**Effects on egg yolk colour of paprika or paprika combined with marigold flower extracts**

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**Abstract**

COLOR-UP® and COLOR-UP YELLOW-S® were fed to a total of 144 twenty-five-week-old Charoenn Pokphand brown laying hens to investigate whether yellow xanthophylls present in marigold flowers in combination with red xanthophylls from paprika fruit can further enhance egg yolk colour. The birds were randomly assigned to three groups: yellow corn basal diet control group (17.50 CP, 2750 kcal/kg ME); the 0.1% dietary paprika group; and the 0.1% dietary paprika plus 0.1% marigold group. Each group contained four replicates with 12 birds. Egg quality was recorded weekly from 25 to 28 weeks of age. Parameters did not show any difference except for egg yolk colour. Egg yolk colour scores were greater in the paprika group than in the control group (11.47 vs 8.64; P<0.05). Egg yolk colour scores of the paprika plus marigold group (12.17) were higher than those of the paprika group (P<0.05). Compared with the control, mean lightness value decreased in the paprika plus marigold group (P<0.05), suggesting a deep egg yolk colour in this group. Redness, yellowness and chroma were improved in both experimental groups (P<0.05). The spectral reflectance wavelength of egg yolk from the experimental groups increased between 600 and 700 nm, suggesting improved chroma. These results indicate that yellow xanthophylls from marigold flower extract (COLOR-UP® adds COLOR-UP YELLOW-S®) in combination with red xanthophylls present in paprika (COLOR-UP®) can further enhance egg yolk colour and chroma.

**Materials and methods**

**Birds and management**

In this study, COLOR-UP® (5 g/kg xanthophylls; Kohkin Chemical Co., Ltd.) was used as the paprika extract and COLOR-UP® YELLOW-S® (20 g/kg xanthophylls; Kohkin Chemical Co., Ltd.) was used as the marigold flower extract.

A total of 144 eighteen-week-old Charoenn Pokphand brown laying hens were obtained from a local commercial company and fed a commercial diet (16 CP, 2850 kcal/kg ME) ad libitum. At 25 weeks of age, chickens producing eggs were divided randomly into three groups: yellow corn basal diet (control group), 0.1% COLOR-UP® (paprika group), and 0.1% COLOR-UP® plus 0.1% COLOR-UP® YELLOW-S® (paprika plus marigold group). Each group had four replicates of 12 chickens. Each replicate was divided further into four large cages (40x40x36 cm), with three birds in each. The basal mash diet was formulated to meet the nutritional requirements for layers (National Research Council, 1994; Table 1). Pigment composition of yellow corn, paprika extract and marigold flower extract (Table 2; Japan Food Research Laboratories, Tokyo, Japan; Kohkin Chemical Co., Ltd., Osaka, Japan) was measured. Room temperature was maintained at a mean of 27°C by an evaporative cooling system. During the entire experimental period, 16 h of constant light was maintained daily. Until 28 weeks of age, egg quality was recorded weekly. Egg quality parameters were determined on randomly selected eggs from each replicate at the end of each week (12 eggs per treatment per week).

**Measurement of egg quality**

After its weight (W) was measured, the egg was cracked and its contents placed on a flat glass plate. Pigmentation of the egg yolk was measured visually using the RYCF scale (Roche Ltd., Basel, Switzerland), and colours were scored according to 15 sample colours ranging from 1 (the lightest) to 15 (the darkest). In addition, yolk colour, yellow index (Y), lightness (L*), redness (a*), yellowness (b*),...
chroma (C) and spectral reflectance of yolks were evaluated mechanically using a spectrophotometer (NF333, Nippon Denshoku Industries Co., Ltd., Tokyo, Japan). YI was calculated using the formula [\(YI=(128X-Yb)^{1/3}\)], where X, Y, and b are values obtained from the spectrophotometer. Chroma was evaluated using the formula [\(Ch=(a^2+b^2)^{1/2}\)]. Albumen height (H) was measured using a micrometer. Haugh units were calculated using a HU formula involving W in grams and H in micrometers (Eisen et al., 1962; Stadelman and Cotterill, 1977). The egg yolk was separated utilising an egg separator and the weight of the yolk, albumen and shell (with inner and outer shell membranes) was measured. Shell thickness (without shell membranes) was measured using a dial thickness gauge (Peacock, Tokyo, Japan) at three places (top, centre and bottom), and the mean of these three measurements represented shell thickness. These egg quality parameters were measured for 36 eggs per group.

Statistical analysis

Egg quality was statistically analysed using one-way analysis of variance of the Statistical Analysis System (SAS, 1997). Differences among treatments were tested using Tukey’s Studentised Range test at P<0.05 (Steel and Torrie, 1980).

