# RELATIVE EFFICIENCIES OF YELLOW CAROTENOIDS FOR EGG YOLK PIGMENTATION

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## Summary

The relative efficiencies of deposition into egg yolk of apo-carotenoic acid ester (APO-E, CAROPHYLL Yellow) and saponified marigold xanthophylls (MX), in the presence of canthaxanthin (CN), were determined using a wheat-based diet. APO-E was deposited with an efficiency of 50 percent and MX with an efficiency between 13 and 20 percent. The dose response relationship for MX was curvilinear with a decreased efficiency at higher concentrations. Canthaxanthin was deposited with an efficiency of 38 percent, irrespective of the source of yellow xanthophyll, up to a dietary concentration of 5.5 mg/kg. At a dietary MX concentration of 8.3 mg/kg the efficiency of deposition of CN declined to 24 percent. The results confirm that the replacement ratio of MX : APO-E is between 3 : 1 and 4 : 1 depending on the dietary inclusion of marigold pigment.

(**Key Words** : Yolk Pigments, Apo-Carotenoic Acid Ester, Marigold Xanthophylls, Canthaxanthin)

## Introduction

Egg yolk colour has long been recognized as one of the most important characteristics of egg quality as judged by consumers of table eggs and products containing eggs (Strieff, 1970; El Boushy and Raterink, 1987). The characteristic golden yellow colour of egg yolks is due to the selective accumulation of carotenoid pigments. However, poultry do not have the ability to synthesize carotenoids *de novo* and accumulated carotenoids are of dietary origin.

There are two distinct phases to commercial pigmentation of eggs to ensure that the yellow/orange colour demanded by consumers is achieved economically and consistently. The first phase is the accumulation of a level of yellow xanthophylls in the yolk approaching visual saturation. During this phase the relative degree of accumulation of the carotenoids is crucial for the achievement of the most economical means of pigmentation. The second aspect of commercial egg yolk pigmentation is a colour phase achieved by including red carotenoids to deepen the colour to yellow/orange. During this phase the rate of accumulation and the colour hue are crucial to acceptable colouration. Usually there are sufficient yellow carotenoids in maize-based rations (>30-35% maize) to satisfy the first saturation phase and these rations normally do not require the addition of yellow carotenoid supplements. Red carotenoids may be used alone to deepen the yolk colour in line with consumer demand (Bird, 1994). In contrast, wheat and barley contain only minimal concentrations of yellow xanthophylls so that a blend of yellow and red carotenoids must be added to the diets of commercial laying hens fed wheat- or barley-based diets.

The two most commonly used sources of yellow carotenoids for egg yolk pigmentation are the chemically synthesized $\beta$-8-apo-carotenoic acid ethyl ester (APO-E) and saponified extracts of marigold flower petals (*Tagetes erecta*). Previous reports have indicated the relative biological efficiency of marigold xanthophylls (MX) as 17-33 with apo-carotenoic acid ester (APO-E) set as standard 100 (Huyghebaert, 1991). Canthaxanthin (CN) is the most common red carotenoid used to enhance egg yolk pigmentation. Numerically the deposition of CN into the yolk has been reported as being 31% of that consumed when 4 mg/kg was supplied in the diet (Schiedt et al., 1987).

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In the present investigation the relative effectiveness of commercial yellow xanthophylls (APO-E and Marigold) under Australian feeding conditions using a wheat-based diet was studied. Analytical determination of the accumulation of the individual carotenoids into the egg yolk was measured using high pressure liquid chromatography.

## Materials and Methods

Sixty, 40-week-old Tegel Super Brown pullets were randomly allocated to individual cages in a commercial layer shed and 15 pullets were fed each of four diets containing wheat as the major cereal. The diet was formulated using (g/kg) wheat (436), sorghum (300), soyabean meal (49), meat and bone meal (100), animal tallow (30), limestone (76), sodium chloride (2), L-lysine (0.9), DL-methionine (1.3) and a vitamin and mineral premix (5). The composition of the premix was given by Usayran and Balnave (1995). The diet contained 160 g crude protein and 11.5 MJ of ME/kg. Fifteen pullets were each fed this diet containing one of four pigment blends (table 1). Diet 1 (Control) contained 50 mg/kg of a commercial blend of CAROPHYLL Yellow (APO-E) and CAROPHYLL Red (CN) in a ratio of 1.28 : 1 providing 2.8 mg/kg of APO-E and 2.2 mg/kg of CN (Colborn Dawes Pty Ltd.). The other three diets were supplemented with differing concentrations of saponified MX (Cromophyl-Oro; Laboratorios Bioquimex S. A.) and CAROPHYLL Red to supply 2.2 mg CN/kg. The concentrations of MX were 2.8 mg/kg (Diet 2), 5.5 mg/kg (Diet 3) and 8.3 mg/kg (Diet 4).

TABLE 1. CAROTENOID COMPOSITION OF DIETS (mg/kg)

| Treatment | Apo-ester | Marigold Xanthophylls | Canthaxanthin |
|---|---|---|---|
| Diet 1 | 2.8 | – | 2.2 |
| Diet 2 | – | 2.8 | 2.2 |
| Diet 3 | – | 5.5 | 2.2 |
| Diet 4 | – | 8.3 | 2.2 |

These diets were fed *ad libitum* for 22 days during which time egg production, egg weight and feed intake were measured. On day 22 six eggs were collected at random from each treatment and analysed for APO-E, total yellow xanthophylls (lutein plus zeaxanthin) and CN using standard HPLC methods (Manz, 1983).

