Pigmentation of Egg Yolk with Yeast Phaffia rhodozyma Containing High Concentration of Astaxanthin in Laying Hens fed on a Low-Carotenoid Diet

Yukio Akiba1, Kan Sato1, Kazuaki Takahashi1, Yoko Takahashi1, Akemi Furuki1, Shigeru Konashi1, Hiroshi Nishida1, Hiroshi Tsunekawa2, Yutaka Hayasaka2 and Hidenori Nagao2

1 Laboratory of Animal Nutrition, Graduate School of Agricultural Science, Tohoku University, Sendai-shi 981-8555
2 Mercian Corporation, Tokyo 104-8305, Japan

The red-pigmented yeast, Phaffia rhodozyma, containing high concentration of astaxanthin (Ax) was evaluated as a dietary pigment source for egg yolk of laying hens fed on a low-carotenoid diet. Diets supplemented with the yeast to provide 0 to 16ppm Ax were fed for 4 weeks, while canthaxanthin (Cx), a synthetic pigment source, was added to diets at 0 to 1.5ppm as the comparing counterpart in 2 experiments. Roche color fan score and a* value on Chroma Meter increased linearly with the increase of logarithmic concentration of dietary Ax and Cx. Cx (Carophyll Red®) was 4 to 5 times as potent as Ax in the yeast for the pigmentation of egg yolk probably because of low digestibility of the yeast. Ax concentrations in plasma and egg yolk increased with dietary Ax concentration and the increases paralleled with the pigmentation of egg yolk. These results suggest that Phaffia yeast containing high concentration of Ax has the potential as natural pigment source for egg yolk of laying hens.


Key words: yeast, astaxanthin, pigmentation, egg yolk, laying hens

Introduction

Visual appearance, especially color, of egg yolks is of major concern to consumers as well as food processors. Since in poultry β-carotene is almost completely converted to vitamin A or is otherwise metabolized, oxycarotenoids (xanthophylls) in feed ingredients play a major role in pigmentation of egg yolk (Karunajeeva et al., 1984). The primary xanthophyll sources have been corn, corn gluten meal and dehydrated alfalfa meal (Bailey and Chen, 1989) which contain lutein, zeaxanthin and cryptaxanthin as the major carotenoids. Otherwise concentrated pigments from natural sources such as marigold and paprika as well as stabilized synthetic carotenoids are efficiently utilized for pigmenting egg yolk (Belyavin and Marangos, 1987).

The preference of consumers for yolk color vary from country to country. While most efforts have concentrated on yellow pigmenting substances, some naturally occurring red substances have been used for modifying yolk color (Lai et al., 1996). Astaxanthin (Ax) isolated from a number of birds, crustaceans and plants has been regarded as a natural occurring carotenoid which imparts red coloration (Schidt et al.,...
A red-pigmented yeast, Phaffia rhodozyma, contains high concentration of Ax has been found to be an excellent dietary pigment source to produce pink flesh in salmonids (Andrewes et al., 1976; Johnson et al. 1977; Johnson and Lewis, 1979). Egg yolks of laying quails and chickens could also be pigmented by feeding Phaffia yeast (Johnson et al., 1980). Dike et al. (1992) noted that feeding laying chickens with 0.3% Phaffia yeast raised Roche color fan value only by 1 point. However, pigmentation potential of Phaffia yeast which has been genetically selected toward high Ax concentration has not been ascertained in laying chickens. We report here the pigmentation of egg yolk and Ax concentrations in plasma and yolks in laying chickens fed on low-carotenoid diet with graded levels of Phaffia yeast.

