Comparative Pigmentation Efficiency of High Dietary Levels of Apo-Ester and Marigold Extract on Quality Traits of Whole Liquid Egg of Two Strains of Laying Hens

F. Sirri,*[1] N. Iaffaldano,† G. Minelli,* A. Meluzzi,* M. P. Rosato,† and A. Franchini*

**Department of Food Science, Alma Mater Studiorum, University of Bologna, 40126 Bologna, Italy; and †Department of Animal, Plant and Environmental Science, University of Molise, 86100 Campobasso, Italy*

Primary Audience: Nutritionists, Egg Producers, Egg Processors, Food Researchers

SUMMARY

This trial was carried out to compare the effect of the dietary supplementation of high doses of either synthetic pigment ethyl ester of β-apo-8′-carotenoic acid (apo-ester) or natural pigments, mainly lutein and zeaxanthin, extracted from *Tagetes erecta*, on egg quality of hens laying brown shell eggs (ISA Brown) and white shell eggs (Hy-Line White W-36). The hens of each strain were divided into 6 groups and fed a corn-soybean basal diet supplemented either with 40, 60, and 80 ppm of apo-ester (APO) or with 120, 180, and 240 ppm of marigold extract (MAR). Egg pigmentation rose linearly and significantly ($P < 0.01$) as the dietary levels of apo-ester increased, but this did not occur when MAR supplementation was used. The amount of β-carotene equivalents in whole liquid egg of MAR treatments was almost constant with varying pigment dietary dose and was significantly lower ($P < 0.01$) than in APO treatments. In both hen strains, whole liquid egg redness (a*) and yellowness (b*) were higher with APO supplementation. The egg component weights were highly affected ($P < 0.01$) by the hen strain, with yolk:egg ratio higher in the Hy-Line. The trial confirms that in spite of the higher level of MAR supplementation, APO has a better efficiency in whole liquid egg pigmentation. The ISA Brown hens showed a better ability to absorb dietary carotenoids than did the Hy-Line White.

Key words: apo-ester, marigold extract, egg quality, whole liquid egg, color, laying hen strain

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DESCRIPTION OF PROBLEM

The pigmentation of the egg yolk is a subject of practical importance for the egg-processing industry, which requires egg products with an appropriate pigmentation level and with a homogeneous distribution of the color to satisfy the demand of the food industry [1, 2].

The yolk color, as well as the broiler skin pigmentation, is related to the amount and type of pigments stored in animal products. In nature, the most widespread group of pigments is the carotenoids. They are represented by carotenes (hydrocarbons) and xanthophylls (oxycarotenoids), but only the latter have a coloring capacity in poultry [3]. Only plants and some microbes

[1]Corresponding author: fsirri@disa.unibo.it

can synthesize carotenoids. Animals, including poultry, absorb carotenoids from the diets [4, 5, 6] and store them after having modified their structure by oxidative metabolism [7, 8]. For this reason, high levels of natural or synthetic pigments are usually added to the diet of commercial layers as yellow or red xanthophylls to achieve the desired yolk color and make the eggs more appealing for consumers and more suitable for the egg-processing industry.

The main yellow pigments used in poultry feeding are marigold extracts and β-apo-8′-carotenoic acid ethyl ester (apo-ester). The first is a natural yellow saponified xanthophyll obtained from marigold flower extracts (*Tagetes erecta*), rich in yellow xanthophylls, mainly lutein and zeaxanthin [9]. It is an effective pigmenter, producing a highly acceptable yellow to yellow-orange color [10]. The apo-ester is obtained by chemical synthesis [11], but it is also isolated in maize [12]. It is well documented that apo-ester shows a better color efficiency than marigold extract. Different studies report that the pigmenting efficiency ratio between apo-ester and xanthophylls of *Tagetes* for egg yolk pigmentation is at least 3:1, irrespective of the physical characteristics of the marigold products and of their lutein:zeaxanthin ratio [6, 13, 14, 15, 16, 17, 18, 19].

The amounts and availability of carotenoids in poultry feed ingredients fluctuate considerably: levels of desired pigments in natural feedstuffs are not always constant, xanthophylls are unstable, and effective levels may decline as a result of denaturation during processing or oxidation during prolonged storage [20, 21].

