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Marigold and Orange Skin as Egg Yolk Color Promoting Agents

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Abstract: The research was conducted with natural ingredients and orange skin in the diet of laying pullets to determine the suitability as pigmenting agents of egg yolk for laying chicken The influence of test ingredients on production characteristics of laying pullets, internal and external quality characteristics of eggs were investigated simultaneously. The experiment covered the proximate analysis of marigold, orange skin meal to determine the nutrient concentrations, xanthophylls contents and 7^{th}-12^{th} weeks feeding trail to investigate the yolk pigment availability of marigold and orange skin as well as laying performance of birds. Orange skin and marigold were used for comparing the pigmenting ability to normal feed containing xanthophylls. Yolk color was improved significantly as compared to control for addition of marigold and orange skin during 4^{th}, 8^{th} and 12^{th} weeks of supplementation. Inclusion of marigold and orange skin in the diet of laying pullets did not cause deterioration in natural and external quality of eggs and there were no significant variations with respect to body weight, hen day egg production egg weight and feed conversion. There was no mortality of birds during study period. The results indicates the conclusion that between two natural ingredients marigold contain more xanthophylls (156.32mg/kg DM) than orange skin (83.02mg/kg DM) and use of 4% marigold meal in the diet of laying pullets is enough to produce eggs with yolk color score 11.00 close to 30 mg synthetic pigment/kg diet at the 12^{th} week of supplementation, whereas the birds that received 4% orange skin in the diet were able to produce eggs with yolk color score 5.0. Moreover, use of marigold and orange skin in diet of pullet has no detrimental effect on internal and external quality of egg as well as egg production characteristics. So, marigold can be used more efficiently for egg yolk pigmentation.

Key words: Marigold and orange skin egg yolk, pigmenting agents, xanthophyll intake

Introduction

The color of egg yolk is very much important for consumers' satisfaction and consumers all over the world usually prefer yolk color ranging from golden yellow to orange, i.e. mid way to high on the Roche Yolk color (RYC) scale as described by Vuilleumiler (1969). Uniformity of yolk color among the eggs laid by a young flock also has a bearing on consumers' preference (Banks and Voss, 1962). This is perhaps due to the influence of food color on human desire or appetite for food and the judgment of its quality (Amerine *et al.*, 1995). Consumers learned by experience to associate quality standards (Pangborne, 1960) and flavours (Kostyla and Clydesdale, 1978) with the color of foods. Yolk color is important in the manufacture of egg products such as liquid frozen and dried whole egg and separated components (Johoson *et al.*, 1980). Thus both industries and consumers of fresh eggs want well-coloured yolk. However, unlike the shell egg market, it is clearly evident that users who favor well coloured products with high pigment content are often prepared to pay a premium (Forsythe, 1968; Fletcher, 1980).

In Bangladesh, rural poultry are reared under scavenging system. So *Desi* laying chicken are able to consume green grasses and other carotenoids and therefore always produce eggs of acceptable yolk color. Commercial poultry farming is expanding Tremendously in all over the country from the last two decades and farmers are rearing their birds under intensive system. Moreover, commercial poultry farmers mostly fed wheat-bast diets to their laying hens which have no contribution for yolk pigmentation. So, pale egg yolk is one of the problems of commercial egg laying farms in our country. Many consumers believe that eggs with pale yolks are neither testy nor nutritious although yolk pigmentation results primarily from xanthophylls, a non nutritive factor having no contribution to taste. But the attitude of the consumers is a matter of concern to egg producers. Currently, poultry industry in many countries of the world is facing a problem of desirable egg yolk pigmentation. Many poultry farms could not obtain desirable degree of egg yolk color (Brahmakshatriya and Shrivastava, 1978). The color of egg yolk is produced by carotenoid pigments, specially by xanthophyll which is present in the natural poultry feeds like maize, Lucerne, grasses, tomatoes, carrots, algae etc. Xanthophyll is also found in marigold and orange skin. Yellow corn, in addition to energy source, also supplies Xanthophyll pigment for chicken. It contains 20-25 mg Xanthophyll/kg (Scott *et al.*, 1968) which can produce yolks with color score ranging 6.5 to 7 on RYC fan if fed to laying birds at 50% dietary levels where it constitutes the only source of Xanthophyll (Saha *et al.*, 1998). In countries like Bangladesh, where the production of yellow corn is very limited, wheat is the