Results and discussion

Egg yolk colour is known to be influenced mostly by the diet of the hen (Colin et al., 2004), and the main colour pigment source in conventional diets is yellow corn. Feeds with 19% yellow corn registered a score of 5.54 on the RYCF scale (Rowghni et al., 2006). Even with the present basal diet containing a high percentage of yellow corn (55.67%), yolk colour score was only 8.64. To score egg yolk colour, paprika and marigold flowers extracts were used because they are rich in xanthophylls (Yeum and Russel, 2002), paprika being a source of red xanthophylls and marigold a source of yellow xanthophylls. In the present study, paprika and paprika plus marigold flower extracts had no significant effects on Haugh units, egg weight, egg shell weight, albumen weight and shell thickness (Table 3). These findings agree with previous trials reporting no change in egg quality following paprika administration (Rowghni et al., 2006; Zhuye et al., 2008). Following paprika supplementation, yolk colour reached a score of 11.47, which was higher than the control score of 8.64 (P<0.05). It is well known that xanthophylls are absorbed from the intestinal tract of the chicken (Gouveia et al., 1996), and incorporated into triglyceride-rich lipoproteins (chylomicrons) that are released into the circulatory system (Salma et al., 2007) and transferred to the yolk (Donald and Williams, 2002). Because, in the current research, hens were fed the same basal diet with the exception of paprika extract, the enhanced yolk colour in the paprika group may have been induced as a result of xanthophylls being deposited in the yolk. The present data are consistent with previous results, indicating that paprika extract significantly improved egg yolk colour (Santos-Bocanegra et al., 2004; Niu et al., 2008). An RYCF score of 11.47 in the paprika group corresponded to a yellow to pale red colour. This score should not be considered as optimal because consumers prefer a red yolk colour. However, red yolk colour was obtained in the paprika plus marigold group whose egg yolk colour score (12.17) was significantly higher than that of the paprika group (P<0.05). This may have been induced by the higher content of xanthophylls in the marigold flower extract (32.51 mg) than that in the paprika extract (5.44 mg).

### Table 1. Composition of the basal diet.

| Parameters                      | Control | Paprika | Paprika-Marigold |
|--------------------------------|---------|---------|------------------|
| Ingredients, %                  |         |         |                  |
| Yellow corn                     | 55.67   |         |                  |
| Defatted rice bran oil          | 6.00    |         |                  |
| Soybean meal                    | 22.98   |         |                  |
| Fish meal                       | 5.00    |         |                  |
| Defatted rice bran oil          | 1.05    |         |                  |
| Oyster shell                    | 7.85    |         |                  |
| Dicalcium phosphate             | 0.45    |         |                  |
| Salt                            | 0.35    |         |                  |
| DL-methionine                   | 0.15    |         |                  |
| Premix*                         | 0.50    |         |                  |
| Total                           | 100     |         |                  |
| Chemical components°            |         |         |                  |
| ME, kcal/kg                     | 2750    |         |                  |
| Crude protein,%                 | 17.50   |         |                  |
| Crude fibre,%                   | 3.76    |         |                  |
| Crude fat,%                    | 8.20    |         |                  |
| Calcium,%                      | 3.51    |         |                  |
| Available phosphorus,%          | 0.35    |         |                  |
| Lysine,%                       | 0.96    |         |                  |
| Methionine,%                    | 0.75    |         |                  |

*Analysis by Japan Food Research Laboratories, 24 November 2009, No. 09023293001-01; °analysis by Kohkin Chemical Co., Ltd.

### Table 2. Pigment composition of yellow corn, paprika and marigold flower.

| Parameters                      | Yellow corn° | Paprika extract° | Marigold flower extract° |
|--------------------------------|--------------|------------------|--------------------------|
| Carotene, mg/g                  | 0.03         | 5.56             | 25.01                    |
| Xanthophylls, mg/g              | 0.04         | 5.44             | 32.51                    |
| Lutein, mg/g                    | 0.005        | -                | 22.56                    |
| Zeaxanthin, mg/g                | 0.01         | 0.54             | 1.65                     |
| Cryptoxanthin, mg/g             | 0.003        | -                | -                        |
| Trans-capsanthin, mg/g          | -            | 2.51             | -                        |
| cis-capsanthin, mg/g            | -            | 0.46             | -                        |

*Analysis by Japan Food Research Laboratories, 24 November 2009, No. 09023293001-01; °analysis by Kohkin Chemical Co., Ltd.

