Comparative pigmentation efficiency of high dietary levels of apo-ester and tagetes on the quality traits of whole liquid egg of two strains of laying hens.

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The yolk pigmentation is considered one of the most important factor in the evaluation of egg quality and different pigment sources such as feed raw materials (maize, maize gluten, alfalfa meal), flower extracts as well as standardised synthesised products can be used in hen feeding for this purpose. A trial was carried out to compare the effects of the dietary supplementation of high doses of either synthetic pigment such as 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 two strains of hens laying brown shell eggs (ISA brown) and white shell eggs (HyLine W36). In total 192 hens (96 + 96) aged 40 weeks were used and the trial lasted 20 weeks. The hens of each strain were divided into six groups and fed a corn-soybean basal diet supplemented either with 40, 60 and 80 ppm of apo-ester or 120, 180 and 240 ppm of Tagetes, since it is widely accepted that the relative efficiency of apo-ester, compared to lutein and zeaxanthin from Tagetes, is 3 to 1. Productive performances of hens (feed intake, feed conversion, rate of laying, mortality), physical properties of eggs (whole egg, yolk and albumen weights) were recorded as well as whole liquid egg colour evaluated by CIE L*, a*, b* system and β-carotene equivalents.
The egg pigmentation raised linearly and significantly ($P<0.01$) as the dietary levels of apo-ester increased (from 66 to 105 ppm for ISAbrown and from 58 to 103 for HyLine), but this did not occur when Tagetes supplementation was used. The amount of β-carotene equivalents in liquid egg of Tagetes groups was almost constant varying the pigment

dietary dose and was significantly lower (P<0.01) than in apo-ester groups. In both hen strains, liquid egg redness (a*) and yellowness (b*) were higher with apo-ester supplementation. Both pigment source and level of inclusion had no significant effects on productive performances. On the contrary physical egg parameters were highly affected (P<0.01) by strain: yolk/albumen and yolk/egg ratio were higher in the HyLine hens. The trial confirms that, in spite of the higher level of Tagetes supplementation, apo-ester has a better efficiency in whole liquid egg pigmentation. Moreover ISA brown hens showed a better ability to absorb dietary carotenoids than the HyLine ones.

Keywords: laying hens; apo-ester; Tagetes; liquid egg colour, egg quality

Introduction

The yolk pigmentation is considered one of the most important factor in the evaluation of egg quality (Hèrnandez et al., 2001): the food industry requires highly pigmented yolks for the production of egg based products more appealing for consumers. Poultry cannot synthesize carotenoids and they must absorb them from the diet (Schiedt, 1998; Blanch, 1999). Different pigment sources such as feed raw materials (maize, maize gluten, alfalfa meal), flower extracts as well as standardised synthesised products can be used in hen feeding for this purpose.

The main yellow pigments used in poultry feeding are Tagetes extracts and beta-apo-8'-carotenoic acid ethyl ester (apo-ester). Different studies report that the efficiency ratio between apo-ester and xanthophylls of Tagetes extracts for table egg yolk pigmentation is at least 3:1, regardless of the physical characteristics (liquid or powder) of the marigold products (Balnave and Bird, 1996; Kluenter, 1998; Blanch, 1999; Steinberg et al., 2000; 2001) and their lutein/zeaxanthin ratio (Hernandez et al., 1999). Little is currently known about the efficacy of high dietary inclusions of pigments for the pigmentation of whole liquid egg used in food industry.

Therefore, a 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, contained in *Tagetes erecta*, on egg quality of two strains of hens laying brown shell eggs (ISA brown) and white shell eggs (HyLine W36). In consequence of the proven higher efficacy in yolk pigmentation of apo-ester in respect to Tagetes, triple concentrations of Tagetes in respect to the corresponding apo-ester treatment were added to the diets (120, 180 240 ppm *vs* 40, 60, 80 ppm, respectively).

Materials and methods

192 pullets 18 weeks old of two different strains, 96 ISA brown and 96 HyLine W36, were randomly housed in California cages (4 hens/cage) and fed a commercial diet (supplemented with 40 ppm of apo-ester or 120 ppm of tagete extract) for 22 weeks. Starting from 40 weeks of age, after a period of two weeks of adaptation to the experimental diets, the hens of each strain were divided in 6 groups of 4 replicates each (16 hens/group) and fed for 20 weeks a corn-soybean basal diet supplemented either with 40, 60 and 80 ppm of β-apo-8 carotenoic acid ethyl ester or 120, 180 and 240 ppm of Tagetes extract. Feed and water were provided *ad libitum*. Productive performances of hens (feed intake, feed conversion, rate of laying, mortality), physical properties of eggs (whole egg, yolk and albumen weights) were recorded. Every 4 weeks, whole liquid egg colour was measured in 6 pools of 4 eggs each per group either by the CIE *L* a* b** system, using a CR-300 Minolta chroma meter, or by spectophotometric analysis of β-carotene equivalents according to the method number 958.05 of the Association of Official Agricultural Chemists (AOAC) (1990).

The data were analysed by one-way and two way ANOVA and means were compared by the Test of Student Newman Keuls using the package of the SAS Institute (1985).