only grain usually considered by the poultry products in the diet formulation. Whwat-based diet usually fails to produce eggs with standard yolk color (color score 1 to 2) on RYC color fan (Saha *et al*., 1998). Since the use of synthetic pigment in the diet increases feed cost, natural carotenoids should be considered as pigmenting agents for egg yolk. Two of such carotenoids available in Bangladesh are marigold and orange skin. Marigold is used only in occasions like Independence day, Victory day, Shahid day, Hindu religious festivals and other purpose. It grows easily everywhere and its production cost is very low. Narahari *et al*. (1981) and Ojeda *et al*. (1983) also reported that marigold petal meal and residue are good sources of xanthophylls and they could be used in layer diets as pigmenting agents for egg yolk coloration. Used marigold and orange skin become useless and just thrown to dustbins. Although they contain xanthophylls pigments, their efficacy in pigmenting egg yolk has never been tested in Bangladesh. Therefore, a study to use marigold and orange skin in layer diets seemed worthwhile to report their pigmenting ability of egg yolk. Considering these facts, the present research work was undertaken to determine xanthophyll and other chemical constituents (proximate components) of marigold and orange skin as well as to assess their efficacy against the control and synthetic carotenoid.

Materials and Methods

This experiment was conducted with laying pullets (Shaver 579) by feeding marigold and orange skin containing formulated diets. Feeding trial was started at the age of 34th weeks and continued up to 40th weeks.

Experimental work: This feeding trial was conducted at Bangladesh Agricultural University Poultry Farm, Mymensingh for a period of six weeks. Proximate analysis, starch, sugar, calcium and phosphorus contents of the feed ingredients, Xanthopyll content of marigold and orange skin were determined at lab. of Poultry Science & Biochemistry, BAU, Mymensingh. Campus available Marigold and Orange skin was collected, air-dried and ground for feed formulation.

Chemical analysis: All feed ingredients 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 Association of Official Analytical Chemists (AOAC, 1990). Starch and sugar contents of marigold and orange skin were determined by the methods of Raghuramula *et al*. (1983). Calcium phosphorus contents of marigold and orange skin were determined by the methods of Page *et al*. (1982).

Determination of xanthophylls: Xanthophyll content of marigold and orange skin were measured using Quackenbush *et al*. (1970) method. Two (2) gm of fine powder sample was used for the extraction of total xanthophyll. The sample was taken in a dry and clean 100 ml volumetric flask. 30 ml of extractant composed for hexane: acetone: ethanol: toluene (10+7+16+7) was added into the volumetric flask and swirled for one minute. The flask was along with its content was left in dark for 16 hours of incubation, 2 ml of 40% methanolic KOH solution was added into the flask and swirled for 1 minute. The flask was then left for 1 hour in dark. Then 30 ml of hexane was added into the flask and swirled for 1 minute. Final volume was made by adding 10% sodium sulfate and vigorously shaken for 1 minute and was left for 1 hour dark. Then column chromatography was done immediately using an aliquot of upper phase. A column of 1.2 × 9cm was constructed by the absorbent (celete and magnesium oxide, 1+1), which was previously dissolved in acetone and degassed. The column was equilibrated by passing hexane-acetone mixture (90+10) 3-4 ml (extracted upper phase) sample was put on the column. After absorption into the column, carotenes were eluted from the column using three-bed volume of hexane-acetone mixture (90+10). After the elution of carotenes, total xanthophylls were eluted from the column with hexane-acetone-methanol mixture (80+10+10). The eluant was carefully collected and a final volume was made by dilution with hexane-acetone-methanol mixture (80+10+10). Optical density of the collected eluant was measured at 474 nm using a junior Spectrometer (Coleman Instrument, Maywood, Illinois, USA).