### Table 3. Effects of paprika or paprika plus marigold flower extracts on egg quality of laying hens during three weeks from 25 to 28 weeks of age (Mean±SE; n=30).

| Parameters                      | Control | Paprika | Paprika-Marigold |
|--------------------------------|---------|---------|------------------|
| Dietary                        |         |         |                  |
| Haugh units                     | 96.75±0.95  | 96.91±1.49  | 96.66±0.69       | 0.524       |
| Egg weight, g                   | 53.92±1.13  | 53.29±0.73  | 51.95±1.18       | 0.219       |
| Egg shell weight, g             | 7.45±0.21   | 7.48±0.16   | 7.58±0.17        | 0.677       |
| Albumen weight, g               | 13.09±0.28  | 12.73±0.32  | 12.67±0.28       | 0.618       |
| Yolk colour                     | 33.38±0.77  | 33.08±0.47  | 31.71±0.88       | 0.187       |
| Shell thickness, mm             | 8.64±0.13   | 11.47±0.28  | 12.17±0.18       | 0.022       |

*Means within a row with no common superscript differ significantly (P<0.05).
Visual yolk colour is the result of three colourimetric variables: hue, \( L^* \) and chroma. Table 4 shows the objective values of yolk colour, YI, \( L^* \), \( a^* \), \( b^* \) and chroma. Although the RYCF score of the paprika group was lower than that of the paprika plus marigold group when measured mechanically by the spectrophotometer, both scores were higher than that of the control (\( P<0.05 \)). However, YI was similar to yolk colour change as measured by the RYCF scale; YI of the paprika plus marigold group was higher than that of the paprika group (121.867 vs 113.370; \( P<0.05 \)). Compared with the control group, the mean \( L^* \) value tended to decrease in the paprika group and was significantly lower in the paprika plus marigold group (\( P<0.05 \)). This resulted in a deeper yolk colour in the paprika plus marigold group. The values of \( a^* \), \( b^* \) and chroma were higher in the paprika group than in the control group (\( P<0.05 \), and tended to increase further in the paprika plus marigold group. These results correspond to those of a previous study, which reports that the \( L^* \) value decreases but the yolk colour scores on the RYCF scale, \( a^* \) and \( b^* \) elevate when dietary paprika levels are increased (Niu et al., 2008).

Figure 1 and Figure 2, respectively, represent the spectral reflectance from the egg yolk at each wavelength and illustrations of egg yolk colour in the control, paprika, and paprika plus marigold groups. At wavelengths between 600 nm (yellow colour) and 700 nm (red colour), both experimental groups showed higher reflectance, suggesting increased chroma in these groups. The results indicate that red yolk colour in laying hens’ eggs may be improved by providing both dietary supplementary marigold flower and paprika extracts.

### Conclusions
Yellow xanthophylls present in marigold flower extracts (COLOR-UP YELLOW-S\(^\circledast\)) can further enhance egg yolk colour and chroma. This has been achieved already using red xanthophylls of paprika (COLOR-UP\(^\circledast\)).

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| Parameters                  | Dietary                        | \( P \) |
|-----------------------------|--------------------------------|--------|
| Yolk colour \( Y_l \)       | Control 8.167±0.211\(^b\)      |        |
|                             | Paprika 12.167±0.167\(^a\)     |        |
|                             | Paprika+Marigold 12.750±0.171\(^a\) |        |
| Yellow index, YI\(^1\)      | Control 90.043±2.987\(^c\)     |        |
|                             | Paprika 113.370±1.727\(^b\)    |        |
|                             | Paprika+Marigold 121.867±1.873\(^a\) |        |
| Lightness, \( L^* \)        | Control 45.193±0.297\(^a\)     |        |
|                             | Paprika 43.583±0.750\(^b\)     |        |
|                             | Paprika+Marigold 42.317±0.741\(^b\) |        |
| Redness, \( a^* \)          | Control -0.365±0.216\(^b\)     |        |
|                             | Paprika 8.980±0.216\(^a\)      |        |
|                             | Paprika+Marigold 10.033±1.448\(^a\) |        |
| Yellowness, \( b^* \)       | Control 36.755±1.653\(^b\)     |        |
|                             | Paprika 42.120±1.054\(^a\)     |        |
|                             | Paprika+Marigold 46.735±1.293\(^a\) |        |
| Chroma, \( C^2 \)           | Control 36.760±1.654\(^b\)     |        |
|                             | Paprika 43.072±1.036\(^a\)     |        |
|                             | Paprika+Marigold 47.807±1.313\(^a\) |        |

\(^a,b,c\) Means within a row with no common superscript differ significantly (\( P<0.05 \)). \(^1\) Yellow index (YI) = (128X-106Z)/\( Y \) where \( X \), \( Y \) and \( Z \) are values obtained from spectrophotometer. \(^2\) Chroma (C) = \((a^*^2+b^*^2)^{1/2}\).

Figure 1. Effects of paprika or paprika plus marigold flower extracts on spectral reflectance wavelength from egg yolk.

Figure 2. Examples of egg yolk colour in the three groups: control (A), paprika extract (B) and paprika plus marigold flower extract (C).
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