SHORT COMMUNICATION

EFFECT OF A TOMATO EXTRACT-SUPPLEMENTED DIET ON EGG YOLK PIGMENTATION AND LYCOPENE TRANSFER EFFICIENCY

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ABSTRACT

The aim of this research was to measure the ability of laying hens fed a tomato extract-supplemented diet to deposit dietary lycopene into the egg yolk, and to investigate the effects of lycopene on yolk pigmentation. Twenty Isa Brown hens were individually caged and fed two dietary treatments: 1) control diet (C) and 2) control diet + 0.08% (w/w) tomato extract dissolved in soybean oil (2% w/w) (TE). The tomato extract-supplemented diet provided a calculated lycopene level of 13 mg/g. Feed and water were provided *ad libitum.* Colorimetrical and HPLC analysis confirmed that the dietary lycopene was incorporated into the egg yolk at values ranging from 0.12 to 0.16 µg/g yolk (as is). Dietary tomato extract affected L*, a* and b* values of yolk and approximately 0.13% lycopene was transferred from the feed to the yolk. The data suggest that the TE diet resulted in a significant lycopene carry over and the intensity of egg yolk colour was influenced by dietary lycopene supplementation.

- Key words: carotenoids, egg, laying hen, lycopene, tomato extract, yolk pigmentation -

INTRODUCTION

Diet optimisation is the most important issue for many human nutritionists. Due to its nutritive excellence, egg is an ideal food and allows nutritional imbalances in modern diets to be overcome (SURAI, 2002).

Life-expectancy is increasing in developed countries and advances are being made in food technology, so consumers are paying more attention to diet as part of a healthy lifestyle. In an effort to re-evaluate animal-derived products, several studies have been performed to increase the enhanced levels of functional substances such as lutein, selenium and n-3 fatty acids in egg. Results have shown that eggs are not only a good nutritional product but are also a good vector for delivering essential nutrients for human health (SURAI, 2000; SURAI *et al.*, 2000; LESKANICH and NOBLE, 1997).

Carotenoids, the most conspicuous and widespread group of pigments in nature, have many physiological functions. Most carotenoids found in food have antioxidant activity, acting as free-radical scavengers. Many authors have studied the dietary carotenoid intake of humans, but there are no data on the optimal levels (HAZELS MITMESSER *et al.*, 2000). Even if no dietary references have been proposed for carotenoids, some authors have described the carotenoid plasma and serum response after tomato product intake (STAHL and SIES, 1996). Lycopene is one of the main components of the carotenoid fraction in tomato and the mean value ranges from 2 to 14 mg/100 g of fresh tomato (ZANFINI *et al.*, 2007). Lycopene exerts peculiar biological effects; in particular, it has the greatest ability to quench singlet oxygen (DI MASCIO *et al.*, 1989). Moreover, it has been shown that the dietary intake of lycopene from tomatoes is inversely related to the risk of certain types of cancer, such as prostate, digestive-tract and lung cancers (GIOVANNUCCI *et al.*, 1995; FRANCESCHI *et al.*, 1994; LE MARCHAND *et al.*, 1989).

The purpose of this work was to investigate the effect of lycopene supplementation on egg yolk pigmentation and to determine the amount of lycopene that crossed over in laying hens fed with a tomato extract-supplemented diet in a view of designing lycopene enriched eggs.

MATERIALS AND METHODS

Chemicals

A lycopene standard was obtained from Sigma (St. Louis, MO, USA). Diethyl ether, hexane, acetone, ethanol and dichloromethane were p.a. grade, while acetonitrile and isopropanol were HPLC grade. All solvents were purchased from Merck (Whitehouse Station, NJ, USA).

Tomato extract

The tomato extract was obtained from *San Marzano* canned peeled tomatoes (10.8 kg). The *San Marzano* cultivar was chosen due to its high carotenoid, lycopene and β-carotene contents (STRAZZULLO *et al.*, 2007). Canned tomatoes were homogenised in a blender for 5 min. The puree was extracted with diethyl ether (w/v 1:2) under stirring, in the dark overnight in order to obtain a high yield of carotenoids in the lipophilic fraction. The upper red layer containing the carotenoids was filtered, concentrated in a rotary evaporator in vacuum (T <35°C) and dried under N_2 as suggested by STRAZZULLO *et al.* (2007). The extract was stored at -30°C pending diet formulation.

Animals and diets

Twenty Isa Brown laying hens (20 weeks old) were individually caged (1,925 cm^2/bird) in two double-deck cage batteries; the mean ambient temperature during the experimental period was 20°-25°C. The birds were randomly divided into two groups, of ten hens each. The control group (C) was fed with a standard layer diet (containing 2% oil w/w) and the test group (TE, tomato extract) was fed the same diet supplemented with 0.08% (w/w) tomato extract (equivalent to 13 μg of lycopene/g diet) previously dissolved in the oil fraction. Soybean oil was used to incorporate the tomato lypophilic extract in the feed. The composition of the diet is reported in Table 1. The trial lasted 14 days and the animals were monitored daily. Feed and water were provided *ad libitum*.