The amount of xanthophyll was collected by the following formula:

$$\text{Total xanthophyll (mg/b)} = OD \times 454 \times f / 236 \times b \times d$$

Where, f = instrument deviation factor = 0.561 / obs. OD
b = light path in cm

d = dilution factor = gm sample × ml. of ext. on column / 50 ml upper phase x final dilution

Determination of metabolizable energy (ME): The ME contents of feed ingredients including marigold and orange skin were determined indirectly using the formula suggest by Wiseman (1987) as follow:

ME (kcal/kg = 35.2 CP + 78.5 EE + 40.5 S + 35.5 Su).

Here,

ME = Metabolizable energy, S = Starch (%), CP = Crude protein (%), Su = Sugar (%), EE = Ether extract (%)

Preparation of house and laying box: The experimental pens of the layer house were properly washed and cleaned by using tap water. Ceiling, walls and floor were cleaned thoroughly. Then disinfection was done by

spraying dilute phenyl solution. After drying, the experimental rooms were divided into 9 separated pens of equal size (2.74 × 1.52 meter). Experimental pens were separated one from another using bamboo materials and wire net. All pens and laying boxes were thoroughly cleaned and further disinfection were done and allowed to dry up as well. Fresh dried sawdust at a depth of 7.62 cm was used on floor as litter material. One feeder trough was hanged at 20 cm and community type next box was provided in each replication having 5 small nests.

Experimental birds and dietary treatment: The experiment was conducted with sixty three Shaver 579 (BAU Poultry Farm hatched) pullets of the same hatch in a Completely Randomized Design (CRD). The birds were randomly divided into 3 dietary treatment groups having 21 pullets in each. Each treatment had 3 replications allocating 7 birds per replication. Initial body weights of birds were adjusted in all treatment groups. The layout of the experiment 1 is shown in Table 1.

Table 1: Layout of experiment

Treatments	No. of birds in each replication			Total No. of birds
	R_1	R_2	R_3	
Control (T_0)	7	7	7	21
4% Marigold (T_1)	7	7	7	21
4% Orange skin (T_2)	7	7	7	21
Grand total				63

Three diets were formulated by using locally available feed ingredients which contained either no xanthophyll source (T_0), 4% marigold (T_1) or 4% orange skin meal (T_2). The diets were formulated by a computer using the Userfriendly Feed Formulation, Done Again (UFFDA). The programme was developed by the University of Georgia, USA 1992 linear programming. The nutrient requirements (ME, CP, Ca, P, Lysine, Methionine, Cystine and Tryptrophan) were satisfied according to breeders recommendation (Shaver-579 Commercial Management Guide). The composition of experimental ration is available in Table 2.

Experimental diet, lighting and medication management: All feed ingredients including test ingredients were ground, crushed and weighed out and hand mixed thoroughly. Fresh water and dry mash feed was supplied *ad libitum* twice a day (morning and afternoon)through out the experimental period. The birds were reared during a photoperiod of 12 hours and an additional artificial light provided for 4 hours to make total lighting period to 16 hours daily. Cosumix plus (Novartis Ltd. Animal health sector, Bangladesh) was added to drinking water for three days continuously to prevent bacterial infection during the first 3 days of

Table 2: Ingredient and chemical composition of diets for experiment

Fee ingredients / Che. Composition	Experimental Diets (amounts in kg)		
	T_0	T_1	T_2
Feed ingredients			
Wheat	52.25	49.73	50.00
Full-fat soyabean	16.78	16.05	18.85
Soyabean meal	2.75	2.19	2.60
Sesame oil cake	6.08	7.69	6.60
Rice polish	9.75	4.00	1.80
Fish meal	2.50	5.36	2.75
Bone meal	1.42	1.70	4.50
Marigold	0.00	4.00	0.00
Orange skin	0.00	0.00	4.00
Oyster shell	7.50	8.33	8.00
Common salt	0.27	0.25	0.23
* Vitamine-mineral-amino acid premix	0.25	0.25	0.25
DL-Methionine	0.20	0.20	0.17
L-Lysine	0.25	0.25	0.25
Chemical composition			
ME (kcal/kg)	2750	2800	2800
CP (%)	17.56	18.00	17.65
Ca (%)	4.12	4.11	4.00
Available P (%)	0.42	0.45	0.45
L-Lysine (%)	0.80	0.86	0.83
Methionine (%)	0.40	0.40	0.42
Tryptophan (%)	0.20	0.20	0.19

