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Evaluation of Xanthophylls Extracted from *Tagetes erectus* (Marigold Flower) and *Capsicum Sp.* (Red Pepper Paprika) as a Pigment for Egg-yolks Compare with Synthetic Pigments

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Abstract: Three experiments were accomplished to compare the efficacy of yellow and red xanthophylls extracted from *Tagetes erectus* and *Capsicum sp.*, with synthetic citranaxanthin, canthaxanthin, and ester of $-apo-8-carotenoic pigments at different concentrations to enhance the yellow color of chicken egg yolk. In each trial brown laying hens were placed in cages, and after seven days of feeding a sorghum-soybean diet without pigments treatments were assigned. Yolk eggs were classified with the Roche yolk color fan (RYCF) or Minolta® refractometer. In the first experiment, 168 hens were randomly distributed to seven treatments with one control group, synthetic pigments, and natural xanthophylls. In the second and third experiments, 100 hens were assigned to four different treatments consisting in the same basal diet with addition of natural xanthophylls, and three combinations of apo-ester and citranaxantin. In all experiments, after four weeks of feeding these experimental diets, the color of 10 egg yolks per treatment was evaluated 10 different times every other day or twice a week. Results indicated that live performance was not affected by treatments, but the egg yolk color changed significantly according to the pigment added. Hens fed with diets with 7.5 ppm of yellow xanthophylls extracted from *Tagetes* and 4.0 ppm of red xanthophylls from *Capsicum* had yolk eggs classified as color 11.7 ± 0.1. *Capsicum* extract levels had linear effect over RYCF. Synthetic carotenoids gave a yolk color that varies from 13 to 14 in the highest concentration, and from 12 (5%) to 14 (43%) at the lowest concentration.

Key words: Feed additive, pigments, egg-yolk color, *Tagetes* and *Capsicum,* xanthophylls and carotenoids

Introduction

Egg yolk color is an important factor for egg marketing in several countries. Yolk pigments are relatively stable and normally are not lost or changed in cooking.

The visual yolk color perceived by the consumer is the result of the deposition and colouring capacity of oxy-carotenoids, called xanthophylls, in the egg yolk. Sources of xanthophylls can be natural or synthetic. They can be mono, dihydro or poly-oxalates. Some xanthophylls such lutein and zeaxanthin are present in common feedstuffs such corn, dried distillers' grains or alfalfa. However, layer diets based on cereals other than corn require pigment supplementation to achieve desired egg yolk color (Ravindran, 1995) due to the high variability in pigment content of feedstuffs. Pigment supplementation allows obtaining consistently the desired color in the final product. Williams (1992) stated that increasing the pigmenting properties of a poultry feed increases costs from $5 to $15/ton. A specific yolk color is achieved by combining different levels of yellow and red xanthophylls. To determine the exact source and dose to add in the layer diet is important since they have variable deposition rates. In the case of the reds, almost 40 to 50% of canthaxantin consumed is deposited in the egg yolk, whereas it is only 10-20% in the case of citranaxanthin (Schoner *et al*., 1990), or 16% in the case of capsanthin (Hamilton, 1992). On the other hand, capsanthin/capsorubin absorbs at a higher wavelength than citranaxanthin, and even higher than canthaxanthin. The final couloring capacity will show that canthaxanthin provides a orange, while capsanthin/capsorubin will give a deep red colour to egg yolks. Presence of mycotoxins, antinutritional factors, and dietary levels of vitamin E, antioxidants, oleic and lauric fatty acids, and calcium affect absorption and deposition of pigments (Gawecki *et al*., 1977). Consumer's preferences for natural organic products in their food are increasing (Williams, 1992). Marigold flower (*Tagetes erectus*) and red pepper paprika (*Capsicum ssp.*) extracts have been used as natural pigments to supplement laying hen diets. There are many commercial products extracted from these plants. Their quality as pigments depends on level of trans isomers, level of saponification, stability against oxidation, and chemical isomerization used to increase concentration of some carotenoids such as zeaxanthin (Delgado-Vargas *et al*., 1998; Soto-Salanova, 2003). The losses in pigmentation capability due to manufacturing or storage vary between 10 and 70%. This explains in

