IJAS Iranian Journal of Applied Animal Science

The Effect of Red Pepper (*Capsicum annuum*) and Marigold Flower (*Tageteserectus*) Powder on Egg Production, Egg Yolk Color and Some Blood Metabolites of Laying Hens

Research Article

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Received on: 27 Apr 2012
Revised on: 17 Jun 2012
Accepted on: 6 Jul 2012
Online Published on: Jun 2013

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Online version is available on: www.ijas.ir

ABSTRACT

An experiment was conducted to investigate the effects of using different levels of Red Pepper powder (RP) and Marigold flower powder (MF) on egg production, egg yolk color and some blood metabolites in laying hens. Ninety, 103-weeks old laying hens were divided into 5 treatments groups of 6 birds in each cage with 3 replicates in a completely randomized design. The birds were fed a corn-soybean meal based diet containing different concentrations of RP (1 and 3%) and MF (1 and 3%) along with a control / untreated group. During 9 weeks of experimental period egg production, egg quality and quantity parameters were recorded. The blood samples were taken at 14, 28 and 42 days of experiment. The white blood cell counts and some blood metabolites were measured. The egg yolk colors were measured using Fan Roche methods. There were no significant differences among treatments for egg quality traits but the yolk color changed in treated group compared with controls ($P<0.01$). The highest yolk color was obtained in 3% RP (with 13.33 Roche Fan score). Effect of RP and MF powder on total blood leukocyte counts was not different significantly in laying hen, but there was a significant effect of RP and MF powder on eosinophil count ($P<0.05$). There was a significant effect of RP and MF powder on triglyceride and total cholesterol ($P<0.05$). The RP and MF did not significantly affected the LDL, HDL, glucose, uric acid, Ca, P, ALT and AST enzymes in treated laying hens ($P>0.05$).

KEY WORDS blood metabolites, egg production, egg yolk color, laying hens.

INTRODUCTION

Researchers initiating a program to screen plants for extra-nutritional benefits are confronted with a number of questions, for example, how to choose the plants to screen, how to collect and store the plants, which parts of the plants to test, whether to test the whole plant or an extract from the plant and, of course, what technique to use to screen the particular characteristics (Vercoe *et al*. 2011). Plants of the Red Pepper (*Capsicum annuum*) family have been widely used in dietary and in traditional oriental medications without any serious adverse reactions. The powder of Marigold flower (*Tageteserectus*), another herbal plant has been extensively used for imparting color and flavor to foods and also for the treatment of a variety of inflammatory conditions and other diseases. Red Pepper and Marigold flower have been found to increase the concentration of some carotenoids such as eaxanthin. The losses in pigmentation capability due to manufacturing or storage vary between 10 and 70%. This explains in part the high variability in results when use of these products is quoted as natural products (Delgado-Vargas *et al.* 1998; Soto-Salanova, 2003). Marigold flower meal is one of the most concentrated sources of lutein (12 g/kg of total xanthophylls, 80 to 90% lutein) and

paprika meal has high levels of capsanthin (4 to 8 g/kg of total xanthophylls, 50 to 70% capsanthin). In marigold and paprika, xanthophylls are usually esterified to fatty acids, which are, attached to fatty acids as the ester. In birds, esterified carotenoids have to be split (saponified) before they are absorbed. So, a previous saponification of xanthophylls may improve its availability to the animals (Quackenbush *et al.* 1972; Hamilton *et al.* 1990). Red pepper, paprika and Marigold flower extracts have been used as natural pigments to supplement laying hen diets. There are many commercial products extracted from these plants and their quality as pigments depends on level of trans isomers, level of saponification, stability against oxidation, and chemical isomerization. This compositional determination is important for the application of marigold extract in nutritional supplements and increases its value as poultry feed colorant because it contains more biologically useful lutein compounds than previously believed (Gurbuz *et al*. 2003). The reduction of total cholesterol, AST, ALT and blood uric acid implied the non-toxic effect of treatments on hepatic and renal tissues (Bocanegra, 2004; Sadeghi, 2008). The objectives of this study were to investigate the efficacy of different levels of RP and MP powders on production performance, egg yolk color, immunity and some blood metabolites, ALT and AST enzymes in laying hens.