T_0 : Control, T_1 = 4% marigold, T_2 = 4% orange skin

acclimatization. Waterers were cleaned with tap water in every morning while feeders were cleaned every week. Moreover, waterers were cleaned and washed thoroughly with detergent in weekly. Faces were removed when necessary and litter was cleaned weekly. Birds in all treatment groups were provided with identical care and management throughout the experimental period. Strict hygienic measures and sanitation programme were taken during this period. Environmental temperature and humidity were recorded twice a day, once in the morning and then in the afternoon.

Data recording: Body weight was recorded at the beginning of the experiment and then at every 3 weeks, interval. Feed supply was recorded weekly. Egg production was recorded daily (replication wise) and the eggs were weighted every day in the afternoon immediately after collection and thereafter change in body weight, feed consumption, feed efficiency, hen-day egg production, hen-housed egg production, egg mash output and survivability were determined as well.

Egg quality characteristics: Egg quality characteristics were measured for the egg laid by birds of different dietary groups. Considering two eggs from each replication were collected during the 4th week of the experimental period. Egg weight, width and length were measured by using egg weighing scale and slide calipers. The egg was then carefully broken on a glass plate (30 cm × 21cm) to measure both internal and external egg quality characteristics.

The internal egg quality characteristics were determined

Table 3: Panel tests of cooked eggs from different diets groups

Points	Total score	Score obtained				
		Sample A	Sample B	Sample C	Sample D	Comments
(A. Panel test for pudding)						
Colour	30					
Flavour	20					
Texture	25					
Taste	25					
B. Panel test for full boiled eggs						
Yolk Colour	30					
Albumen colour	15					
Yolk Texture	15					
Albumen texture	15					
Taste	25					

Table 4: Chemical composition of marigold and orange skin

Ingredients	DM%	Composition on DM basis (%)							Total xanthophylls mg/kg
		ME kcal/kg	CP	EE	CF	Ash	Ca	Total p	
Marigold	88.30	3322	12.5	6.7	20	6.7	0.50	0.50	156.32
Orange skin	87.40	1353	5.6	3.7	20	3.0	0.45	0.30	83.02

Table 5: Supplemental xanthophylls consumed by different diet groups

Dietary groups	Weeks of supplem-entation	Xanthophylls* consumed through diets (mg/hen/day)
Control diet (Wheat based)	4	0
4% Marigold	4	0.745
4% Orange skin	4	0.397

* Calculated on the basis of xanthophylls concentrations found in marigold and orange skin from the average feed consumption of birds in the particular week.

by estimating the albumen dry matter, albumen weight, albumen index, Haugh unit, yolk weight, yolk dry matter, yolk index and yolk color. The external egg quality characteristics were measured by estimating the egg shape index, egg breaking strength, shell thickness, dry shell weight, percent shell, dry membrane weight, and percent membrane. Yolk color score was determined by comparing with the Roche yolk color (RYC) fan. Yolk index was calculated as the ratio of average yolk height to average yolk width following removal of the yolk from the albumen. Egg shape index was calculated for each egg from its average length and width.

Egg shape index =
[Average width of egg / Average length of egg] x 100

The albumen index was calculated by dividing the average height of thick albumen by its width. After drying shell thickness was measured by means of an eggshell thickness meter (Ogawa Seiki Co. Ltd; Tokyo, Japan). To reduce error, 3 measurements were taken for each eggshell. One on large end, one on the small end and one on the equator region of the eggshell. The mean of these three measurements was considered as the shell thickness of a particular egg. The 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 egg breaking was calculated by using a formula cited by Arad and Marder (1982).

$$BS = 50.86 \times (EW)^{0.915}$$

Where, EW = Egg weight (g). Finally, the following calculations were made for different components as suggested by Chowdhury (1988).