Results and discussion

The effect of increasing dietary levels of apo-ester and Tagetes on whole liquid egg colour within each strain is showed in table 1 whereas the separated effects of strain and pigment and their interaction are given in table 2. Increasing the supplementation levels of apo-ester, the pigmentation of whole liquid eggs raised linearly and significantly ($P<0.01$); indeed β-carotene equivalents ranged from 66 to 105 ppm for ISA brown and from 58 to 103 for HyLine, but the same was not observed with Tagetes supplementation. The contents of β-carotene equivalents in whole liquid egg of Tagetes groups were almost constant varying the pigment dietary dose and were significantly lower ($P<0.01$) than in apo-ester groups. In both hen strains liquid egg redness (a*) and yellowness (b*) were higher with apo-ester supplementation.. Furthermore, the strain markedly influenced the pigmentation of whole liquid eggs as well as the physical properties ($P<0.01$) (*Table 3*). The eggs laid by HyLine hens had lower weights but heavier yolk in comparison with ISA brown hens, therefore yolk/albumen and yolk/egg ratios resulted higher in the former strain ($P<0.01$). Hen's

performances, including mortality, were neither affected by the pigment source nor by the level of dietary supplementation (data not shown).

Table 1. Effect of increasing dietary levels of apo-ester and Tagetes on whole liquid egg colour (CIE-lab system) and on β-carotene equivalents determined during the overall experimental period within each strain.

	L*	a*	b*	β-carotene equivalents (ppm)
ISA brown				
Apo-ester 40	61.70 Bb	8.64 C	66.54 Aa	66.45 Cc
Apo-ester 60	61.40 Bbc	10.22 B	66.17 Aab	84.97 Bb
Apo-ester 80	60.78 Bc	12.07 A	66.72 Aa	104.09 Aa
Tagetes 120	64.90 Aa	1.71 E	64.81 ABabc	41.59 De
Tagetes 180	64.55 Aa	2.31 DE	63.95 ABbc	46.61 Dd
Tagetes 240	64.42 Aa	2.69 D	62.62 Bc	48.18 Dd
SE	0.24	0.23	0.68	1.71
HyLine white				
Apo-ester 40	64.27 Cc	6.85 C	67.55 A	57.99 C
Apo-ester 60	62.80 Dd	10.56 B	67.82 A	84.36 B
Apo-ester 80	61.49 Ee	12.03 A	66.94 AB	102.91 A
Tagetes 120	66.91 Aa	0.77 E	64.04 B	37.72 E
Tagetes 180	65.97 ABb	1.84 D	65.11 AB	43.41 DE
Tagetes 240	65.40 Bb	2.48 D	65.12 AB	47.02 D
SE	0.27	0.28	0.71	2.06

a, b, c, d, e, f: P<0.05; A, B, C, D, E: P< 0.01.

Table 2. Effect of strain and dietary levels of apo-ester and Tagetes on colour (CIE-lab system) and on β-carotene equivalents content of whole liquid egg.

	L*	a*	b*	β-carotene equivalents (ppm)
Strain				
HyLine white	64.47 A	6.27 A	66.10 a	62.23 B
ISA brown	62.96 B	5.75 B	65.13 b	65.31 A
SE	0.11	0.10	0.28	0.77
Pigment (ppm)				
Apo-ester 40	62.98 Cc	7.74 Cc	67.05 A	62.22 C
Apo-ester 60	62.10 Dd	10.39 Bb	66.99 A	84.66 B
Apo-ester 80	61.14 Ee	12.05 Aa	66.83 A	103.50 A
Tagetes 120	65.90 Aa	1.24 Ef	64.42 B	39.65 E
Tagetes 180	65.26 ABb	2.07 De	64.53 B	45.01 D
Tagetes 240	64.91 Bb	2.58 Dd	63.87 B	47.60 D
SE	0.18	0.18	0.49	1.34
Strain	< 0.01	< 0.01	< 0.01	< 0.01
Pigment	< 0.01	< 0.01	< 0.01	< 0.01

Strain*Pigment	< 0.01	< 0.01	n.s.	n.s.

a, b, c, d, e, f: P<0.05; A, B, C, D, E: P< 0.01; n.s.= not significant.

Table 3. Effect of strain and dietary levels of apo-ester and Tagetes on physical properties of eggs.

	Egg weight (g)	Yolk weight (g)	Albumen weight (g)	Yolk/ albumen	Yolk/ egg
Strain					
ISA brown	62.29 A	16.06 B	40.53 A	0.40 B	0.26 B
HyLine white	59.92 B	16.52 A	37.93 B	0.44 A	0.28 A
SE	0.21	0.07	0.16	0.002	0.0008
Pigment (ppm)					
Apo-ester 40	61.29 ABb	16.39	38.26 B	0.42 A	0.27 A
Apo-ester 60	62.59 Aa	16.30	40.75 A	0.40 B	0.26 B
Apo-ester 80	60.61 Bb	16.14	38.95 B	0.42 A	0.27 A
Tagetes 120	60.52 Bb	16.19	38.88 B	0.42 A	0.27 A
Tagetes 180	60.33 Bb	16.24	38.55 B	0.42 A	0.27 A
Tagetes 240	61.36 ABb	16.47	39.25 B	0.42 A	0.27 A
Se	0.36	0.11	0.29	0.004	0.002
Strain	<0.01	<0.01	<0.01	<0.01	<0.01
Pigment	<0.01	n.s.	<0.01	<0.01	<0.01
Strain*Pigment	<0.01	<0.05	<0.01	<0.05	<0.05

a, b: P < 0.05; A, B: P < 0.01; n. s.= not significant.

This study confirms the better pigmentation efficiency of apo-ester in comparison with Tagetes, even when very high pigment doses were added to the diet. One interesting observation is that the strain affects not only the egg physical parameters, but also the pigmentation levels of the eggs: the ISA brown showed higher levels of pigments in the whole liquid eggs in spite of their lower weight of yolk. These findings are in accordance with Fletcher et al., (1977) who, comparing the colour of egg yolks of several hen strains, found different genetic capabilities to absorb and deposit pigments in the yolk.

These results might be useful for egg processors to optimise the rate of pigmentation of egg products in order to satisfy the demand of food industry and consumers for very high pigmented eggs.

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