Published reports on the effects of yellow sources of pigments, such as marigold extract and apo-ester, on egg yolk or whole liquid egg pigmentation are referred to low or moderate levels of dietary inclusion, whereas there is a lack of comparable literature on the efficacy of high dietary inclusions of pigments for the pigmentation of whole liquid egg. The only paper dealing with this topic tested the effects of dietary supplementation of apo-ester and marigold extract at 40 and 120 ppm, respectively [19]. Moreover, little attention has been paid to investigate the effect of these pigments when administered at very high concentrations for a long period on productive performances of hens belonging to different genetic strains. Therefore, the objectives of this trial were to compare the effects of long-term dietary supplementations to white or brown hens with high doses of either apo-ester or marigold extracts on whole liquid egg pigmentation, egg component weights and ratios, and hen productivity.

Table 1. Composition of the basal diet

Ingredients	g/kg
Corn (CP 8%)	555.8
Soybean meal (CP 47%)	248.1
Wheat (CP 12%)	93.3
Soybean oil	18.0
Limestone	56.9
Dicalcium phosphate	16.0
Sodium bicarbonate	3.4
Met	1.6
Choline chloride	2.7
Antioxidant (butylated hydroxytoluene)	0.2
Vitamin and mineral premix[1]	4.0
Calculated analysis	
DM	876.6
CP	169.9
Crude fat	44.4
CF	23.1
Ash	125.5
Ca	37.4
Available P	3.78
Natural xanthophylls[2] (mg/kg)	10.6
ME (kcal/kg)	2,830

[1]Provided the following per kilogram of diet: vitamin A (retinyl acetate), 11,000 IU; cholecalciferol, 3,000 IU; DL-α-tocopheryl acetate, 40 IU; menadione sodium bisulfite, 3.3 mg; riboflavin, 6.0 mg; pantothenic acid, 11.0 mg; niacin, 30 mg; pyridoxine, 4 mg; folic acid, 1 mg; biotin, 0.05 mg; thiamine, 2.5 mg; vitamin B_{12}, 20 μg; Mn, 15 mg; Zn, 50 mg; Fe, 30 mg; Cu, 6 mg; I, 1.5 mg; Se, 0.2 mg; and ethoxyquin, 100 mg.
[2]Determined concentration.

MATERIALS AND METHODS

Experimental Design

Ninety-six ISA Brown [22] and 96 Hy-Line White W-36 [23] laying hens (28 wk old) were housed in an environmental controlled poultry facility in conventional cages (550 cm^2/hen floor space) according to the European directive laying down minimum standards for the protection of laying hens (1999/74/CE). The hens of each strain were divided in 2 groups, both receiving the same corn-soybean basal diet for 12 wk (Table 1) supplemented either with 40 ppm of apo-

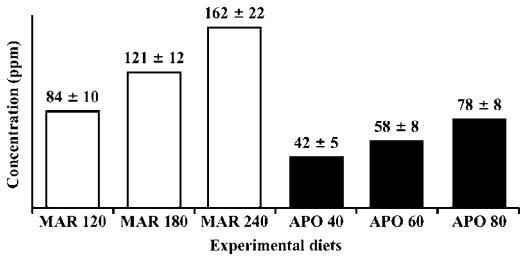


Figure 1. Mean values (± SD) of the analyzed concentrations of apo-ester (APO) and xanthophylls from marigold extract (MAR) of the experimental diets.

ester [24] (APO) or 120 ppm of marigold extract [25] (MAR) until the trial began.

At the age of 40 wk, the hens of each group were divided in 3 experimental subgroups of 4 replicates of 4 hens each for a total of 16 hens per experimental subgroup. The hens were fed the basal diet supplemented with 40, 60, and 80 ppm of apo-ester (APO 40, APO 60, APO 80) or 120, 180, and 240 ppm of marigold extract (MAR 120, MAR 180, MAR 240) calculated from the active substances and considering that it is widely accepted that the relative efficiency of apo-ester vs. marigold is 3:1. Feed and water were provided on an ad libitum basis throughout the 20-wk experiment.