**Materials and Methods**

*Birds and diets*

White Leghorn hens were housed individually in cages. These pullets were randomly assigned into 6 or 7 groups with 2 replicates of 8 birds each and had free access to experimental feed and water at all times. A daylength of 14 hours was provided. Low-carotenoid diet shown in Table 1 was given to all the pullets for 7 days prior to the start of each experiment following feeding a commercial-type diet containing yellow corn as a pigment source.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn, yellow</td>
<td>10.00</td>
</tr>
<tr>
<td>Grain sorghum</td>
<td>20.00</td>
</tr>
<tr>
<td>Wheat</td>
<td>39.83</td>
</tr>
<tr>
<td>Defatted rice bran</td>
<td>3.00</td>
</tr>
<tr>
<td>Soybean meal (46% protein)</td>
<td>11.88</td>
</tr>
<tr>
<td>Fish meal (60% protein)</td>
<td>3.71</td>
</tr>
<tr>
<td>Yellow grease</td>
<td>2.50</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.14</td>
</tr>
<tr>
<td>Calcium phosphate (tribasic)</td>
<td>1.03</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>7.58</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.16</td>
</tr>
<tr>
<td>Vitamin mixture¹</td>
<td>0.20</td>
</tr>
<tr>
<td>Calculated analysis</td>
<td></td>
</tr>
<tr>
<td>Energy, ME kcal/g</td>
<td>2.80</td>
</tr>
<tr>
<td>Protein, %</td>
<td>16.0</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>3.50</td>
</tr>
<tr>
<td>Phosphorus, %</td>
<td>0.65</td>
</tr>
</tbody>
</table>

¹ Vitamin mix provided per kilogram diet: vitamin A, 4000 IU; vitamin D₃, 200 ICU; tocopherol acetate, 12 mg; thiamine chloride, 2 mg; riboflavin, 3 mg; niacin, 9 mg; calcium pantothenate, 3 mg; folic acid, 0.1 mg; vitamin B₁₂, 0.06 mg; and choline chloride, 150 mg.
Experiments 1 and 2

In experiment 1, 96 hens aged 196 days were divided into 6 groups. The treatments consisted of 5 levels of Phaffia yeast (0, 2, 4, 8 and 16 ppm based on the Ax concentration) and one level (1 ppm) of canthaxanthin (Cx, Carophyll Red®). All 6 experimental diets were fed to the pullets in the mash form for 4 weeks.

In experiment 2, 112 hens aged 238 days were randomized into 7 dietary treatments. Phaffia yeast was added at 3 levels with 1.5, 3 and 6 ppm based on the Ax concentration. Cx (reagent-grade) was added at 0.375, 0.75 and 1.5 ppm in diets. Control group received a diet with no pigment source. The feeding schedule was essentially the same as in experiment 1.

Phaffia yeast and canthaxanthin

Yeast, Phaffia rhodozyma, fermented in a jar were freeze-dried at a room temperature. The yeast contains 22.4% protein, 26.9% fat, 3.6% ash, 4.7% water and 4.58 mg phosphate/g. The total carotenoid and Ax concentrations were 3,521 and 2,340 ppm. Thus the yeast was supplemented at 0, 0.064, 0.085, 0.128, 0.171, 0.256, 0.342 and 0.683% in the low-carotenoid diet so as to give 0, 1.5, 2, 3, 4, 6, 8 and 16 ppm Ax in experiments 1 and 2. Carophyll Red® (Roche Japan, Tokyo, Japan) containing 10 g Cx/100 g was added to supply 1 ppm Cx in diet in experiment 1. In experiment 2, reagent-grade Cx (Sigma Chemical Company, St. Louis, MO, USA) was added in amount to supply 0.0375, 0.075 and 1.5 ppm Cx in diet.

Measurements

Body weight and feed consumption of laying hens were recorded every week for a total of 4 weeks. Eggs were collected daily and the weight was recorded. Blood was collected from wing vein with haparin, as anticoagulant, and centrifuged for 15 min at 1,500×g. The plasma was stored at -20°C for later analysis.

Yolk color analysis

For yolk color analysis, 2 eggs from 1 bird were collected during the last 4 days of the feeding period. The eggs were opened and the yolk was transferred to a disposable petri dish (60×15 mm). The intensity of yolk color was determined visually using a Roche color fan (VUILLEMIER, 1969). The lightness (L*), redness (a*) and yellowness (b*) of the yolk were measured using a Minolta CR-200 Chroma Meter (Minolta, Tokyo, Japan).

Analysis of carotenoids and trans-astaxanthin

Plasma (0.5 ml) was extracted with 0.5 ml acetone and agitated with 1.0 ml hexane followed by centrifugation for 10 min at 1,500×g. The extraction was made twice and the combined hexane layer was dried up in vacuo, dissolved into acetone and centrifuged for 10 min at 12,000×g. Egg yolk (0.5 g) was extracted with 4 ml acetone, centrifuged for 10 min at 1,500×g, the supernatant was concentrated, extracted with 0.4 ml acetone, centrifuged for 10 min at 12,000×g and filtrated. The supernatants were provided for high-performance liquid chromatography (Shimazu SPD-Å AV) using a column (YPC-Pack) with ODS-A-312. The solvent system consisted of CH₃CN : MeOH = 15 : 3 and CH₃CN : MeOH : water = 15 : 3 : 2. Samples were monitored at 474 nm for trans–Ax and 478 nm for carotenoids. The retention time was standardized by
Statistical analysis