Panel test with pudding and full boiled egg: Panel test of pudding and full-boiled eggs was conducted to know the yolk color of processed eggs collected from different treatment groups and their subsequent effect of processing. For proper judgment 10 professors were selected from different departments. Obtained score was measured by the following scorecard.

Excellent : 90 to 100
Very good : 80 and above but below 90
Good : 70 and above but below 80
Poor : 60 and above but below 70

Statistical analysis: Data for different variables were subjected to analysis of variance in a Completely Randomized Design (Steel and Torri, 1980). If treatment effects were significant the least significant difference (LSD were calculated to compare treatment means.

Results and Discussion

Chemical composition of marigold and orange skin: The Chemical composition of marigold and orange skin is shown in Table 4. The DM, CP, CF, Ca, total Phosphorus and Ash contents of marigold were found to be close to the values previously reported by Narahari *et al.* (1981) and Ojeda *et al.* (1983). The EE and xanthophyll content was lower to the findings of Narahari *et al.* (1981). Dry matter, CF, Ca contents of orange skin was more or les similar to the values of marigold found in the present study. But Ash, EE, CP, total P, ME were less than half of marigold whereas xanthophyll content of orange skin was 83 mg as against 156 mg/kg for marigold.

Supplemental xanthophyll intake and yolk color: The differences in yolk color scores were highly significant between marigold and other (orange skin and control diet) dietary groups ($p<0.01$). Yolk color score of the eggs laid by birds fed marigold based diet was highest of all (8.2) and differ significantly from orange skin and control group (Table 6 and Plate-2). However, the yolk color scores of orange skin based diet group (3.3) did not differ significantly with control (1.2). Daily supplemental xanthophyll intake and yolk color score of different groups of pullets fed on different diets are shown; Table 5 and 6 respectively. The tables show that birds consumed 0.745 and 0.397 mg supplemental xanthophyll from 4% marigold and 4% orange skin respectively. The yolk color scores were found to the 8.2 and for those groups.

Sikder *et al.* (1998) found 1.87 yolk color score from 62% wheat based die fed for 3 weeks. Although the differences in Roche yolk color values of control and 4% orange skin dietary groups were non-significant ($p>0.05$), the color score indicates that the use of orange skin in the diet improved yolk coloration. This was because total xanthophyll consumption in the orange skin group was higher than that of control diet (Table 5). The yolk color of 4% marigold was higher than that of 4% orange skin group because the birds on 4% marigold group diet consumed more xanthophylls. Reently, Sikder *et al.* (1998) reported a yolk color value of 3.12 during 3rd week of 4% dried carrot meal diet supplementation. This value was very close of the value obtained for 4% orange skin group at 4th week of supplementation. On the other hand, Khaton *et al.* (1999) found 8.12 yolk color value during 8th week of 15% azolla meal diet supplementation, which was similar to the values obtained for 4% marigold group supplementation at 4th week in this experiment.

Performance of laying pullet: The performance data of laying pullets fed three experimental diets are shown in Table 7. The body weight of experimental birds during 6 weeks of study period showed no significant variation among dietary groups. This non-significant difference agreed well with the result of the previous reports by Sikder *et al.* (1998) and Akhter (1995) who worked with carotenoid containing natural feed ingredients carroot (*Daucaus carota*) and duckweed (*Lemna minor*) respectively.

Table 6: Effect of marigold and orange skin on yolk pigmentation

Dietary treatment	Yolk color score
Control diet	1.2^{B}
4% marigold	8.2^{A}
4% orange skin	3.3^{B}
LSD value with level of significant	2.53* *

Means sharing uncommon superscripts differed significantly * * $P<0.01$

Although layers fed marigold and orange skin had slightly higher egg production the differences were found to be statistically non-significant in comparison with control. Sikder *et al.* (1998) reported similar pattern of results from dried carrot meal diets. This result suggests that the use of 4% marigold and 4% orange skin in the diet of laying pullets had no detrimental effect on the rate of egg production.