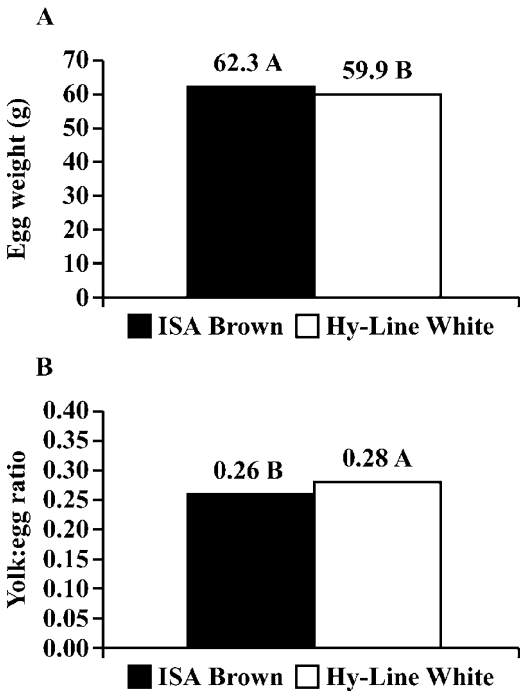


Figure 2. Effect of hen strain on egg weight and yolk:egg ratio. ^{A,B}Values with no common letter differ significantly ($P < 0.01$).

Diet Preparation

The experimental diets were prepared every 2 wk for a total of 10 times to minimize losses of pigments during storage. Moreover, both the sources of pigment were split in 10 parts, vacuum-packed, and stored in the dark at 4°C until use. The concentrations of apo-ester and total xanthophylls in the feed were analyzed in 3 samples for each experimental diet at every mixing session by an HPLC procedure according to the methods of Weber [26, 27].

Productive Traits and Color Measurements

Productive performances, such as feed consumption, feed conversion rate, egg production, and mortality, were evaluated and calculated on a 14-d basis, whereas the egg components were individually weighed every 4 wk in 24 eggs per group and then yolk:egg ratio was determined.

To evaluate the whole liquid egg pigmentation and color, every 4 wk, 6 pools of 4 eggs per group were collected and analyzed according to method number 958.05 of the Association of Official Analytical Chemists [28] for β-carotene equivalent determination and reflectance colorimetry methods [29] for the color assessment and were reported as L*, a*, and b* values for lightness, redness, and yellowness, respectively.

The deposition rate of the pigment was calculated as follows:

Deposition rate = (deposited pigment/pigment ingested) × 100

where deposited pigment (mg/egg per d) = mean daily whole liquid egg mass (g) × concentration of pigment in whole liquid egg (mg/g) and pigment ingested (mg/d) = mean daily feed ingested (g) × concentration of pigment in feed (mg/g).

Statistical Analysis

The data were analyzed by 1-way ANOVA using the GLM procedure of the SAS package [30], with the main factor being the dietary treatment of productive performance and color data and the hen strain for the egg weight and components. Regression model (r^2) and probabilities

Table 2. Effect of increasing dietary levels of apo-ester (APO) and marigold extract (MAR) on whole liquid egg color (CIE-Lab system) and on β-carotene equivalents determined during the overall experimental period (from 40 to 60 wk of hen age)

Item	β-carotene equivalents (ppm)	Lightness (L*)	Redness (a*)	Yellowness (b*)
ISA Brown				
MAR 120	41.6[d]	64.9[a]	1.7[e]	64.8[ab]
MAR 180	46.6[d]	64.6[a]	2.3[de]	64.0[ab]
MAR 240	48.2[d]	64.4[a]	2.7[d]	62.6[b]
APO 40	66.5[c]	61.7[b]	8.6[c]	66.5[a]
APO 60	85.0[b]	61.4[b]	10.2[b]	66.2[a]
APO 80	104.1[a]	60.8[b]	12.1[a]	66.7[a]
SE	1.71	0.24	0.23	0.68
Hy-Line White				
MAR 120	37.7[e]	66.9[a]	0.8[e]	64.0[b]
MAR 180	43.4[de]	66.0[ab]	1.8[d]	65.1[ab]
MAR 240	47.0[d]	65.4[b]	2.5[d]	65.1[ab]
APO 40	58.0[c]	64.3[c]	6.9[c]	67.6[a]
APO 60	84.4[b]	62.8[b]	10.6[b]	67.8[a]
APO 80	103.0[a]	61.5[e]	12.0[a]	66.9[ab]
SE	2.06	0.27	0.28	0.71
Strain				
ISA Brown	65.31[a]	62.96[b]	5.75[b]	65.13[b]
Hy-Line White	62.23[b]	64.47[a]	6.27[a]	66.10[a]
SE	0.77	0.11	0.10	0.28

[a–e]Values within a column and strain with no common letter differ significantly ($P < 0.01$).