Table 1: Diet composition and nutrient content

Ingredient	g/kg
Sorghum	65.61
Rice	8.20
Soybean meal	12.76
Meat-bone meal	5.00
Calcium carbonate	7.32
Tricalcium phosphate	0.49
Salt	0.18
Sodium bicarbonate	0.05
DL-Methionine	0.16
Lysine	0.08
Vitamin and mineral premix[1,2]	0.15
	100.00
Nutrient content	
Metabolizable energy, (kcal/kg)	2,867
Crude protein, %	15.61
Calcium, %	3.50
Total phosphorus, %	0.63
Available phosphorus, %	0.42
Lysine, %	0.71
Methionine + cysteine, %	0.65

[1]Vitamin premix provides per kg of product: 4,000 IU vitamin A; 900 IU vitamin D_3; 2,500 mg vitamin E; 234 mg vitamin K_3; 99 mg thiamin; 1,920 mg riboflavin; 249 mg pyridoxine; 3,250 mg vitamin B_{12}; 3,325 mg pantothenic acid, 4,900 mg niacin, 1,470 mg antioxidant.
[2]Trace mineral mix provides per kg of product: Mn ($MnSO_4$•H_20) 28,600 mg; Zn ($ZnSO_4$•$7H_20$) 24,300 mg; Fe ($FeSO_4$•$7H_20$) 25,000 mg; I (Ca $(IO_3)_2$•H_20) 276 mg; Cu ($CuSO_4$•$5H_20$) 3,010 mg; Co 50 mg; Se 76 mg; 1.000 vehicle Q.S.P.

part the high variability in results when used these products quoted as natural products (Soto-Salanova, 2003). The present experiments aimed at comparing the efficacy to enhance egg yolk color of the yellow and red xanthophylls extracted from *Tagetes erectus* and *Capsicum ssp.*, with the synthetic citranaxanthin, canthaxanthin, and ester of $-apo-8-carotenoic ethyl ester pigments.

Materials and Methods

Three trials were conducted with 24 wk old ISA-Brown layers. Hens were placed in groups of four per cage. A sorghum-soybean diet without pigments was used as basal diet (Table 1). This diet was fed in all trials during seven days previous to initiate the experimental period. Egg yolks were classified with the Roche Yolk Colour Fan (RYCF) (Vuilleumier, 1969). There was no yolk that had RYCF values higher than seven at the end of this pre-experimental period. In the first experiment, 168 hens were randomly distributed in 42 cages with six experimental units for each one of the seven treatments with a control group, two combinations of synthetic pigments, and three combinations of natural xanthophylls (*Tagetes erectus*) with increasing levels of *Capsicum sp.* extract (Table 2). In the second and third experiments, 100 hens were assigned to five treatments consisting in the same basal diet with addition of natural red or yellow xanthophylls, and three combinations of synthetic carotenoid pigments (Table 3). In each experiment, after 4 weeks of feeding the experimental diets, yolk colors were evaluated 10 different times every other day or twice a week in 10 eggs per treatment. Experiments lasted 10 weeks. RYCF was used in all experiments, and in the first one, the Minolta® refractometer was also used. Redness (a) and yellowness (b) detected by refractometer were analyzed. Egg production, feed intake, and feed conversion (feed:egg mass) were also recorded. Eggs were collected daily and classified according to weight. Data was analyzed as a complete randomized design. All percentage data, including percentages of acceptable yolk colors was transformed to square root of n+1 prior to ANOVA analysis in the SAS system (2002).

Results and Discussion

Egg production, feed intake and feed conversion were not affected by any of the treatments in all experiments ($P > 0.05$), but the egg yolk color changed significantly ($P < 0.001$) according to the pigments and concentrations used. Pigment supplementation had not been associated with changes in production (Angeles and Scheideler, 1998; Garcia *et al*., 2002; Soto-Salanova, 2003). In all three experiments, the yolk color was recovered after one week of dietary supplementation with any of the pigments. Similar results had been observed by Halaj *et al*. (1999) and Garcia *et al*. (2002) at evaluating synthetic pigments. Lower values were observed for natural pigments after the first week of evaluation. The deposition of natural pigments in the yolk is slower than with the synthetic ones. Gawecki *et al*. (1977) observed improvements in egg yolk colors only after two weeks of supplementation with natural pigments.

