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Evaluation of Marigold Flower and Orange Skin as Sources of Xanthophyll Pigment for the Improvement of Egg Yolk Color

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The efficacy of ground marigold flower and orange skin was evaluated for egg yolk coloration. Two feeding trials were conducted. The first trial was carried out with 63 laying pullets (Shaver 579) that received 0, 40 g marigold flower and 40 g orange skin per kg of mixed feed in three dietary treatments. Each of the treatments comprised of 21 birds distributed to three replicate groups each of seven. The second trial was conducted with 84 laying pullets of the same genetic source that received 0, 40 g marigold flower, 40 g orange skin and 30 mg synthetic pigment in four dietary treatments each having number of birds and replicates similar to trial 1. All birds received identical care and management including diets of more or less similar in composition. Yolk color scores were measured using Roche yolk color fan and other egg quality characteristics and production performance were recorded simultaneously. The noteworthy findings are i) egg yolk color significantly improved and reached to a level of consumers' standard by feeding 40 g/kg marigold flower and ii) egg yolk color was slightly improved by feeding 40 g/kg orange skin as compared to wheat-based control diet. It was concluded that yolk color in laying pullets may be improved by feeding both orange skin or marigold flower but the latter ingredient appeared to be most effective.

Key words: marigold flower, orange skin, yolk color, egg quality, laying performance

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Introduction

The color of egg yolk is one of the important factors of egg quality that should receive attention of the poultry producers as per expectation of the consumers. Consumers generally prefer yolk color ranging from golden yellow to orange (Vuilleumier, 1969) because, such a color of egg yolk is essential for their satisfaction. Yellow color of egg yolk is also important in manufacturing egg products such as liquid frozen and dried whole eggs or separated egg components (Bartov and Bornstein, 1974; Johnson *et al.,* 1980). Food processors usually rely on egg yolks to impart color on various products such as noodles, pasta, cake, cheese etc. Moreover, uniform and good yellow color of egg yolk is an indication of healthy flock of a laying farm from which eggs are produced. Although the issue of yolk coloration is less important from nutritional point of view, the attitude of consumers has become a matter of concern to egg producers. Because, many consumers believe that eggs with pale yolk color is neither tasty nor nutritious. The scavenging hens always produce eggs with acceptable yolk color because of their free access to green plants that contain huge xanthophyll pigments. In contrast, most of the commercial poultry farms use high energy concentrated feed which are naturally low in pigment particularly in diets containing inadequate amounts of yellow corn. Therefore, commercial poultry production in many countries faces the challenging issue of desirable yolk pigmentation when limited amounts of xanthophyll containing ingredients are considered. Poultry farms fail to obtain the standard level of egg yolk color if proper attention is not given on this issue (Brahmakshatriya and Shrivastava, 1978). Thus, the desirable yolk color is an important concern to both poultry producers and consumers.

Yolk pigmentation results primarily from carotenoid pigments, specially xanthophylls, a non-nutritive factor having no contribution to taste. Since birds do not synthesize pigments in their physiological system (Marusich and Bauernfeind, 1981), incorporation from external sources in the diet is necessary for proper yolk pigmentation.

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Xanthophyll is a carotenoid pigment present in many food items of human being and animals such as yellow corn, tomatoes, carrots, lucerne, green grasses, algae or various aquatic weeds. The most commonly used ingredient for yolk pigmentation is yellow corn which, in addition to energy, supplies 20–25 mg/kg xanthophylls. Previous report suggests that layer diet based on cereal grains other than yellow corn requires additional pigment supplementation to achieve desirable yolk color (Ravindra, 1995). There are some countries in the world where production and availability of yellow corn is very limited, and the poultry producers are dependent on other grains such as wheat, barley, oat etc in diet formulation. Wheat-based diet fails to produce eggs with standard yolk color because of its deficiency of desirable pigment (Saha *et al.,* 1998). Use of synthetic pigments in poultry diet is an alternative measure for egg yolk coloration. Of course, this increases overall feed cost and its inclusion is often prohibited, in some countries, by government regulations. In recent years, organic poultry farming is gaining momentum and the important legislative regulations stipulated in organic poultry production is that all yolk pigments must be of natural sources. Moreover, consumer's preference for natural organic products in their food is increasing (Williams, 1992). Consequently, poultry nutritionists should think for the alternative sources of natural carotenoids as pigmenting agents for egg yolk. Two of such feed ingredients those contain carotenoids may be non-traditional ingredients like marigold flower and orange skin.