MATERIALS AND METHODS

Ninety five, 103 weeks old single comb white leghorns Hyline (W-36) were divided into five treatments groups. Each treatment had three replicates of 6 hens kept in each cage, provided with 16 hours of daily light. All birds were fed isocaloric mash diets for 9 weeks. The diets were formulated to meet or exceed the nutrient requirements of laying hens for age older than 103 weeks and egg production percentage was less than 70% (Hy Line International, 2007). Treatments were RPM1 (1% Red pepper powder), RPM3 (3% Red pepper powder) and MF1 (1% of Marigold flower powder), MF3 (3% Marigold flower powder) and no supplementation (control). The composition of the experimental diets is shown in Table 1. Feed consumption was recorded weekly and feed efficiency was calculated during the 9 weeks experimental period. Daily egg production and egg yolk color egg were recorded. Egg quality traits including shell quality, egg shape index, and egg yolk color were measured during 6 weeks. The egg yolk colors were measured using Fan Roche methods.

Blood samples were collected from each bird weekly, beginning at the 7 d of experiment prior to feeding, from the wing vein using sterilized syringes and needles. Blood samples were centrifuged and serum was separated 2 to 3 hour after blood collection. The white blood cell counts and some blood metabolites were measured. Serum samples were maintained at -20 ˚C for up to 3 d until biochemical analysis. Blood serums were analyzed for serum total cholesterol, glucose, uric acid, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), Ca and P using an auto analyzer [Technicon RA 1000, Bayer Diagnostics] and using the commercial diagnostic kits [Pars Azmun Co. INC]. Data were analyzed in a one-way ANOVA using the General Linear Models procedure of SAS based on completely randomized design (CRD). From each pen 3 birds were used as the experimental unit for performance and serum chemistry data. Differences among diets, when significant, were also ordered using Duncan test.

RESULTS AND DISCUSSION

Serum metabolites of hens fed diets containing Red Pepper and Marigold flower powder are shown in Table 2. Glucose and uric acid were not affected by dietary supplementation of different levels of Red Pepper powder and Marigold flower. Some studies reported that the supplementation of different levels of RP powder and Marigold flower decreased glucose in rats. The addition of 1% MF decreased total cholesterol in comparison with controls ($P<0.05$) but there was no significant effect of Red Pepper powder and Marigold flower treatments on other parameters (Table2). There was a significant effect of RP and MF powders on triglyceride level ($P<0.01$). The lowest level was obtained for 1% MF group. There is profound evidence that dietary consumption of Red Pepper powder and Marigold flower improved the total cholesterol and triglyceride as compared with untreated control group in rats (Kawado *et al.* 1986). They have also stimulating effect on peptic juices, such as gastric juice, bile, pancreatic and intestinal juices in rats was discovered. Malekizadeh *et al*. (2011) using some herbal medicine suggested that curcumin and related antioxidants may complement the well established anti atherogenic action of tocopherol. The curcumin antioxidants might be especially useful as anti atherogenic agents in those processes linked to a marked increase in blood lipid peroxidation such as myocardial infraction. Moreover, dietary supplementation of Red Pepper powder and Marigold flower were found to improve antioxidant status of rats and broiler chickens (13, 20). The HDL and LDL were not affected by dietary supplementation of different levels of RP and MF ($P>0.05$). Also different levels of RP powder and MF did not affect the concentration of blood calcium and phosphorous (Table 2). Effect of Red Pepper powder and Marigold flower on total blood leukocyte counts was not different significantly on lymphocyte, heterophil, monocyte and basophilic. But there was a significant effect of RP and MF powder on eosinophil in laying hens (Table 3).

Table 1 Composition of experimental diets (%)

	C	RPM 1	RPM 3	MF 1	MF 3
Ingredient					
Corn	65.62	65.25	64.50	65.25	64.49
Soybean meal	13.35	13.39	13.33	13.38	13.47
Wheat bran	2.03	1.28	-	1.31	-
Fish meal	5.00	5.00	5.00	5.00	5.00
Soybean oil	1.70	1.70	1.70	1.70	1.70
RP	-	1.00	3.00	-	-
MF	-	-	-	1.00	3.00
Dicalcium phosphate	0.59	0.66	0.69	0.60	0.63
Oyster shell	10.87	10.87	10.86	10.87	10.86
Vitamin-mineral mixture[1]	0.25	0.25	0.25	0.25	0.25
Common salt	0.38	0.34	0.34	0.38	0.34
DL-methionine	-	0.01	0.01	-	-
L-lysine	-	-	0.06	-	0.01
Nutrient contents					
ME (kcal/kg)	2796	2763	2771	2804	2815
Crude protein (%)	14.64	14.92	14.71	14.71	14.98
Calcium (%)	4.48	4.57	4.61	4.52	4.53
Available Phosphorous (%)	0.33	0.30	0.33	0.31	0.34
Methionine (%)	0.29	0.30	0.31	0.30	0.32
Lysine (%)	0.78	0.73	0.75	0.72	0.76