In each experiment, a SAS application package was used for statistical calculations (SAS, 1982). Group data for multiple comparisons were analyzed by ANOVA using a general linear models procedure followed by Duncan's multiple range test. The data of egg yolk pigmentation in experiment 1 was subjected to regression analysis (REG Procedure of SAS) following transformation of dietary Ax concentration using natural logarithm. Statements of statistical significance were based on \( P < 0.05 \) otherwise indicated in the text and legends.

Results

Experiment 1

The average feed intake and body weight gain of 6 groups of pullets during the 28-day experimental period were 106 g/d and 55 g/28 days, respectively. Egg production rate and egg weight were averaged 94.8% and 55.9 g and not significantly different among treatments.

Yolk color evaluation and Ax and total carotenoids concentrations in plasma and yolk are presented in Table 2. Yolk color score (Y) estimated using Roch color fan

| Table 2. Effects of feeding Phaffia yeast and canthaxanthin on egg yolk color and concentrations of astaxanthin and carotenoids in yolk and plasma of laying hens fed on a low-carotenoid diet* (Experiment 1) |
|---------------------------------|------------------|------------------|------------------|------------------|
|                                  | Control          | Phaffia yeast (Aastaxanthin, ppm) | Canthaxanthin (ppm) |
| Yolk color score (Y)            | 6.6±0.1a         | 7.7±0.1d         | 9.8±0.1c         | 11.5±0.1b        | 12.7±0.1a       | 9.9±0.1c         |
| Yolk color value (L* - a* - b*) | 54.2±0.4a        | 54.4±0.4a        | 52.9±0.3b        | 52.1±0.3b        | 50.7±0.5a       | 52.7±0.5a        |
|                                 | 1.8±0.1f         | 0.3±0.1f         | 2.3±0.2d         | 4.8±0.2b         | 9.0±0.4a        | 4.0±0.2c         |
|                                 | 35.8±0.9a        | 29.4±0.1d        | 34.7±0.8ab       | 32.2±0.6bc       | 31.9±1.0b       | 34.3±0.8abc      |
| Yolk (µg/g)                     | ND               | 0.39±0.04d       | 0.82±0.12c       | 1.57±0.42b       | 3.38±0.46a      | —                |
| Astaxanthin (µg/ml)             | 13.8±2.1b        | 14.7±2.2a        | 15.3±1.9ab       | 16.0±2.4ab       | 18.6±2.5c       | 19.8±4.2a        |
| Plasma (µg/ml)                  | 0.022±0.011d     | 0.087±0.042c     | 0.128±0.076c     | 0.217±0.062b     | 0.362±0.101a    | —                |
| Astaxanthin (µg/g)              | 1.46±0.62        | 1.23±0.37        | 1.56±0.83        | 1.70±0.31        | 2.09±0.57       | 2.10±0.60        |

1) Body weight at commencement of the experiment was 1.70±0.03 kg.
2) Carophyll Red.
3) Yolk color score was estimated with aid of Roche color fan. Mean±SD with 32 observations.
4) Yolk color value was determined by Chroma Meter. Mean±SD with 32 observations.
5) Mean±SD with 6 observations.
6) Trans-astaxanthin.
7) ND: Not detected. -: Not analyzed.
8) Means with different superscripts in row are significantly different (P<0.05).
increased linearly ($Y = 5.567X + 6.25, r = 0.993, P < 0.001$) with the logarithmic concentration of Ax ($X$) in diets. Yolk color score of 1 ppm Cx fed group was significantly higher than the control counterpart and roughly comparable to that of 4 ppm Ax fed group. L* value on Chroma Meter measurements tended to decrease with the increase of dietary Ax concentration. Redness ($a^*$ value) of yolk increased in a same manner with the yolk color score and significant increase was detected in hens fed diet with 2 ppm Ax. The significant increase of $a^*$ value was observed in Cx (Carophyll Red®) fed hens. Yellowness ($b^*$ value) of yolk tended to decrease in groups receiving Ax as compared with control group while that in Cx fed group was comparable to the control. Trans-Ax concentrations in plasma and egg yolk increased linearly ($P < 0.01$) with increase of Ax content in diets and attained 0.36μg/ml and 3.38μg/g, respectively, when hens received diet with 16 ppm Ax. Total carotenoid concentrations in plasma and egg yolk were in a trend to increase with addition of dietary pigments.