## Results and Discussion

Mean daily feed intakes over the 3-week study were 132, 129, 123 and 130 g/d, respectively, for pullets fed Diets 1-4. Corresponding mean daily egg mass outputs were 52, 53, 51 and 52 g/d. Two eggs from pullets fed Diet 2 and one egg from pullets fed Diet 4 were broken prior to analyses. The carotenoid contents of the remaining eggs are shown in table 2.

TABLE 2. CAROTENOID CONTENT ($\mu$g/g) OF YOLKS (Mean ± SD)

| Treatment | Apo-ester | Xanthophylls | Canthaxanthin |
|---|---|---|---|
| Diet 1 | 10.8±2.4 | – | 6.5±1.3 |
| Diet 2 | – | 4.4±1.2 | 6.7±2.4 |
| Diet 3 | – | 6.9±1.3 | 6.6±1.6 |
| Diet 4 | – | 8.6±2.3 | 4.2±1.9 |

APO-E was deposited more efficiently in egg yolk than were the MX. There was a dose-related increase in the xanthophyll content of the egg yolks laid by pullets fed the marigold pigment. However, even at the highest dosage (8.3 mg/kg) the concentration did not exceed that of the control birds receiving the APO-E in Diet 1. The APO-E : CN ratio in the egg yolk of pullets fed Diet 1 was 1.66 : 1 compared with the dietary ratio of 1.28 : 1 indicating a slightly greater efficiency in the deposition of the APO-E compared to CN. At the similar dietary xanthophyll : CN ratio in Diet 2 the corresponding ratio in the egg yolk was only 0.66 : 1 indicating a much lower efficiency of deposition of MX relative to APO-E.

Extrapolation of the dose-response of the MX to the dietary APO-E concentration of 2.8 mg/kg indicates that for an equivalent degree of yolk pigmentation approximately 4 g of MX were required to replace 1 g of APO-E. However, it is evident that the efficiency of deposition of MX declined with increasing dietary concentration. At typical dietary inclusion levels of 4-5 mg yellow xanthophylls/kg the ratio approaches 3 : 1 given the more effective utilization of MX at lower dietary concentrations. This conclusion agrees with the results of other studies which suggest that for equivalent egg yolk pigmentation between 3 and 4 g of MX are required to replace 1 g of APO-E in the feed (Huyghebaert, 1991). The CN concentration in the egg yolks was approximately three-fold that of the diets except for Diet 4 where a two-fold increase was observed.

Deposition rates for the yellow xanthophylls and CN were calculated as the percentages of consumed carotenoids which were deposited in egg yolk. Individual yolk weights and an average feed intake of 146 g for each egg produced were used in this calculation. The results are shown in figures 1 and 2.

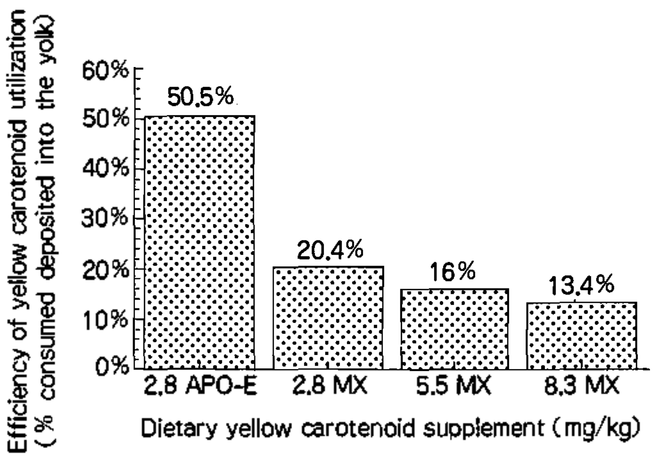


Figure 1. Efficiency of the utilization of marigold xanthophylls (MX) and apo-carotenoic acid ethyl ester (APO-E) by laying hens.

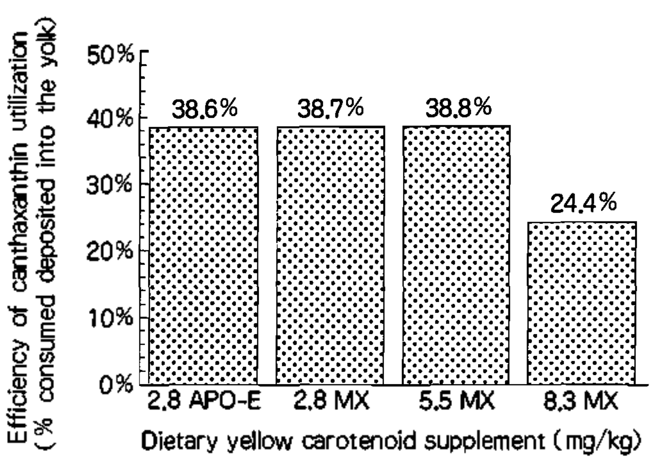


Figure 2. Efficiency of the utilization of canthaxanthin by laying hens supplemented with either apo-carotenoic acid ethyl ester (APO-E) or marigold xanthophylls (MX) in the feed.

APO-E was the most efficiently deposited carotenoid with 50 percent of the pigment consumed being deposited in the yolk. MX were less efficiently deposited at between 13 and 20 percent. CN showed an intermediate efficiency at 38 percent for Diets for Diets 1-3 and 24 percent for Diet 4. This implies that the deposition of CN was unrelated to the source of yellow xanthophyll at dietary concentrations up to 5.5 mg/kg. However, its utilization may be impaired at higher dietary concentrations of MX.

These results indicate a replacement ratio of MX : APO-E of between 3 : 1 and 4 : 1 depending on the dietary inclusion rate of the marigold pigment. The efficiency of deposition of the CN may be reduced at higher dietary concentrations of MX.

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