The common marigold is familiar to everyone, with its pale-green leaves and golden orange flowers. It is a scented herb of the genus *Tagetes* that grows abundantly in many countries of the world with minimum agronomic care. Hence, its production cost is very low. Marigold flower that usually used in different occasions like National Days, religious festivals and other purposes in many countries is being wasted just after the festivals. Currently, marigold plants are commercially grown in Mexico, Peru and India for pigment production (Bosma *et al.,* 2003). Although lutein and zeaxanthin are the main xanthophylls in egg yolk (Karunajeeva *et al.,* 1984), the most significant sources of xanthophylls in marigold is lutein (Delgado-Vergas and Paredes-Lopez, 1996; Hadden *et al.,* 1999). Marigold petal and residues were also reported to be good sources of xanthophylls (Narahari *et al.,* 1981; Ojeda *et al.,* 1983). In a recent study, Santos-Bocanegra *et al.* (2004) observed a value of yolk color score of 11.7 in Roche Yolk Color (RYC) fan with an inclusion of 7.5 ppm yellow and 4 ppm red xanthophylls extracted from marigold and Red Pepper Paprika, respectively. Although a few studies have evaluated the efficacy of xanthophylls extracted from marigold flower on egg yolk pigmentation, to authors' knowledge information regarding the inclusion of sun-dried marigold flower and orange skin is lacking in the literature. These non-conventional feed ingredients have not been considered for inclusion in the diet with the objective of determining their efficacy in yolk coloration. Thus, a study of using marigold flower and orange skin in layer diets seemed worthwhile to evaluate pigmenting ability of these ingredients. Considering these facts, the present study was undertaken with the objectives to determine xanthophylls and some other chemical constituents of marigold and orange skin and to assess their efficacy in egg yolk coloration as being natural sources of xanthophylls.

Materials and Methods

Collection, Drying and Storage of Marigold Flower and Orange Skin

Marigold flower and orange skin were collected from Bangladesh Agricultural University (BAU) campus, Mymensingh Medical College Hospital (MMCH), Mymensingh and local markets around the BAU, Bangladesh. They were air-dried, ground and subsequently stored separately in plastic bags until used for feed formulation.

Chemical Analysis of Marigold Flower, Orange Skin and Other Feed Ingredients

The test ingredients (marigold flower and orange skin) and other feed ingredients used for feed formulation were subjected to chemical analysis for the determination of dry matter (DM), crude protein (CP), ether extract (EE), nitrogen free extract (NFE) and ash by following standard methods as suggested by the Association of Official Analytical Chemists (AOAC, 2000). Starch and sugar contents of marigold flower and orange skin were determined by the methods of Raghuramula *et al.* (1983) whereas calcium and phosphorus contents of these ingredients were determined by the method suggested by Page *et al.* (1982). Xanthophylls content in marigold flower and orange skin were analyzed by the method suggested by Quackenbush *et al.* (1970).

Experimental Protocol

Trial 1: Experimental House, Birds and Dietary Treatments

An experimental room was divided into nine separate small pens of equal size (2.74×1.52 meter). Bamboo materials and wire net were used for the separation of pens from each other. Fresh sawdust at a depth of 7.6 cm was spread on the floor as litter material. Sixty three Shaver 579 pullets, 34-week old, were considered from the same hatch and randomly divided into three dietary treatment groups each of 21 pullets. Each treatment had three replications each of seven birds. Initial body weight of the birds was adjusted in all treatments to minimize the variation among the groups. The control diet was a wheat-based diet formulated with locally available feed ingredients that contained no xanthophyll. The other two diets were prepared by adding either 40 g ground marigold flower or 40 g ground orange skin per kg of mixed feed. All diets were supplemented with additional vitamin-mineral and amino acid premix at the rate of 2.5 g/kg of mixed feed. A computer assisted programme namely Users Friendly Feed Formulation, Done Again (UFFDA, 1982) was considered for balancing nutrients of the dites. The nutrient requirements (ME, CP, Ca, P, lysine, methionine, cystine and tryptophan) were satisfied close to the breeder's recommendations of the strain (Shaver-579 Commercial Management Guide, 2000). Chemical compositions of the experimental diets fed to laying hens are shown in Table 1.