Experiment 1: The basal diet did not support RYCF values higher than 4. The distribution of acceptable yolk colors according to treatment is presented in Table 4. All treatments supplemented with pigments achieved average values higher than 11 in RYCF. Hens fed with diets supplemented with synthetic pigments had higher uniformity than any of the groups fed diets that contained natural pigment extracts. Both synthetic pigment combinations supported consistently RYCF values higher than 12, without being significantly different. The redness and yellowness of yolk eggs evaluated with Minolta refractometer showed similar values. The addition of *Capsicum* extract increased linearly the values of RYCF (Table 5). However, that trend was not observed in the values of Minolta refractometer, and no significant differences were observed with this method among the *Tagetes* and *Capsicum* combinations. But

Table 2: Treatment distribution in the first experiment

Treatment	Yellow base	Dose		Red base	Dose	
		Active base	Product		Active base	Product
		g/ton feed			g/ton feed	
1	None - Control	-	-	None - Control	-	-
2	Apo- carotenoic	2.5	25	Canthaxantin	1.5	15
3	Apo- carotenoic	2	20	Citranaxanthin	3	30
4	*Tagetes erecta*	7.5	375	*Capsicum sp*.	2.5	500
5	*Tagetes erecta*	7.5	375	*Capsicum sp*.	3.0	600
6	*Tagetes erecta*	7.5	375	*Capsicum sp.*	3.5	700
7	*Tagetes erecta*	7.5	375	*Capsicum sp.*	4.0	800

Table 3: Treatment distribution in the second and third experiments

Treatment	Yellow base	Dose		Red base	Dose	
		Active base	Product		Active base	Product
		g/ton			g/ton	
1	*Tagetes erecta*	7.5	375	*Capsicum sp*.	4.0	800
2	Apo- carotenoic	1.5	15	Citranaxanthin	3.5	35
3	Apo- carotenoic	1	10	Citranaxanthin	3.5	35
4	Apo- carotenoic	1	10	Citranaxanthin	3.0	30

Table 4: Averages of egg yolk color redness (a), yellowness (b), Roche yolk color fan (RYCF) number, and percentages of egg yolks with acceptable market color observed in experiment 1 in hens fed diets supplemented with combinations of levels of apo-carotenoic ethyl ester, canthaxanthin, and citranaxanthin, or *Tagetes* and *Capsicum*

Treatments	Redness (a)		Yellowness (b)		RYCF		Acceptable yolk color	
							11 or less	12-14
							%	
Control	-7.45[c]	± 0.35	34.61[b]	± 1.35	3.73[d]	± 0.24	-	-
Apo-ester, 2.5 ppm + Canthaxanthin, 1.5 ppm	0.02[a]	± 0.35	40.50[a]	± 0.94	12.46[a]	± 0.17	7.53	92.47
Apo-ester, 2.0 ppm + Citranaxanthin 3.0 ppm	0.63[a]	± 0.35	40.26[a]	± 0.96	12.61[a]	± 0.09	0.05	99.95
Tagetes, 7.5 ppm + *Capsicum*, 2.5 ppm	-2.84[b]	± 0.45	42.37[a]	± 0.74	10.49[c]	± 0.27	65.84	34.16
Tagetes, 7.5 ppm + *Capsicum*, 3.0 ppm	-3.33[b]	± 0.44	42.42[a]	± 0.90	10.77[bc]	± 0.11	32.51	67.49
Tagetes, 7.5 ppm + *Capsicum*, 3.5 ppm	-2.98[b]	± 0.44	40.63[a]	± 0.93	11.17[b]	± 0.20	60.00	40.00
Tagetes, 7.5 ppm + *Capsicum*, 4.0 ppm	-2.19[b]	± 0.35	42.66[a]	± 1.17	11.70[ab]	± 0.12	12.50	87.50
P-value	<0. 0001		<0. 0001		<0. 0001			
CV %	39.8		7.4		5.2			

[abcd] Means within a column with different superscript indicate significant differences with $p < 0.05$ (Tukey's test)

Table 5: Effect of levels of *Tagetes* and *Capsicum* extracts over the yolk color classified by Roche color fan

Type of model	Observed trait	b_o	b_1	b_2	P-value	R^2
Linear	Redness	-4.286 (1.133)[1]	0.443 (0.346)		0.209	0.13
	Yellowness	42.761 (2.808)	-0.236 (0.857)		0.785	0.02
	RYCF	8.417 (0.544)	0.805 (0.166)		< 0.001	0.42
Quadratic	Redness	-4.575 (1.112)	0.411 (0.336)	1.286 (0.742)	0.109	0.13
	Yellowness	42.324 (2.838)	-0.284 (0.858)	1.943 (1.895)	0.575	0.03
	RYCF	8.362 (0.555)	0.798 (0.168)	0.245 (0.370)	0.060	0.42