Data on feed consumption on different diet groups were close to each other hand and statistically non-significant. It appears that the use of 4% marigold and 4% orange skin in the diet of laying pullet could not affect palatability and therefore feed intake was found to be more or less uniform. Results on feed consumption agreed well with the results of Khaton *et al.* (1999) who reported that azolla meal upto 10% dietary level had no effect on the consumption of mixed feed.

The average egg weight obtained in different dietary groups in this experiment was more or less similar and did not differ significantly (Table 7) Sikder *et al.* (1998) reported non-significant difference in egg weight from feeding diets containing 50% maize and upto 8% carrot meal in the layer diet. This result also agreed well with Akhter (1995) and Haustein *et al.* (1990) who also found non-significant differences among layers fed diets containing 15% Lemna species or a diet containing 15% Wolffia species respectively. It is interesting to note that carrot meal and both Lemna and Wolffia species are also able to produce well colored yolks. In addition, it is clear from this study that the inclusion of 4% marigold and 4% orange skin in the diet of layer diet had no harmful effect on egg weight.

The differences in feed conversion ratios of different dietary groups were not significant. The most efficient utilizer of feed was those birds which received 4% marigold and the lowest efficient group was the control group. Sikder *et al.* (1998) also found no significant difference in feed efficiency of laying hen fed either control 50% maize, 4% or 8% carrot meal. A similar non-significant response was also reported by Johir and Sharma (1979) with "duckweed"for chick starter or broiler ration at 10% level. It appears that the use of 4%