**Experiment 2**

The average feed intake and body weight gain of the 6 groups of pullets during the 28-day experimental period were 108 g/d and 64 g/28 days, respectively. Egg production rate and egg weight were averaged 93.5% and 59.0 g and not significantly different among treatments.

Yolk color score and redness ($a^*$ value) increased linearly ($P < 0.01$) with the

<table>
<thead>
<tr>
<th>Table 3. Effects of feeding Phaffia yeast and canthaxanthin on egg yolk color and concentrations of astaxanthin and carotenoids in yolk and plasma of laying hens fed on a low-carotenoid diet1 (Experiment 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Yolk color score2</td>
</tr>
<tr>
<td>Yolk color value3</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Yolk (μg/g)5</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Total carotenoids</td>
</tr>
<tr>
<td>Plasma (μg/ml)6</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

1 Body weight at commencement of the experiment was 1.75±0.04 kg.
2 Yolk color score was estimated with aid of Roche color fan. Mean±SD with 32 observations.
3 Yolk color value was determined by Chroma Meter. Mean±SD with 32 observations.
4 Mean±SD with 6 observations.
5 Trans-astaxanthin.
6 ND: Not detected. —: Not analyzed.

Means with different superscripts in row are significantly different ($P < 0.05$)
logarithmic concentrations of Ax or reagent-grade Cx in diets (Table 3). Yolk color score of 1.5 ppm Cx (reagent-grade Cx) fed group was significantly higher than the control counterpart and roughly comparable to that of 1.5 ppm Ax fed group. No significant differences among treatments were observed in lightness (L* value). Yellowness (b* value) of yolk in Cx fed groups were higher than that in Ax fed groups. Trans-Ax concentrations in plasma and egg yolk increased by Ax addition. Significant increases of Cx concentration in plasma and yolk were observed in hens fed diet with 1.5 ppm Cx. There were no significant differences among treatments in total carotenoid concentrations in the egg yolk.

Discussion

The present data showed that pigmentation of egg yolks linearly increased with increase in logarithmic concentration of dietary Ax (1.5–16 ppm) in laying hens fed on a low-carotenoid diet. Roche color fan score was low (6.6–6.8) in the control hens because of feeding of low-carotenoid diet and significantly raised by feeding Phaffia yeast even when the dietary Ax concentration was 1.5 ppm. DIKE et al. (1992) noted that feeding laying chickens with 0.3% Phaffia yeast for 8 months increased Roche color fan score only by 1 point. JOHNSON et al. (1980) showed that dominant wavelength, the main measurement parameter of color, increased from 572 nm to 592 nm in egg yolk of laying hens fed on a diet containing 20 ppm Ax. Our data evidenced that the least dietary Ax concentration for pigmenting egg yolk was approximately 1.5 ppm when Phaffia yeast was provided as the Ax source and the linear increase of redness (a*) value with logarithmic concentration of dietary Ax substantiated the pigmenting potential of Phaffia yeast. On the other hand, yellowness (b* value) in the Ax fed groups were relatively low as compared to the control group, whereas b* value tended to increase in hens fed Cx. This is due to differences in pigmenting characteristics that Ax causes originally red color while Cx causes yellow as well as red color. NELSON and BAPTIST (1968) reported that lutein combined with added Ax was more effective than lutein alone in the pigmentation of egg yolk.

The pigmentation (Roche color fan score and a* value) of egg yolks was increased with an increase of Cx concentration in diets in experiment 2. However, the extent of pigmentation of Cx in experiment 2 was less than that in experiment 1. As reagent-grade Cx was used in experiment 2, present results may show that the availability of Cx is dependent on the source in which the pigmentation of reagent-grade Cx is less potent than commercially available Cx (Carophyll Red®).

The linear regression equations were given between yolk color score (Y1) or a* value (Y2) and logarithmic concentration of dietary Ax (X) in experiment 1 (Y1 = 5.567 X + 6.25, r = 0.993, P < 0.001; Y2 = 9.533 X – 3.25, r = 0.985, P < 0.001). Based on the equations, pigmenting potential of Cx was estimated to be 4.4 times that of Ax for yolk color score and 5.5 times for a* value. It is inferable, therefore, that efficacy of Cx in the pigmentation is about 4 to 5 times that of Ax. Otherwise, since digestibility of yeast has been understood to be low because of the hard cell wall, the low pigmenting efficiency of Ax may be due to the low availability of yeasts in digestive tract of
chickens. In this context only 40.7% of Ax present in the Phaffia yeast was extracted by acetone in the present study.