[1]Standard error of the coefficient

Table 6: Egg yolk color classified by Roche color fan (RYCF) and distribution of yolk colors of hens fed with diets supplemented with *Tagetes* and *Capsicum* extracts, and apo-carotenoic ethyls ester and citranaxanthin

Treatments	Second experiment				Mean	± SEM	Third experiment		
	RYCF color distribution						RYCF color distribution		
	11	12	13	14			≤ 10	Acceptable colors	
								10 - 12	13 - 15
			%					%	
Tagetes, 7.5 ppm + *Capsicum*, 4.0 ppm	100				11.6^{d}	0.15	31.94^{a}	65.91^{a}	2.15^{d}
Apo-ester, 1.5 ppm + Citranaxanthin 3.5 ppm	-		86	14	13.8^{a}	0.23	19.03^{b}	11.33^{b}	69.64^{a}
Apo-ester, 1.0 ppm + Citranaxanthin 3.5 ppm	5	10	15	70	13.1^{b}	0.60	24.06^{ab}	22.29^{b}	53.65^{abc}
Apo-ester, 1.5 ppm + Citranaxanthin 3.0 ppm	-	5	52	43	12.4^{c}	0.25	15.37^{c}	25.87^{b}	58.76^{ab}
P-value					<0.01		<0.001	<0.001	<0.001
CV %					5.2		15.8	12.9	17.5

^{abcd} Means within a column with different superscript indicate significant differences with $p < 0.05$ (Tukey's test)

yellowness values of natural pigments were significantly lower than the ones observed with the synthetic pigments (Table 4). Hernandez *et al.* (1999) had concluded that natural xanthophylls from *Tagetes* have a relative yolk pigmenting efficiency 3 times lower than the synthetic apo-ester. Garcia *et al*. (2002) concluded that to obtain yolks classified as 14 in RYCF it was necessary to add 6 ppm of pure canthaxanthin. This concentration is higher than the one used in the work presented, however similar RYCF values were observed. Soto-Salanova (2003) presented data of yolk colour produced with combinations of 5.9-6.1 ppm of apo-ester and 1.7 -2.0 ppm of canthaxanthin, or different levels of *Tagetes* and *Capsicum*. Those RYCF and Minolta colorimeter values are similar to the ones presented herein. In the present experiment, with addition of 375 ppm of *Tagetes* product and 800 ppm of *Capsicum* product was possible to obtain a consistent RYCF value of 11. Similar observations have been reported by Kluenter *et al*. (1998) and Hoppe (1998).

Experiments 2 and 3: The average values observed with RYCF and their distribution is presented in Table 6.

The highest concentration of *Tagetes erectus* and *Capsicum sp.* was evaluated in the second and third experiment. In both experiments, this combination supported RYCF values of 11. All combinations of synthetic pigments supported RYCF values higher than 12. Lower (1.0 ppm) levels of yellow pigment (apo-ester) reduced significantly the orange-golden appearance in the yolk (13.1 vs 13.8), but affected the uniformity in the egg yolk colour in both experiments. Reduction of citranaxanthin to 3.0 ppm reduced RYCF to 12.4, but the uniformity was better, i.e., lower percentage of eggs had RYCF values 10 or lower. Even though, the same treatments were tested in the second and third experiments slight changes in results were observed. Several dietary factors, processing and storage conditions might affect the pigmenting properties of the different products. These observations indicate once again that under commercial conditions is important to have a constant evaluation of the RYCF values as part of egg quality control to guarantee the yolk color desired by the specific market. The supplementation with natural pigments is more expensive than the synthetic sources (Bird, 1996; Hoppe, 1998). Commercial utilization depends on consumer acceptability and willingness to pay an extra price for a natural product.

Marigold flower (*Tagetes erectus*) and red pepper Paprika (*Capsicum sp.*) xanthophylls at rates of 7.5 and 4 g/ton of feed can support consistently RYCF value of 11. It is necessary at least three times more xanthophylls than synthetic pigments in the feed to obtain the same RYCF values. Each combination of natural or synthetic pigments changes the average and the distribution of egg yolk colors. Average RYCF values achieved with natural pigments are always slightly lower (11) than with synthetic pigments (12–13).

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