Trial 2: Experimental House, Birds and Dietary Treatments

Experimental house was similar to those described in trial 1 except that the number of pens used in this trial was 12. Eighty four Shaver-579 commercial layers from the same hatch and same genetic group were reared to investigate the efficacy of test ingredients in yolk coloration. Chemical compositions of three different diets i.e. control, 40 g marigold/kg, 40 g orange skin/kg were similar to trial 1, and the fourth diet contained 30 mg synthetic pigment/kg of mixed feed. The synthetic pigment Carophyll® Yellow manufactured by F. Holfmann-La Roche Ltd., (Basel, Switzerland) was procured from the local representative of Jayson Agrovet Ltd. (Dhaka, Bangladesh). The purpose of evaluating synthetic pigment was to give a comparative profile in egg yolk pigmentation with the natural sources of xanthophylls considered in this study.

Measurements of Egg Quality Characteristics

For the measurements of egg quality characteristics, two eggs from each replication were considered. Samples were randomly taken after 4 weeks of supplementation 38 weeks of age during trial 1 and after 8 and 12 weeks of supplementation 42 and 46 weeks of age respectively during trial 2. Among the internal qualities, egg yolk color was measured using RYC Fan (F. Holfmann-La Roche Ltd., Basel, 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. Although the

Table 1. **Composition of experimental diets (Trial 1 and 2)**

Feed ingredients/Chemical composition	Experimental diets (amount in 1000 g)			
	Control	40 g/kg marigold	40 g/kg orange skin	30 mg/kg synthetic pigment[1]
Feed ingredients				
Wheat	522.5	497.3	500.0	522.5
Full-fat soybean	167.8	160.5	188.5	167.8
Soybean meal	27.5	21.9	26.0	27.5
Sesame oil cake	60.8	76.9	66.0	60.8
Rice polish	97.5	40.0	18.0	97.5
Fish meal	25.0	53.6	27.5	25.0
Bone meal	14.2	17.0	45.0	14.2
Marigold	0	40.0	0	0
Orange skin	0	0	40.0	0
* Synthetic pigment	0	0	0	0.3
Oyster shell	75.0	83.3	80.0	75.0
Common salt	2.7	2.5	2.3	2.7
** Embavit L (vitamin-mineral-amino acid premix)	2.5	2.5	2.5	2.5
DL-methionine	2.0	2.0	1.7	2.0
L-lysine	2.5	2.5	2.5	2.5
Chemical composition				
ME (kal/kg)	2750	2800	2800	2750
CP (g/kg)	175.6	180.0	176.5	175.6
Ca (g/kg)	41.2	41.1	40.0	41.2
Available P (g/kg)	4.2	4.5	4.5	4.2
Lysine (g/kg)	8.0	8.6	8.3	8.0
Methionine (g/kg)	4.0	4.0	4.2	4.0
Tryptophan (g/kg)	2.0	2.0	1.9	2.0

Trial 1 = Control, 40 g/kg marigold and 40 g/kg orange skin.
Trial 2 = Control, 40 g/kg marigold, 40 g/kg orange skin and 30 mg/kg synthetic pigment.
* Synthetic pigment: One gram contains 100 mg β-Apo-8′-Carotenoic Ethyl Ester (F. Holfmann-La Roche Ltd., Basel, Switzerland).
[1] Considered in trial 2 only.
** Embavit L was added at the rate of 2.5 g/kg mixed feed which contained Vitamin-A 12500 IU, Vitamin-D_3 2500 IU, Vitamin E 20 mg, Vitamin K_3 4 mg, Vitamin B_1 2.5 mg, Vitamin B_2 5 mg, Vitamin B_6 4 mg, Nicotinic acid 40 mg, Pantpthemic acid 12.5 mg, Vitamin B_{12} 0.01 mg, Folic acid 0.8 mg, Biotin 0.1 mg, Cobalt 0.4 mg, Copper 10 mg, Iron 60 mg, Iodine 0.4 mg, Manganese 60 mg, Zinc 50 mg, Selenium 0.5 mg, DL-Methionine 100 mg, Choline chloride 300 mg, L-lysine 60 mg.