On days 1, 7 and 14 of feeding, the eggs laid in each experimental unit were collected (n=10), weighed and cracked. Shells, albumen and yolks were separated and weighed and the egg yolk colour was measured. Egg yolks and albumens were then lyophilized and stored at -20°C for further analyses.

Yolk measurement and analysis

The yolk colour was determined (n=10) at room temperature (20°C) by using a portable Minolta Colorimeter CR-331C (Minolta Camera, Osaka, Japan) with D_{65} illuminant and the 2° standard observer. The results are expressed in terms of lightness (L*), redness (a*) and yellowness (b*) in the CIELAB colour space model (CIE, 1978). Additional reflectance data such as chroma (C*), and hue angle (h*) were also calculated. The colorimeter was calibrated throughout the study using a standard white ceramic tile. Colour difference (ΔE*) was calculated as follows: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, where the ΔL*, Δa*, and Δb* values are the differences in colour co-ordinates between treatments.

Yolk and albumen dry matter, ether extract

Table 1 - Ingredients and calculated composition of basal diet[1].

Ingredients	g/kg	
Corn		590
Wheat roughage		15
Soybean meal		180
Alfalfa meal		30
Corn gluten		40
Oil		20
Calcium carbonate		82
Dicalcium phosphate		23
NaCl		5
DL-Methionine		3
L-Lysine		2
Vitamin and mineral premix[2]		10
Calculated composition		
ME	MJ/kg	11.51
Crude protein %		16.60
Ether extract %		4.55
Crude fiber %		4.19
Ash %		13.00
Dry matter %		87.06
Calcium %		3.79
Phosphorus %		0.74
Methionine %		0.57
Lysine %		0.85

[1]Diet was fed at 120 g/bird/day.
[2]Premix provided (mg/kg of diet): vitamin A, 1500 IU; vitamin D, 800 IU; vitamin E, 10 mg; vitamin K, 0.5 mg; riboflavin, 3.6 mg; pantothenic acid, 10 mg; vitamin B_{12}, 0.1 mg; choline chloride, 200 mg; niacin, 17.0 mg; manganese sulfate, 66 mg; zinc sulfate, 60.5 mg;
iron sulfate, 49.5 mg; copper sulfate, 49.5 mg; calcium iodate, 0.26 mg;
sodium selenite, 0.10 mg.

and ash were assessed by AOAC methods (1990) (n=5). Crude protein was determined by using "Rapid N" (FARINA and BEDETTI, 2007) (n=5).

The yolk lycopene value was determined by HPLC (n=5). Lyophilized yolk (600 mg) was extracted with 40 mL of hexane-acetone-95% ethanol (50:25:25 v/v/v). The mixture was kept on ice and agitated continuously on a magnetic stirrer plate for 15 min at 150 RPM until all the lycopene was completely extracted. Agitation was continued at room temperature for another 5 min after adding 6 mL of deionised water. After decanting, the upper non-polar layer (NPL) containing lycopene was filtered and then, concentrated in a rotary evaporator in vacuum (T < 35°C) and dried under N_2.

The HPLC apparatus consisted of a Dionex P680 pump (Dionex, Sunnyvale, CA,USA) equipped with a Rheodyne Model 7725i injection valve (Rheodyne, Rohnert Park, CA, USA), a Dionex UVD-170/U UV-vis detector (λ=470 nm), a Dionex thermostatted column compartment TCC-100, and a Chromeleon® 6 data handling system (Dionex, Sunnyvale, CA, USA).

An Ascentis™ C18 column (25 cm × 4.6 mm, 5 μm particles) (Supelco, Bellefonte, PA, USA) was used for the analysis. Twenty μL of NPL dissolved in 1 mL of dichloromethane were injected into the chromatographic system by a full loop injection system. The system was run isocratically with a mobile phase containing acetonitrile-isopropanol (90:10 v/v) for 25 min at a flow rate of 2.5 mL/min. The column temperature was kept at 30°C for all of the chromatographic runs. The identification and quantification of lycopene were obtained through the combined use of the retention time, and co-chromatography with standard.

The lycopene yield (expressed in terms of percentage) from feed to egg yolk were calculated by the following equation:

Lycopene transfer efficiency = Lycopene deposition in egg yolk (A) × 100 / Lycopene consumption by feed (B)
where:

A = yolk weight (g) ×
× yolk lycopene concentration (μg/g)
B = feed consumption (g/d/bird) ×
× feed lycopene concentration (μg/g).

Statistical analysis

Data were analyzed by one-way ANOVA using SPSS (SPSS, 2008) considering the dietary treatment as the only factor for each sampling day. Statistical significance was accepted at $P < 0.05$. Results are presented as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Diets were isonitrogenous and isoenergetic (Table 1) and satisfied the energy requirements of the laying hens

The quality traits of eggs on days 1, 7 and 14 are reported in Table 2. Dietary treatment did not affect these parameters. This supports the findings of LEESON and CASTON (2004), who observed production parameters were not influe ced by diet in laying hens fed with different inclusion levels of lutein in corn-soy diets.