Ax was transported through blood circulation and the plasma taken from hens 4 weeks after Phaffia yeast feeding contained Ax at 0.04 to 0.36 µg/ml. In a similar manner, plasma Cx concentration increased with an increase of dietary Cx concentration. Schaeffer et al. (1988) noted that serum lutein concentration was 1.5 µg/ml in hens fed 20 ppm lutein for 4 weeks. Nelson et al. (1990) noted that serum xanthophyll concentrations did not exhibit significantly diurnal variations in laying hens. Ax accumulated in eggs with an increasing manner (ranging from 0.4 µg to 3.4 µg/g yolk) in response to the increase of dietary Ax level which is similar to yolk Cx concentration in the present study. These increase in yolk Ax or Cx concentrations paralleled with increases in Roche color fan score and a* values on Chroma Meter, substantiating the observation that the degree of yolk pigmentation is due to the dietary carotenoid concentration. On an assumption that yolk accounts for 30% of total egg weight, Ax in egg yolk amounted to 25 µg against 691 µg in daily trans-Ax intake in laying hens fed a diet with 8 ppm Ax, implying that the deposition efficiency is about 3.6% in the present study. This is in a good accordance with report of Johnson et al. (1980) that the deposition efficiency of carotenoids in Phaffia yeast into egg yolk was 3 to 4% in laying quails. The low deposition efficiency of Ax in Phaffia yeast may be partly due to the low digestibility of yeasts because of the hard cell wall. Hencken (1992) reported, on the other hand, that the deposition in egg yolks was 30 to 45% for Cx, 14% for Ax and 25% for zeaxanthin in laying hens. Balnave and Bird (1996) noted that Cx was deposited with an efficiency of 38% in laying hens. This conflict findings awaits further investigation.

Acknowledgment

The authors thank Michishige Harada, Hiroshi Mori and Katsumi Suzuki for technical assistance.

References


Hencken, H. (1992) Chemical and physiological behavior of feed carotenoids and their effects on
低カロチノイド飼料を給与した産卵鶏における高濃度のアスタキサンチンを含む赤色酵母（Phaffia rhodozyma）
給与による卵黄着色

秋葉征夫1・佐藤　幹1・高橋和昭1・高橋洋子1・古木明美1・小梨　茂1・
西田浩志1・恒川　博2・早坂　豊2・長尾秀則2

1 東北大学大学院農学研究科、仙台市青葉区　981-8555
2 メルシャン株式会社、東京都中央区　104-8305

低カロチノイド飼料を給与した産卵鶏を供試し、カロチノイドの一種であるアスタキサンチンを高濃度に含む
赤色酵母の卵黄着色効果を検討した。飼料中のアスタキサンチン濃度が0から16 ppmになるように赤色酵母を
添加した飼料およびカスタキサンチン（Carophyll Red® および試薬用）を0から1.5 ppm含む飼料を産卵
鶏に4週間給与した。

ロッシュカラーファンスコアおよびChroma Meter
によるa*値はアスタキサンチンおよびカスタキサンチンの添加濃度にしたがって有意に上昇した。また、ロッ
シュカラーファンスコア（Y1）およびa*値（Y2）と飼
料中アスタキサンチン濃度（log 値）の間に正の有意の
一次回帰式が求められた（Y1 = 5.567X + 6.25, Y2 =
9.533 X − 3.25)。これらの回帰式からカタキサンチン
（Carophyll Red®）の卵黄着色効果は赤色酵母中のアス
タキサンチンの約4-5倍と計算されたが、これは酵母
の消化性が低いことが関与しているものと推定された。
血漿中および卵黄中のトランス型アスタキサンチン濃度
も飼料中濃度にしたがって上昇し、その上昇は卵黄の着
色効果と比例していた。

以上の結果より、赤色酵母は天然の卵黄着色に飼料と
して有用であることが明らかとなった。

（家禽会誌，37：77-85，2000）

キーワード：赤色酵母，アスタキサンチン，卵黄着色，
産卵鶏