"standard" of yolk color score ranges from 7–9 in RYC Fan, a range from 6–12 is usually considered as acceptable for consumers' satisfaction. The other internal egg quality characteristics were determined by estimating albumen dry matter, albumen weight, albumen index, Haugh unit, yolk weight, yolk dry matter and yolk index. External egg quality characteristics were determined by measuring egg shape index, egg breaking strength, shell thickness, shell dry weight, percent shell, dry membrane weight and percent membrane. Weight of different egg components namely yolk weight, albumen weight, shell dry weight and membrane dry weights were also measured in accordance with Chowdhury (1988). The objective of measuring associate internal and external egg quality characteristics were to examine whether the inclusion of such pigment-containing feed ingredients had any inimical effect on the table egg quality. Finally, a panel test was conducted by supplying egg pudding and full-boiled eggs to a 10-member panel board for the evaluation of egg yolk color, taste of final food products and overall attitudes of consumers to egg quality. The eggs used for panel test were collected from the experimental birds after feeding the test ingredients (marigold, orange skin or synthetic pigment). Both egg pudding and full boiled eggs were evaluated by the panel board within 100 scores considering color, flavor, texture and overall taste independently. The original nomenclature of the samples was kept unknown to board members in order to avoid the introduction of bias.

Record Keeping and Data Analysis

Records for the yolk color score and other quality characteristics were kept during the experimental period. Productive characteristics of layers were also noted. Necessary calculations for variables were done where required. In trial 1, ANOVA was performed in accordance with Completely Randomized Design (Steel and Torrie, 1980). In trial 2, two-way ANOVA was performed with two main factors (treatments X time (weeks)) using MSTAT statistical package to observe the effect of the length of supplementation on egg yolk coloration. Finally, Least Significant Differences (LSD) were calculated to compare treatment means.

Results

Chemical Composition of Marigold Flower and Orange Skin

The chemical composition of marigold flower and orange skin is shown in Table 2. Results of the chemical analysis revealed that DM, CF and Ca contents of orange skin and marigold flower were more or less similar. The composition of remaining components shows that the xanthophyll, CP, EE, total P and ash contents in marigold flower were nearly double than the orange skin. The calculated ME concentration of marigold flower was also nearly 2.5 times higher than the orange skin.

Trial 1

Improvement of Egg Yolk Color

The yolk color score of birds fed marigold flower was highest (8.2) in RYC Fan and differed significantly ($P<0.01$) from that of orange skin (3.3) and wheat-based control diet (1.2) (Table 3). Each laying hen consumed 0.749 and 0.397 mg/day xanthophyll following inclusion of 40 g/kg marigold and 40 g/kg orange skin in the diet, respectively. Yolk color of control group fed wheat-based diet was quite pale although the data did not show significant difference from the orange skin-fed group.

Performance Characteristics of Laying Pullet

Table 4 shows that all variables examined in the current

Table 2. **Chemical composition of marigold flower and orange skin**

Ingredients	DM (g/kg)	Composition on DM basis (g/kg)							Total xanthophylls (mg/kg)
		ME kcal/kg	CP	EE	CF	Ash	Ca	Total P	
Orange skin	874.0	1353	56.0	37.0	200.0	30.0	4.5	3.0	83.02
Marigold flower	883.0	3322	125.0	67.0	200.0	67.0	5.0	5.0	156.32

Table 3. **Effect of marigold, orange skin and synthetic pigment on egg yolk pigmentation**

