest value (12.55) were observed for basal diet and 3% RPM, respectively. It appears that a RYCF value of 10.0 or close to it, could be produced by feeding 1.2% MF, 0.5 and 1% RPM or 0.6% CP. Hens feed diets supplemented with different levels of RPM had significantly ($p<0.01$) higher RYCF values than the other diets, with no significant difference between 0.6% CP and 0.5% RPM. As the level of RPM in the diet increased, the visual color changed from almost yellow to orange to red. However, addition of 0.5% RPM resulted in RYCF value (9.67), which was very close to the optimum RYCF value (10.0) preferred by consumers and was similar to RYCF value produced by CP (9.57). When RPM was used as color additive with basal diet at 2 and 3%, reddish or red egg-yolk colors with RYCF higher than 12 was obtained which are preferred in cake industry (Marusich and Bauernfeind, 1970; Fletcher and Halloran, 1981; Papa *et al*., 1985). These results were in agreement with the earlier findings of Marusich and Bauernfeind (1970); Fletcher and Halloran (1981) and Kirkpinar and Erkek (1996). Although yellow corn is used at high levels in the practical diets of laying hens, it does not enhance the red pigmentation of yolk properly. This was the case in the present study with basal diet with very low RYCF (5.54) which was found to be insufficient at the color for consumer, hence adding the natural or artificial color additives to the basal diet is necessary (Kirkpinar and Erkek, 1996), to obtain orange egg-yolk color preferred by consumers. Since some of the artificial color additives have cancerous effects in consumers (Oktay and Olgun, 1972), the natural color additives are preferred for improving egg-yolk pigmentation. Present study showed that RPM could be used as natural color additive at 0.5 or

1% to basal diet to enhance egg-yolk pigmentation (with RYCF values 9.67 and 10.93, respectively) in the favor of orange yolk color, because both very yellow (produced by SP and basal diet) or reddish yolk (produced by 2 and 3% RPM) color are not preferred by consumers. The supplementation of the laying hens diet with synthetic pigments is more expensive than home grown natural coloring sources since the former should be imported. These results indicated that, the addition of MF or RPM to basal diet instead of CP can be used to increase dietary xanthophylls levels and potentially enhance resulting yolk color. Relatively small quantities of RPM resulted in a much greater color response than did the MF (Fletcher and Halloran, 1983; Bocanegra *et al*., 2004). The most important findings was that SP which is an oil industry by-product in Iran and is available in large quantities and is useless, produced undesirable egg-yolk colors. What is needed now is comprehensive studies aimed at using this by- product with other coloring sources such as red pepper or marigold flower for obtaining the optimum yolk color which is preferred by consumers and will also result in an added value to the farm economy. In addition, RPM which is available in sufficient amount and cheap in Iran, could be used up to 3% as egg-yolk coloring additive in the diet of laying hens to produced a wide range of yolk colors desired by the specific market.

REFERENCES

Bartov, I. and S. Bornsteins, 1980. Studies on egg yolk pigmentation. Effect of ethoxiquin on xanthophyl within and among genetic sources. Poult. Sci., 59: 1460-1461.

Belyavin, C., 1981. The evaluation of egg yolk pigmentation. Poult. Process. Kemin, Belgium.

Bocanegra, E.S., X.O. Osorio and E.O.O. Rondon, 2004. Evaluation of *Tagets erectus* (marigold flower) and *Capsicum* sp. (red pepper paprika) as a pigment of egg yolks compared with synthetic pigments. Intl. J. Poult. Sci., 3: 685-689.

Couch, J.R. and F.M. Farr, 1971. The effect of adding canthaxanthin and beta-apo-8-carotenal to laying diets containing yellow corn and alfalfa on egg yolk pigmentation. Br. Poult. Sci., 12: 49-55.

De Groote, G., 1964. Comprative yolk pigmenting values of new carotenoid sources and synthetic carotenoids. J. Agric. Vet. Chem., 5: 9-20.

El Baushly, A.R. and R.V. Raterink, 1989. Various aspects of egg yolk pigmentation explored. Feed Stuffs., 30: 41-43.

Erkek, R. and A.M. Talug, 1990. Yumurta tavugu ve kasaplik pilic karma yemlerinde renk maddeleri kullanimi. Yem Sanayi Dergisi., Ankara, 66: 30-37.

Fletcher, D.L. and R.H. Halloran, 1981. An evaluation of a commercially available marigold concentrate and paprika oleoresin on egg yolk pigmentation. Poult. Sci., 60: 1846-1854.