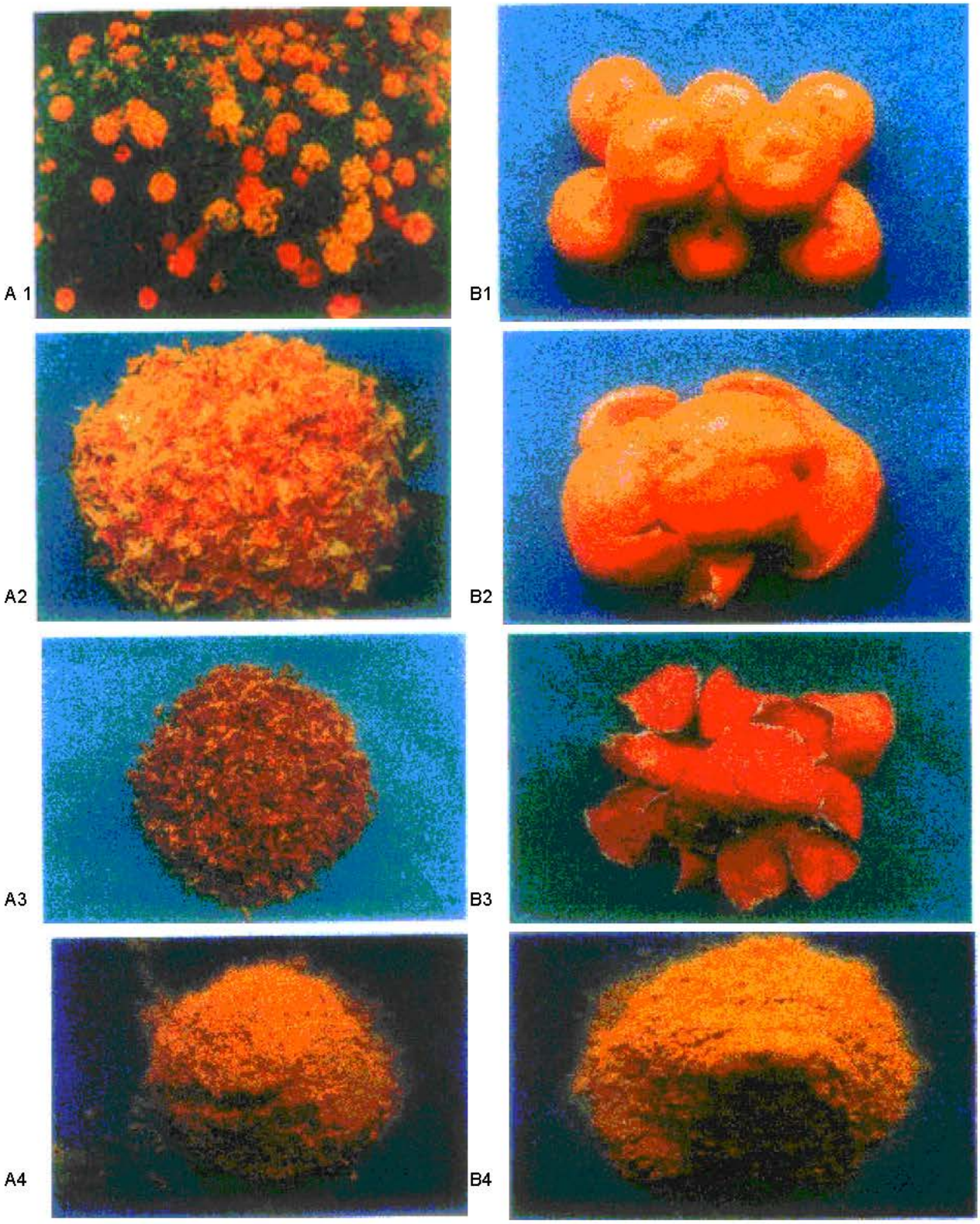

Plate 1: A_1) Marigold in garden, A2) Marigold ray florets before drying, A3) Marigold ray florets after drying, A4) Marigold ray florets after grinding, B1) Orange, B2) orange skin before drying, B3) Orange skin after drying, B4) Orange skin after grinding.

Table 7: The performance characteristics of laying pullets fed marigold or orange skin in the diets (1-6 week)

Variable	Dietary treatments			SED
	Control	4% Marigold	4% Orange skin	
Body weight (g/bird)	1624.0	1685.0	1609.0	38.64
Hen-day Production (%)	69.5	71.3	71.3	6.175
Feed consumption g/day/bird	119.0	118.0	119.0	0.660
Egg weight (g)	58.3	58.9	57.7	0.627
Feed conversion	3.2	2.8	2.9	0.261
Egg mass output egg/hen/day	37.7	42.2	41.3	3.589
Livability (%)	100	100	100	-

All variables showed non-significant differences (P>0.05)

Table 8: External egg quality characteristics of laying pullets fed either marigold or orange skin containing diets

Variables	Dietary treatment			SED
	Control	4% Marigold	4% Orange skin	
Sample egg weight (g)	61.8	61.8	62.8	2.78
Shape index	73.2	74.1	74.4	0.745
Shell dry weight (g)	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)	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

All variables showed non-significant differences (P>0.05)

Table 9: Internal egg quality characteristics of laying pullets and fed diets containing either marigold or orange skin

Variables	Dietary treatment			SED
	Control	4% Marigold	4% Orange skin	
Albumen index	0.087	0.087	0.081	0.006
Fresh albumen weight (g)	36.39	38.75	35.74	1.88
Dry albumen weight (g)	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)	14.56	14.31	14.56	0.379
Dry yolk weight (g)	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 non-significant differences (P>0.05)

marigold and 4% orange skin laying pullets diet did not influence feed conversion ratios of layers.

Birds fed marigold and orange skin showed slightly higher egg mass yield than control but the data did not differ significantly (P>0.05) from that of control. It indicates that the use of 4% marigold and 4% orange skin in the diet had no effect on daily egg mass output. Of course, it appears that slightly higher egg mass output in marigold and orange skin was probably a reflection of slightly higher egg production of those groups. Saha *et al.* (1998) conducted experiment with yellow corn and azolla meal separately they reported similar trends in the result for egg mass output.

All birds were healthy and there was no mortality in this experiment, which was supported by Sikder *et al.* (1998), Akhter (1995) and Hamid *et al.* (1993). This result clearly indicated that the use of 4% marigold and 4% orange skin in the diet had no effect on the health of laying birds.

External and internal quality characteristics of eggs: The results of external quality characteristics of eggs are shown in Table 8. It appears from the table that sample egg weight, shape index, dry shell weight, percent shell, shell thickness, dry membrane weight, percent membrane, and breaking strength of eggs laid by birds during 4 weeks of supplementation of 4% marigold and 4% orange skin in the diet did non vary significant (P>0.05). It indicates that different treatment groups in the diet as used in this study had no influence on external quality of eggs.