Dietary groups	Consumption of xanthophyll (mg/hen/day)	Xanthophyll/kg mixed feed (mg)	Trial 1		Trial 2			
			Weeks of feeding	Yolk color score	Weeks of feeding	Yolk color score	Weeks of feeding	Yolk color score
Control diet	0	0	4	1.2[b]	8	1.00[c]	12	1.00[d]
40 g/kg marigold	0.749	6.25	4	8.2[a]	8	9.47[a]	12	11.00[b]
40 g/kg orange skin	0.397	3.32	4	3.3[b]	8	4.40[b]	12	5.00[c]
30 mg/kg synthetic pigment (trial 2)	2.830	30.00	—	—	8	7.16[ab]	12	11.71[a]

Means of yolk color score bearing uncommon superscripts in a column differ significantly ($P<0.01$).

study (body weight, hen-day production, feed consumption, egg weight, feed conversion ratio (FCR), egg mass output, livability) showed no significant differences among the treatment groups. This indicates that there was no detrimental effect of such natural ingredients on productivity.

Egg Quality Characteristics

The external egg qualities such as egg weight, shape index, dry shell weight, percent shell, shell thickness, dry membrane weight, percent membrane and breaking strength of eggs during 4 weeks of supplementation of 40 g/kg marigold and 40 g/kg orange skin did not vary significantly ($p > 0.05$) (Table 4). Likewise, the internal egg quality characteristics namely the albumen quality (albumen index, albumen weight, albumen dry matter and Haugh unit) and yolk quality (yolk index, yolk weight, yolk dry matter) except yolk color score also did not differ significantly among the treatment groups.

Trial 2

Improvement of Egg Yolk Color

Table 3 shows the improvement of yolk color by feeding diets containing 40 g marigold flower, 40 g orange skin and 30 mg synthetic pigment per kg of mixed feed. Following 8 weeks of supplementation, the highest yolk color score in RYC Fan was observed in birds fed marigold (9.47) followed by synthetic pigment (7.16) and orange skin (4.40) respectively. Whereas, after 12 weeks of supplementation, the yolk color score fed synthetic pigment was significantly higher (11.71) than the marigold (11.00) and orange skin diets (5.00). The efficiency of yolk coloration fed orange skin diet, however, was higher than the wheat-based control diet, as would be expected. The results of factorial analysis (two main factors: dietary supplementation X weeks) showed that the yolk color was significantly improved at 12th week as compared to 8th week of supplementation for all test ingredients when supplemented independently.

Productive Performance and Egg Quality Characteristics

The results of productive performance also showed no significant difference among the dietary treatment groups (Table 5). Like trial 1, both external and internal egg quality characteristics except that of yolk color score did not show significant differences.

Scores of Egg Pudding and Boiled Eggs

Table 6 shows the scores averaged for egg pudding and boiled eggs of different treatments as evaluated by the panel board. It was surprising to note that the products prepared with the eggs produced after feeding both marigold and orange skin scored exceptionally higher than the

Table 4. **Performance characteristics of laying pullets fed marigold flower or orange skin in the diets (Trial 1)**

Variables		Dietary treatments: Control	40 g/kg marigold	40 g/kg orange skin	SEM
Productive performance	Body weight (g/bird)	1624.0	1685.0	1609.0	38.64
	Hen-day egg production (%)	69.5	71.3	71.3	6.175
	Feed consumption (g/birds/day)	119.0	118.0	119.0	0.660
	Egg weight (g/egg)	58.3	58.9	57.7	0.627
	Feed conversion	3.2	2.8	2.9	0.261
	Egg mass output (g egg/henday)	37.7	42.2	41.3	3.589
	Livability (%)	100	100	100	—
External egg quality characteristics	Sample egg weight (g/egg)	61.8	61.8	62.8	2.78
	Shape index	73.2	74.1	74.4	0.745
	Shell dry weight (g/egg)	6.1	5.9	5.9	0.189
	Percent shell	10.0	9.7	9.9	0.347
	Shell thickness (mm)	0.42	0.38	0.39	0.025
	Membrane dry weight (g/egg)	0.15	0.17	0.15	0.018
	Percent membrane	0.23	0.28	0.26	0.026
	Egg breaking strength	2215.4	2208.9	2138.4	30.48
Internal egg quality characteristics	Albumen index	0.087	0.082	0.081	0.006
	Fresh albumen weight (g/egg)	36.39	38.75	35.74	1.88
	Dry albumen weight (g/egg)	4.90	5.19	4.86	0.295
	Albumen dry matter (%)	13.50	13.39	13.60	0.178
	Yolk index	0.42	0.42	0.41	0.011
	Fresh yolk weight (g/egg)	14.56	14.31	14.56	0.379
	Dry yolk weight (g/egg)	7.56	7.51	7.60	0.258
	Yolk dry matter (%)	53.10	52.40	52.20	1.608
	Haugh unit	81.00	80.00	79.00	1.80