Fletcher, D.L. and R.H. Halloran, 1983. Egg yolk pigmentation properties of a marigold extract and paprika oleoresin in practical type diet. Poult. Sci., 62: 1205-1210.

Fredriksson, S., K. Elwinger and J. Pickova, 2006. Fatty acid and carotenoid composition of egg yolk as an effect of microalgae addition to feed formula for laying hens. Food Chem. (In Press).

Garcia, E.A., A.A. Mendes, C.C. Pizzolante, H.C. Goncalves, R.P. Olivera and M.A. Silva, 2002. Effect of canthaxantina levels on performance and egg yolk quality of laying hens. Braz. J. Poult. Sci., 4: 1-4.

Gawecki, K., A. Potkanmski and H. Lipinska, 1977. Effect of carophyll yellow and carophyll red added to commercial feeds for laying hens on yolk colour and its stability during short-term refrigeration. Ruczniki Akademii Rolniczez W Poznaniu., 94: 85-93.

Guenthner, E., C. Wendel Carlson, O.E. Olsen, G.O. Kohler and A. Lyle Livingston, 1973. Pigmentation of egg yolk by xanthophylls from corn, marigold, alfalfa and synthetic sources. Poult. Sci., 52: 1787-1798.

Halaj, M., P. Halaj, F. Valasek, F. Moravick and M. Melen, 1999. The effect of synthetic pigment addition to feed on the color of hen egg yolk. Czech J. Anim. Sci., 44: 187-192.

Henken, H., 1992. Chemical and physical behavior of feed carotenoids and their effects on pigmentation. Poult. Sci., 71: 711-717.

Hilton, C.F., J.L. Fry and R.H. Harms, 1974. Influnce of xanthophyl free pullet grower diet on subsequent egg yolk pigmentation. Poult. Sci., 53: 223-225.

Karunajeewa, H., 1980. The deposition of synthetic oxycarotenoids in egg yolks. World Poult. Sci., 36: 219-222.

Kirkpinar, F. and R. Erkek, 1996. Beyaz misir ve bugday temeline dayali karma yemiere ilave edilen Bazi Dogal ve sentetik Renk maddeleriinin yumurta sari rengi ve verim uzerine. J. Vet. Anim. Sci., 23: 9-14.

Mackay, E., G.J. Mountney and E.C. Naber, 1963. Yolk color resulting from different levels of paprika extract in the ration. Poult. Sci., 42: 32-37.

Marusich, W.L., E.D. Ritter and J.C. Bauernfeind, 1960. Evaluation of carotenoid pigments for coloring egg yolks. Poult. Sci., 39: 1338-1345.

Marusich, W.L. and J.C. Bauernfeind, 1970. Oxycarotenoid in poultry pigmentation. Yolk Studies. Poult. Sci., 49: 1555-1561.

NRC., 1993. Nutrient Requirement of Poultry. 1st Edn., National Academy Press, Washington, DC., pp: 200.

Oktay, E. and H. Olgun, 1972. Klrmlzl biberin New-Hampshire tavuklarynda yumurta verimi, yumurta kalitesi ve kulucka verimine etkisi. 4. Bilim Kongresi. Lalahan, Ankara, pp: 43-57.

Papa, C.M., D.L. Fletcher and H.R. Halloran, 1985. Utilization and yolk colouring capacity of xanthophylls from synthetic and high xanthophylls concentrates. Poult. Sci., 64: 1464-1469.

Saunders, C., 1964. Feeding for egg yolk colour. Proc. Soc. Adv. Anim. Nutr. (S. Africa), 1: 81-98.

SAS., 1997. SAS User's Guide. SAS Institute Inc., Cary, NC., USA.

Soto-Salanova, M.F., 2003. Natural Pigments:Practical Experiences. In: Garnsworthy, P.C. and J. Wiseman (Eds.), Recent Advances in Animal Nutrition. Nottingham University Press. Nottingham, UK., pp: 67-75.

Taweesak, N., 2000. Use of marigold petal meal (*Tagets erecta*) as a source of xanthophyll in layer diets. J. Sci. Technol., 22: 169-176.

Vdedibie, A.B.I. and C.C. Opera, 1998. Responces of growing broilers and laying hens to the dietary inclusion of leaf meal from *Alchornia cordifolia*. Anim. Feed Sci. Technol., 71: 157-164.

Vuilleumier, J.P., 1969. The Roche colour fan-an instrument for measuring yolk colour. Poult. Sci., 35: 226-227.