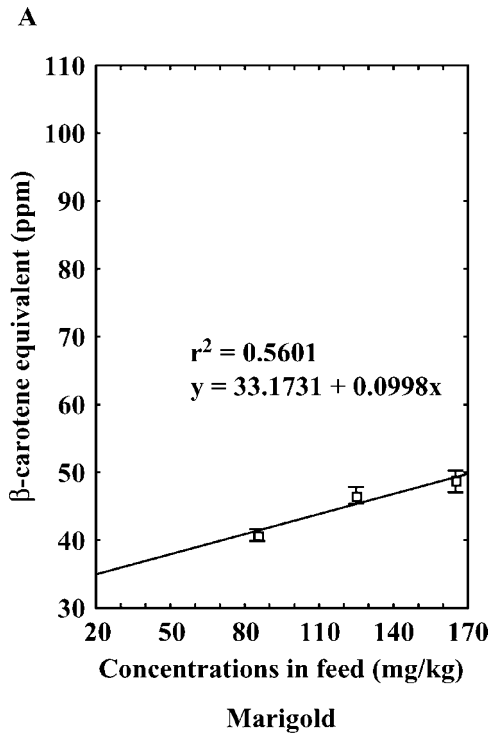
were analyzed to assess the relationship between the β-carotene equivalents in egg and the pigment amount in feed. Pearson's correlation coefficients and probabilities were calculated to evaluate the relationships between the color parameters (L*, a*, b* and β-carotene equivalents) of whole liquid egg and pigment content of feed.

RESULTS

Determinations of the content of xanthophylls in 180 samples of feed showed very little variations in respect to theoretic values for apo-ester compared with marigold extracts, which were 30% lower than the theoretic ones (Figure 1). This result is partly due to the loss of xanthophylls that occurred during storage; in fact, the concentration of the commercial products at the beginning of the trial was 14.7 g/kg instead of 20 g/kg and dropped to 10.4 g/kg after 10 wk and 8.9 g/kg after 20 wk.

The different dietary treatments did not significantly affect feed intake, feed conversion rate, egg production, egg mass, and mortality of either strain (data not shown). In general, some differences among genetics arose, but the values are in accordance with the performance goals of the strains. Moreover, ISA Brown, compared with Hy-Line White, laid significantly ($P < 0.01$) heavier eggs (62.3 vs. 59.9 g) with a lower yolk weight and a lower yolk:egg ratio (0.26 vs. 0.28; Figure 2).

The effect of increasing dietary levels of apo-ester and marigold extracts on whole liquid egg color within each strain is shown in Table 2. Increasing the supplementation levels of apo-ester caused the β-carotene equivalents to be raised linearly and significantly ($P < 0.01$) in both strains, ranging from 66 to 104 ppm for ISA Brown and from 58 to 103 ppm for Hy-Line. On the contrary, the contents of β-carotene equivalents in whole liquid egg of MAR treatments were almost constant with varying pigment dietary dose and were significantly lower ($P < 0.01$) than those of the APO treatments, both in the ISA Brown and Hy-Line hens. Similarly, redness (a*) was higher ($P < 0.01$) in both strains when apo-ester was used, and the values linearly increased ($P < 0.01$) along with the dietary supplementation. In particular, the redness of APO treatment eggs was roughly 5 times higher than in MAR treatments.