All variables showed no significant differences among dietary treatments.

Table 5. **Performance characteristics of laying pullets fed marigold, orange skin and synthetic pigment in diets (Trial 2)**

	Variables	Weeks of feeding	Dietary treatments				SEM
			Control	40 g/kg marigold	40 g/kg orange skin	30 mg/kg synt. pigment	
Productive performance	Body weight (g/bird)	—	1788.8	1760.7	1676.4	1688.0	49.0
	Hen-day egg prod (%)	—	80.3	81.1	85.4	82.7	3.30
	Feed consumption (g/bird/day)	—	119.7	119.6	119.4	119.0	0.233
	Egg weight (g/egg)	—	61.3	62.6	60.1	60.9	0.604
	Feed conversion	—	2.6	2.3	2.4	2.3	0.129
	Egg mass output (g egg/henday)	—	46.0	51.5	49.3	50.4	2.51
	Livability (%)	—	100	100	100	100	—
External egg quality characteristics	Sample egg weight (g/egg)	8	60.3	61.1	59.3	61.4	7.16
		12	60.5	63.3	61.4	60.1	7.42
	Shape index	8	71.4	73.4	72.1	73.2	0.617
		12	72.9	73.6	73.2	73.0	0.875
	Shell dry weight (g/egg)	8	5.9	5.8	5.7	5.8	0.225
		12	5.8	5.9	5.5	5.8	0.273
	Percent shell	8	9.7	9.6	9.6	9.5	0.303
		12	9.7	9.4	9.1	9.8	0.323
	Shell thickness (mm)	8	0.410	0.378	0.385	0.379	0.012
		12	0.386	0.375	0.378	0.393	0.013
	Membrane dry weight (g/egg)	8	0.119	0.117	0.124	0.150	0.011
		12	0.100	0.117	0.100	0.117	0.012
	Percent membrane	8	0.197	0.221	0.187	0.236	0.022
		12	0.164	0.184	0.162	0.195	0.022
	Egg breaking strength	8	2167.2	2193.1	2151.9	2212.2	74.9
		12	2167.8	2260.5	2184.9	2160.9	52.8
Internal egg quality characteristics	Albumen index	8	0.094	0.096	0.087	0.088	0.00
		12	0.092	0.096	0.088	0.097	0.009
	Fresh albumen weight (g/egg)	8	36.047	37.277	35.027	38.130	1.73
		12	36.830	38.017	36.990	36.820	1.70
	Dry albumen weight (g/egg)	8	4.870	4.727	4.830	5.007	0.363
		12	4.947	5.247	4.897	4.807	0.231
	Albumen dry matter (g/egg)	8	13.517	13.747	13.753	13.123	0.272
		12	13.423	13.260	13.210	13.030	0.278
	Yolk index	8	0.437	0.439	0.424	0.430	0.009
		12	0.424	0.424	0.445	0.422	0.018
	Fresh yolk weight (g/egg)	8	14.537	15.150	14.837	14.693	0.387
		12	14.557	15.590	14.940	15.100	0.590
	Dry yolk weight (g/egg)	8	7.390	7.857	7.843	7.663	0.196
		12	7.373	7.917	7.657	7.670	0.321
	Yolk dry matter (g/egg)	8	51.670	52.510	52.443	52.150	12.63
		12	51.197	51.617	51.273	50.887	0.839
	Haugh unit	8	88.00	88.00	85.00	85.00	2.50
		12	85.00	84.00	83.00	80.00	2.20

All variables showed no significant differences among dietary treatments.