The results of internal quality characteristics obtained from experiment 1 are shown in Table 9. It appears from the table that albumen quality (albumen index, albumen weight and albumen dry index) and yolk qualities (yolk index, yolk weight, yolk dry matter and Haugh unit) except yolk color score did not vary significant (P>0.05). Previous experiments with yellow corn, carrot meal, azolla meal and duck weed meal reported similar trend

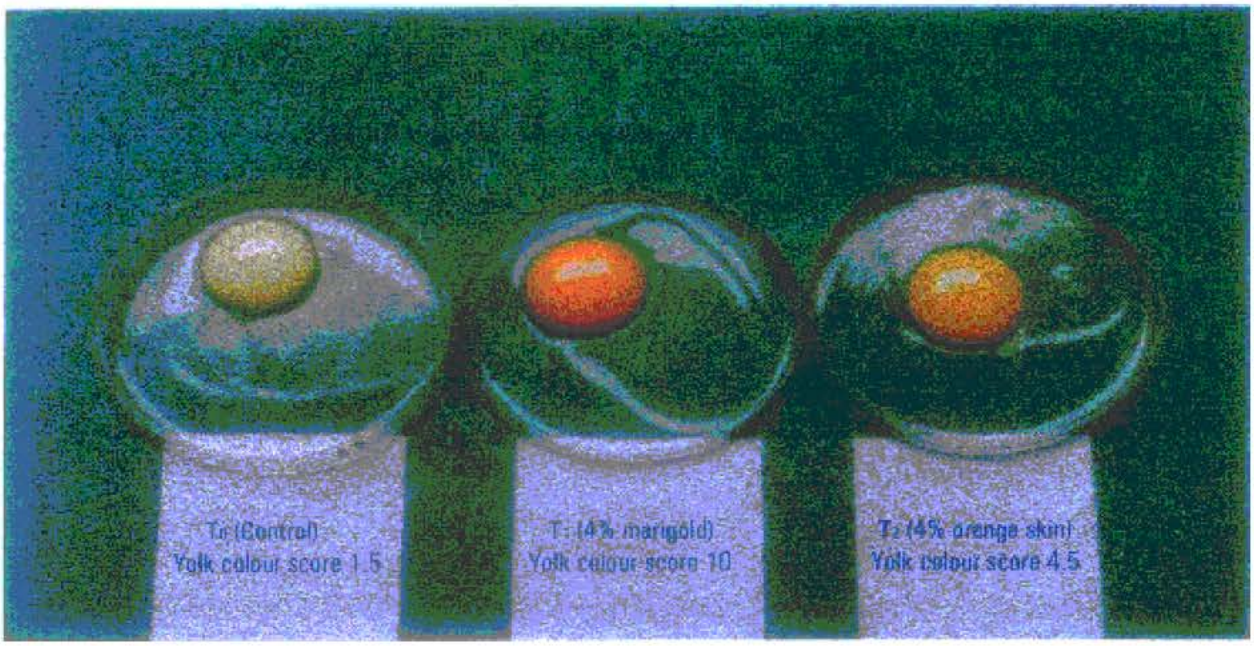

Plate 2: Improvement in yolk colour (scores represent the highest value) as affected by different dietary treatments after 4 weeks

in the results for both albumen and yolk quality characteristics of eggs (Saha *et al*., 1998; Sikder *et al*., 1998; Khaton *et al*., 1999 and Akhter, 1995).

Conclusions: Inclusion of marigold and orange skin in the diets of laying pullets did not cause deterioration in internal and external quality of eggs and there were no significant variation with respect to body weight, hen day egg production, egg weight and feed conversion. There was no mortality of birds during the study period as well. Use of 4% marigold meal in the diet of laying pullets can produce eggs with color score 11.0 close to 30 mg/kg synthetic pigment diet at 12th week of supplementation, whereas the birds that received 4% orange skin in the diet were able to produce eggs with color score 5.0 during 12th week of supplementation. So, marigold can be used more efficiently for egg yolk pigmentation.

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