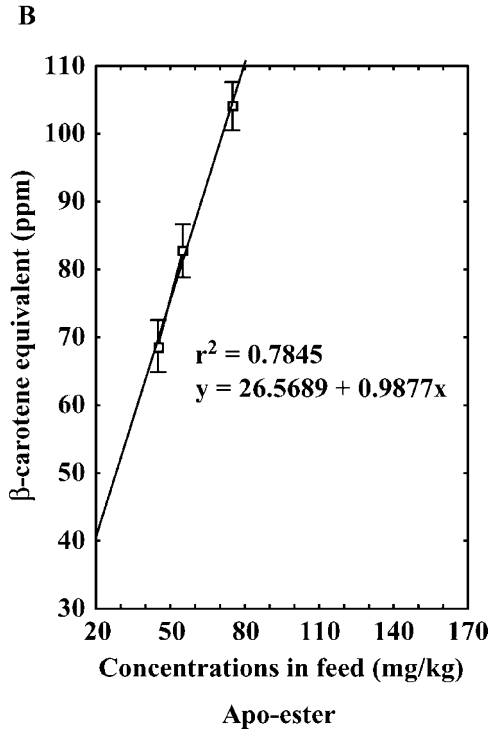
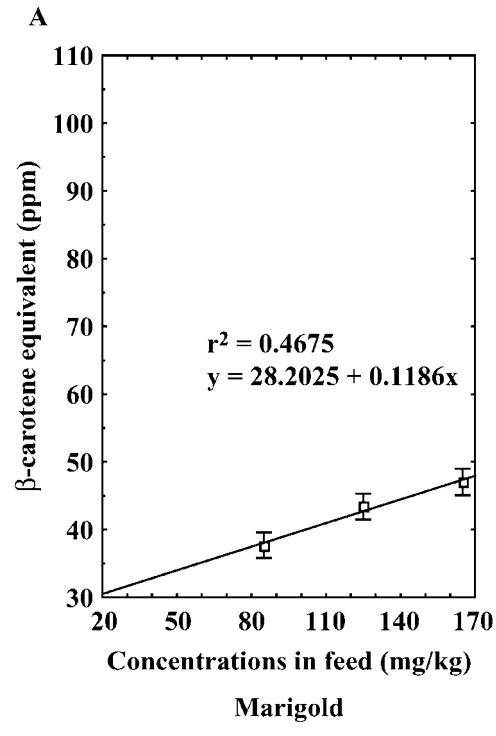


Figure 3. Effect of levels and sources of yellow pigments on β-carotene equivalents in whole liquid egg of ISA Brown hens.



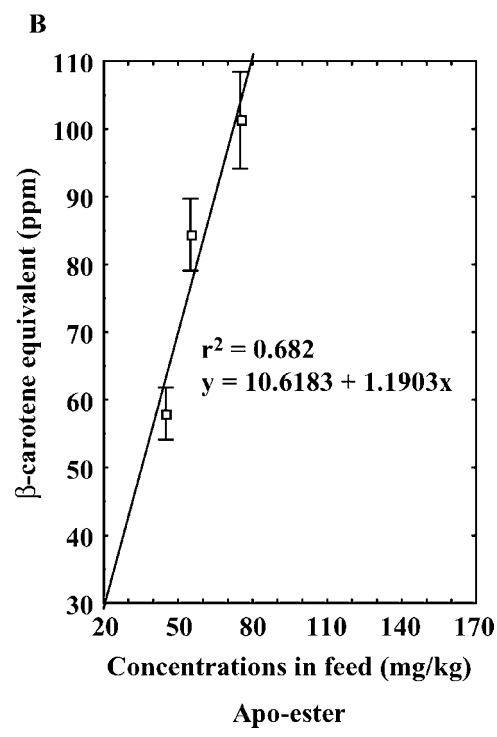


Figure 4. Effect of levels and sources of yellow pigments on β-carotene equivalents in whole liquid egg of Hy-Line White hens.

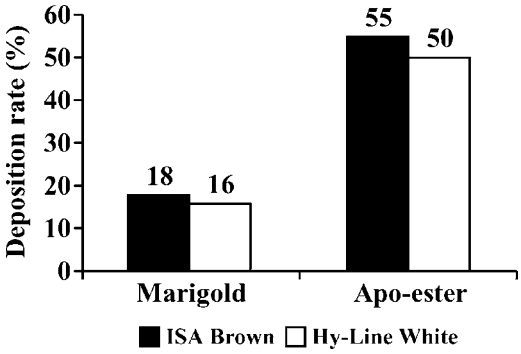


Figure 5. Deposition rate of pigments from diets to eggs in relation to the hen strain and type of pigments.