Table 6. **Score of panel test for pudding and boiled eggs prepared from the egg samples collected from different experimental diets (Trial 2)**

Egg samples collected from experimental diets	Scores of panel test	
	Egg pudding	Boiled eggs
Control	73.9	69.4
40 g/kg marigold flower	81.1	85.9
40 g/kg orange skin	74.4	82.3
30 mg/kg synthetic pigment	60.0	64.4

control and synthetic pigment receiving groups. Thus the products prepared with the eggs after feeding natural pigments are more acceptable to the consumers as evident from the decision of the judges of the panel board.

Discussion

The data on chemical composition of DM, CP, CF, Ca, total P and ash contents of marigold were close to the values previously reported by Narahari *et al.* (1981) and Ojeda *et al.* (1983). The contents of EE and xanthophyll, however, were found lower than the values reported by Narahari *et al.* (1981). It is notable that the xanthophyll contents in marigold was almost double than that of orange skin. Therefore, significant improvement of yolk colors by feeding marigold in both the trials was observed because of the high concentration of xanthophyll in the diets.

Although the egg yolk color score in RYC fan was not statistically different between orange skin and wheat-based diets, inclusion of orange skin in the diet slightly improved overall yolk coloration. Previously, Sikder *et al.* (1998) found 3.12 yolk color score at 3rd week of supplementation of 40 g/kg dried carrot meal (another natural ingredient) which was very close to the value obtained for 40 g/kg orange skin group. On the other hand, Khatun *et al.* (1999) found 8.12 color score where they fed 150 g/kg azolla, an aquatic weed, for eight weeks. This result was close to the values obtained in the current study for 40 g/kg marigold flower after 4 weeks of supplementation. Thus the supplementation of 40 g/kg marigold for four weeks in layer diet is equivalent to 150 g/kg azolla supplementation for eight weeks in terms of yolk color score that may be considered standard and therefore acceptable. Following 8 and 12 weeks of supplementation, yolk color was also significantly improved in birds fed marigold as compared to orange skin because of higher level of xanthophyll concentration in marigold. The yolk color in birds that received 40 g/kg orange skin was very close to the yolk color obtained previously from 500 g/kg yellow corn in layer diet at 3rd week of supplementation (Sikder *et al.* 1998).

It appears that for each of the coloring agents group, yolk color score was also significantly improved after 12 weeks of supplementation as compared to 8 week, and the interaction between dietary supplementation and time was also positive (data not shown). Moreover, the yolk color score at 4th week in trial 1 (data was not analyzed statistically with trial 2 because of an independent experiment) showed lower values as compared to 8 and 12th weeks for each of the dietary supplementation. These results however indicate a usual manner of yolk color improvement in association with the increase of supplementation period. Since the birds are capable of storing pigment materials in their biological systems, the gradual improvement of yolk color during the entire experimental period with the same level of supplementation might be an eventual fact of the stored xanthophylls. It is notable that 40 g/kg orange skin in layer diet, although improved yolk color but failed to reach the acceptable range of color score. Therefore, feasibility for the improvement of yolk color by feeding orange skin with an inclusion level higher than the tested level in the current study should be examined. Productive performances of layer particularly feed consumption, and feed utilization should also be taken into account when high level of orange skin would be considered for inclusion. No apparent effects on other egg quality traits and productivity suggest that inclusion of 40 g/kg orange skin or marigold does not have any adverse effect on these productive performance and egg quality characteristics. Scores of panel test also go in favor of including natural pigments for consumers' satisfaction.

We have reported here the improvement of egg yolk color by feeding marigold flower and orange skin to laying hens. The noteworthy findings are: i) egg yolk color significantly improved and reached to a level of consumers' standard by feeding 40 g/kg marigold flower, ii) egg yolk color is slightly improved by feeding 40 g/kg orange skin as compared to wheat-based control diet. In conclusion, yolk color in laying hen may be improved by feeding both orange skin or marigold flower but the latter ingredient appeared to be most effective.

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