[1] Provided per kilogram of diet: vitamin A (retinyl palmitate): 1200 IU; Cholecalciferol: 2500 IU; vitamin E (DL-*α*-tocopheryl acetate): 20 IU; vitamin K3: 4 mg; Thiamin: 1.5 mg; Riboflavin: 50 mg; Pantothenic acid: 10 mg; Niacin: 30 mg; Pyridoxine: 4 mg; Choline chloride: 250 mg; Folic acid: 0.5 mg; Biotin: 220 µg; vitamin B12: 12 µg; BHT: 250 mg; Manganese: 48 mg; Zinc: 40 mg; Iron: 24 mg; Copper: 16 mg; Iodine: 0.6 mg; Selenium: 0.12 mg and DL-methionine: 50 mg.

Table 2 Serum chemistry of hens fed diets containing Red Pepper and Marigold flower powder

Parameters	Treatments					SEM
	RP 1%	RP 3%	MF 1%	MF 3%	Control	
Total cholesterol (mg/dL)	206.33 [a b]	184.67 [a b]	146.00[b]	184.00 [a b]	234.00[a]	10.54
HDL-cholesterol (mg/dL)	36.33	39.00	39.00	33.33	33.33	1.47
LDL-cholesterol (mg/dL)	48.67	62.67	50.33	53.67	54.67	1.84
Triglyceride (mg/dL)	1600 [b]	1700 [b]	1027[c]	1750[b]	2749[a]	162.7
Glucose (mg/dL)	193.00	201.00	215.667	204.33	214.67	3.127
Calcium (mg/dL)	31.51	31.53	28.70	30.41	31.89	0.41
Phosphorous (mg/dL)	4.77	5.37	5.67	5.27	5.17	0.13
Uric acid (mg/dL)	4.90	6.20	5.37	5.50	6.13	0.189
AST (U/L)	234.00	214.00	239.67	239.67	214.00	5.05
ALT (U/L)	8.20	11.00	8.50	5.33	10.33	0.83

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
Means represent 6 pens per treatment, 3 birds per pen average of 6 sampling every week in experimental period.

In the present study, the RP and MF powder had no significant effects on haugh unit score (HU), egg weight, egg shell weight, yolk weight and shell thickness (Table 4). These findings are similar to previous trials reporting no change in egg quality following *paprika* administration (Zhuye *et al*. 2008). Many researchers observed that the pigment supplementation had not been associated with the changes in production performance (Angeles and Scheideler, 1988; Gonzalez *et al*. 1999; Garcia *et al*. 2002; Soto-Salanova, 2003). Feed efficiency and egg weight were not affected by dietary supplementation of different levels of RP and MF. There were no significant differences among treatments for other egg quality traits (Table 4).

Egg yolk color is known to be influenced most by the diet of the hens. The yolk color changed in treated groups as compared with control group significantly (P<0.01). The brightest yolk color was obtained in 3% RP (with 13.33 Roche Fan score) and the lowest yolk color was in control group (with 7 Roche Fan score) (Figure 1). These findings agree with previous trials of Zhuye *et al*. (2008), who reported that following *Paprika* supplementation; yolk color reached a score of 11.47. The natural colors of the egg yolk are a result of xanthophylls. *Paprika* (*Capsicum annuum*) is a source of red xanthophylls; containing 4-8 g/kg of total xanthophylls which intensify egg yolk color (Bocanegra *et al*. 2004).

Table 3 Total blood leukocyte counts of hens fed Red Pepper or Marigold flower powder

Parameters (%)	Treatments					SEM
	RP 1%	RP 3%	MF 1%	MF 3%	Control	
Lymphocyte	59.78	62.00	65.78	63.89	70.67	1.39
Heterophil	36.55	34.22	31.11	34.22	25.33	1.43
Monocyte	1.56	1.56	1.11	1.00	1.56	0.105
Basophilic	1.22	1.24	1.22	0.65	0.78	0.165
Eosinophil	0.33^{b}	0.78^{b}	0.67^{b}	0.33^{b}	1.55^{a}	0.146
H:L	0.67	0.60	0.49	0.56	0.40	0.037

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
Means represent 6 pens per treatment, 3 birds per pen average of 6 sampling every week in experimental period.