The regression equations between dietary pigment supplementation and pigment concentration in eggs of ISA Brown and Hy-Line White hens are shown in Figures 3 and 4, respectively. The slope values of the regression equations are 10-fold higher for the APO treatment in respect to the MAR treatment in the ISA Brown hens. This indicates that the increase of the dietary level of apo-ester in the diet produces a 10-fold higher increment of pigments in the whole liquid egg compared with the marigold supplementation. Similar results were recorded for the Hy-Line White hens.

The transfer efficiency of apo-ester from the diets to the liquid egg was 55 and 50% for ISA Brown and Hy-Line, respectively (Figure 5). The transfer efficiency for marigold was 18 and 16% for ISA Brown and Hy-Line, respectively. In particular, in both ISA Brown and Hy-Line White eggs, the deposition rate for marigold decreased as the dietary dose was increased; on the contrary, for apo-ester, the pigment transfer was not affected by the dietary doses (Figure 6).

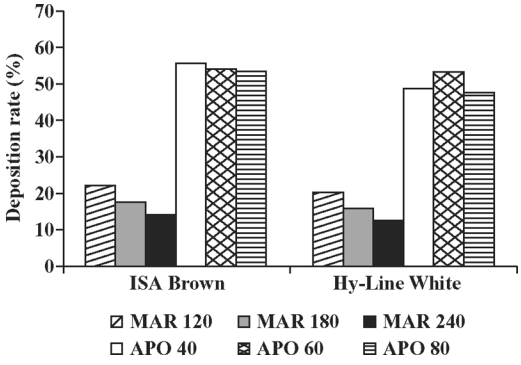


Figure 6. Deposition rate of pigments from diets to eggs in relation to the hen strain and source and doses of pigments. MAR = marigold extract; APO = apo-ester.

In Tables 3 and 4, the correlation coefficients of whole liquid egg color attributes, β-carotene equivalents, and concentration of pigment in feed are reported. The pigment concentration of feed is correlated with the whole liquid egg β-carotene equivalents and with lightness (L*) and redness (a*), whereas yellowness (b*) is insensitive. The whole liquid egg β-carotene equivalents are negatively and positively correlated, respectively, with lightness and redness. In general, the correlation coefficients are higher for APO treatments than for MAR.

DISCUSSION

The increasing supplementation levels of pigments in laying hen diets resulted in intensified pigmentation of whole liquid eggs, but this effect was more evident with inclusion of apo-ester than with marigold extract. Our results agree with those observed in different studies performed to compare the pigmentation efficiency of apo-ester vs. xanthophylls obtained from marigold products. Balnave and Bird [15] compared apo-ester (in powder form) and marigold xanthophylls (in liquid form) across a wide range of supplementation levels. Their results demonstrated that the deposition rate of apo-ester was 3 times that obtained with marigold extract. These outcomes were confirmed by Kluenter et al. [16] in 2 different experiments using different dietary inclusions of apo-ester and marigold xanthophylls. In the first experiment, apo-ester efficiency was compared with that of a powder marigold product containing 92% lutein and 8% zeaxanthin. In the second experiment, a liquid marigold product containing 70% lutein and 30% zeaxanthin was used. The results of both trials indicated that 3 times the level of apo-ester as marigold extract in the feed was required to achieve similar yolk color and carotenoid content in the yolk. Also, Blanch [6] and Steinberg et al. [19], comparing at corresponding dose levels the apo-ester concentration and the total xanthophyll concentrations in the whole liquid eggs measured as β-carotene equivalents, found that the efficiency ratio was about 3:1 for the dietary inclusion of marigold and apo-ester. In our trial, we found a

Table 3. Correlation coefficients among color attributes and β-carotene equivalents of whole liquid egg (WLE) and concentrations of marigold extract in feed (mg/kg)

Item	L*	a*	b*	β-equivalent WLE	Marigold in feed
Lightness (L*)	1.00				
Redness (a*)	–0.72*	1.00			
Yellowness (b*)	0.24	0.08	1.00		
β-equivalent WLE	–0.49*	0.79*	0.03	1.00	
Marigold in feed	–0.30*	0.57*	–0.05	0.68*	1.00

*$P \leq 0.001$.

transfer efficiency of 50 to 55% and 16 to 18%, respectively, for apo-ester and marigold extract, with a ratio between them close to 3:1, confirming the results of the above-mentioned authors using lower supplementation doses than those used in this experiment.

Moreover, with very high doses of dietary pigments, we found different regression equations between pigment concentration in feed and whole liquid egg. Indeed, a high value of slope for APO treatments compared with MAR treatments confirmed the higher capability of apo-ester in depositing the pigments in egg.

The pigment deposition rate from the diet to the egg was 10% higher in ISA Brown both for marigold and apo-ester. The higher pigmentation as β-carotene equivalents found in the ISA Brown eggs compared with Hy-Line eggs may be due to different genetic capabilities to absorb and deposit pigments in their yolk, as shown by Fletcher et al. [31] comparing the color of egg yolks of several hen strains. Therefore, considering that ISA Brown laid heavier eggs with a lower yolk:egg ratio, we assume that the concentration of pigments in the yolk should be higher than in Hy-Line egg yolk.

Yellowness of whole liquid egg was almost stable with the dietary inclusion of either apo-ester or marigold, whereas redness constantly increased according to the dietary pigment dose. Steinberg et al. [19] found that the yellowness reached a plateau at dietary doses of 20 and 40 ppm of apo-ester added to a basal diet containing 14.4 ppm natural xanthophylls, whereas for marigold, no plateau was found when 15, 30, 60, and 120 ppm were used in the diet.

Our data are partially consistent with those of the previous authors; indeed, adding increasing doses of apo-ester over 40 ppm to a basal diet containing 10.6 ppm natural xanthophylls caused no change of yellowness, whereas with marigold inclusion, we did not observe any improvement in yellowness above 120 ppm.

Several authors reported that yellowness tends to plateau at dietary concentrations of 15 to 20 ppm of yellow xanthophylls in feed, whereas the redness still responds to high dietary levels, especially for apo-ester [14, 19]. Similarly, in our trial, the supplementation of increasing doses of apo-ester as well as of marigold enhanced the redness (a*) values of whole liquid egg, whereas lightness and yellowness (b*) were little affected. However, the great increments of redness obtained with apo-ester are particularly important when the whole liquid egg is used for pasta production, as usually occurs in Italy.

Table 4. Correlation coefficients among color attributes and β-carotene equivalents of whole liquid egg (WLE) and concentrations of apo-ester in feed (mg/kg)

Item	L*	a*	b*	β-equivalent WLE	Apo-ester in feed
Lightness (L*)	1.00				
Redness (a*)	–0.71*	1.00			
Yellowness (b*)	0.20	0.08	1.00		
β-equivalent WLE	–0.63*	0.89*	–0.03	1.00	
Apo-ester in feed	–0.49*	0.75*	–0.07	0.84*	1.00

*$P \leq 0.001$.

Using a monopigment source of color, such as apo-ester or marigold, the evaluation of β-carotene equivalents appeared adequate for the whole liquid egg color assessment, because the correlation coefficients between β-equivalents and lightness and redness were significantly high for both sources of pigment. These findings confirm the results obtained by Fletcher [32].

CONCLUSIONS AND APPLICATIONS

1. The dietary inclusion of increasing levels of apo-ester linearly enhances the concentrations of pigments and the redness (a*) of whole liquid egg, whereas lightness (L*) and yellowness (b*) were little affected.
2. The supplementation of very high concentrations (120 to 240 ppm) of marigold extract produces very small increments in whole liquid egg pigmentation.
3. The relative efficiency in egg pigmentation between apo-ester and marigold extract was close to 3:1 both in ISA Brown and Hy-Line White W-36 hens.
4. The ISA Brown hens showed a better capability to transfer the pigments from the diet to the egg compared with the Hy-Line White. The deposition rate was 10% higher either with apo-ester or marigold supplementation. These findings are of particular interest considering that ISA Brown laid eggs with a lower yolk:egg ratio than Hy-Line White.
5. During feed storage, 30% of pigment losses occurred when marigold extract were used, whereas minor losses were recorded for apo-ester.
6. From the present outcomes and considering the pigment efficacy of both the sources, the recommended dietary supplementation levels for whole liquid egg pigmentation can be 120 ppm for marigold extracts, whereas no recommended levels are suggested for apo-ester, because its dietary inclusion varies in relation to the desired whole liquid egg color.

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