Table 4 Mean egg quality traits of lying hens during the experiment

Parameters (%)	C	MF 3	MF 1	RP 3	RP 1	SE
Egg index shape (%)	75.12	74.06	74.10	74.16	74.44	0.492
Yolk index shape (%)	42.68	42.35	41.81	42.55	42.30	0.299
Haugh unit (%)	72.80	72.34	73.04	71.34	72.70	0.691
Shell weight (g)	6.68	6.91	6.90	6.44	6.20	0.156
Shell diameter (Mm×10^{-2})	34.50	34.40	35.00	34.17	34.27	0.759
Egg weight(g)	65.17	64.99	64.86	65.71	66.16	0.42
Egg mass(g)	33.25	31.10	29.90	35.42	34.12	1.27
Feed efficiency	2.62	2.95	2.85	2.63	2.68	0.08

Egg mass= (egg production×egg weight) / 100.
Feed efficiency ratio= feed intake / egg mass (g/g).

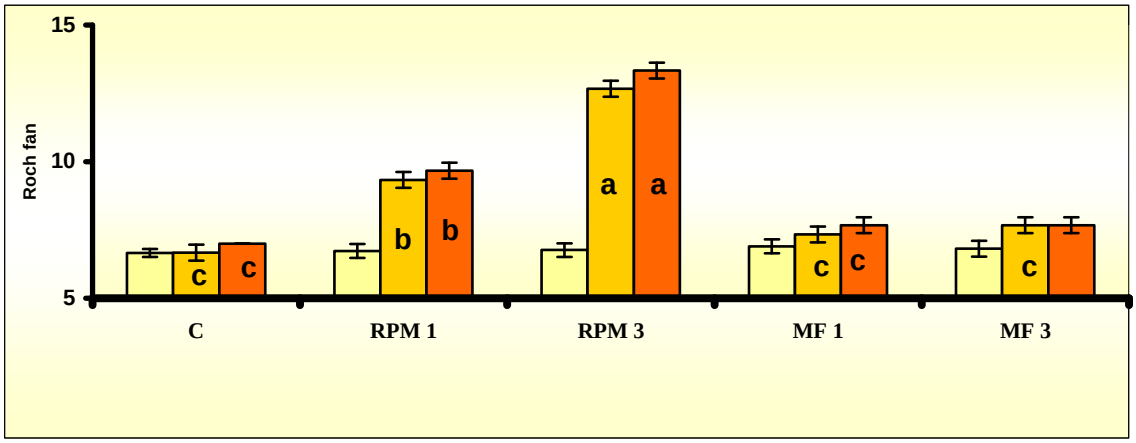

Figure 1 Mean egg yolk colors of treatments on days 1, 15 and 30 of experiment
The means within the same treatments with at least one common letter, do not have significant difference (P>0.05)

Marigold is also a good source of yellow xanthophylls, which also enhance egg yolk color. Egg yolk color in current study changed significantly (P<0.001) according to the sources and concentration of pigment sources (Figure 1). Bornstein and Bartov (1966) reported that there was linear relationship between dietary carotenoids and visual scoring of egg yolk. Egg yolk color increased about one unit of Roche color fan score for every 10 mg/kg of total carotenoids from Sano increased. However, the highest level of carotenoids from Sano (30 mg/kg) gave lower yolk color than corn-soy basal group that contained only 12 mg/kg of total carotenoids from corn. Gonzalez *et al*. (1999) and Karadas *et al*. (2006) reported that in egg yolk lutein has highest deposition efficiency and followed by zeaxanthin and β-cryptoxanthin, respectively. Some studies indicated that the Sano (*Sesbania javanica* Miq) had beneficial effect on increasing yolk color score. Adding 10 mg/kg of total carotenoids from Sano in carotenoids-free diet or in combination with cantaxanthin can elevate approximately one unit of Roche's color fan scale.

CONCLUSION

Supplemental of RP and MF as herbal additives might have some positive effects on production performance and egg yolk color of the laying hens. The addition of RP improved the egg yolk color especially at the level of 3% RP. As a results of this study, supplementation of RP and MF as

herbal additives might have some positive effects on production performance, immunity and some blood parameters of the laying hens. Further studies would be helpful by adding different levels of RP and MF to clarify the nutritional, therapeutic and physiological effects of these herbal additives on production and immunity of broiler and laying hens.

ACKNOWLEDGEMENT

This work was supported by Razi University.

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