The chemical composition of yolk and albumen was not influenced by dietary treatment as illustrated in Table 3; the dry matter, crude protein, ether extract and ash values were in agreement with the literature (CEROLINI *et al.*, 2008; THAPON and BOURGEOIS, 1994).

Colorimetrical data (Table 4) showed that yolks from hens fed with the supplemented tomato extract diet were darker than those of the control group. On days 7 and 14, the values of yolk colour parameter a* of the TE group were significantly higher ($P < 0.01$), and the L* values were lower ($P < 0.05$ and < 0.01, respectively) as were the b* values ($P < 0.05$). As expected, tomato extract contributed to egg yolk colour because lycopene, a red pigment, is darker than the yel-

Table 2 - Effects of incorporating tomato extract in the diet for 2 weeks on egg quality (n=10, mean±SD).

		Day 1			Day 7			Day 14		
		C	TE	P	C	TE	P	C	TE	P
Egg weight	g	55.74±4.78	59.06±4.17	NS	58.99±5.01	56.40±2.70	NS	60.95±8.12	57.12±3.40	NS
Shell weight	g	7.09±0.79	6.70±0.50	NS	7.19±0.42	6.95±0.31	NS	6.71±0.69	6.89±0.69	NS
Yolk weight	g	13.48±1.45	13.93±1.32	NS	14.35±1.08	12.91±1.72	NS	15.15±0.85	14.51±1.21	NS
Albumen weight	g	34.10±2.93	37.22±2.84	NS	37.79±4.02	36.36±2.25	NS	38.97±4.65	36.32±2.13	NS

C= Control group; TE = Tomato extract group.

Table 3 - Proximate analysis of egg yolks and albumen (n=5, mean±SD).

		Day 1			Day 7			Day 14		
		C	TE	P	C	TE	P	C	TE	P
Yolk										
Dry matter	%	51.15±0.87	51.27±0.43	NS	52.07±0.68	51.41±0.77	NS	51.32±0.56	51.69±0.60	NS
Ash	%	1.50±0.36	1.51±0.33	NS	1.51±0.15	1.35±0.63	NS	2.16±0.95	1.82±0.35	NS
Ether extract	%	29.58±0.76	29.49±0.40	NS	27.82±2.18	26.27±0.75	NS	34.20±9.02	30.21±1.67	NS
Crude protein	%	16.58±0.59	16.37±0.65	NS	16.21±1.48	16.27±0.40	NS	16.49±4.79	16.11±0.82	NS
Albumen										
Dry matter	%	21.14±2.47	21.51±1.13	NS	21.03±2.16	20.14±1.20	NS	20.83±1.90	20.30±2.01	NS
Ash	%	0.60±0.07	0.73±0.16	NS	0.55±0.12	0.48±0.37	NS	0.64±0.26	0.53±0.10	NS
Ether extract	%	0.04±0.02	0.05±0.02	NS	0.03±0.01	0.04±0.12	NS	0.04±0.01	0.04±0.01	NS
Crude protein	%	11.84±2.01	12.52±2.43	NS	9.29±1.24	9.28±0.39	NS	11.31±3.90	9.90±1.56	NS

C= Control group; TE = Tomato extract group.

low lutein and zeaxanthin pigments normally present in egg yolk (OLSON *et al.*, 2008).

In a previous study with Isa Brown laying hens, FERRANTE *et al.* (2003) showed that a tomato extract-supplemented diet resulted in a higher a* value in egg yolk. The difference in the h* co-ordinate value ($P < 0.01$) between groups C and TE increased from day 7 to 14. Moreover, tomato extract addition resulted in a detectable colour difference; the variation was higher on day 7 than on day 14 (ΔE^*= 4.93 and 3.71, respectively). Compared to a* and b*, the L* parameter, influenced the calculated colour variation (ΔE) the most. In fact, on days 7 and 14 the ΔL^* values were 4.08 and 2.77; Δa^* were 2.39 and 2.06 and Δb^* were 1.38 and 1.35, respectively.

The influence of lycopene on egg yolk colour was also observed by KARADAS *et al.* (2006). In their trial on adult Japanese quails fed a diet containing tomato powder, the Roche colour fan showed a well defined orange colour in the egg yolk which was the result of a combination of yellow xanthophylls and lycopene.

The carotenoid content in egg yolk is a reflection of their dietary provision, as previously reported by SURAI *et al.* (1998), SURAI and SPEAKE (1998) and SURAI (2002). In this study, lycopene transfer was further confirmed by HPLC, measuring the lycopene incorporated in the egg yolk. No lycopene content was detected in the eggs on day 1. The lycopene content in the yolk, increased in the TE group: 0.12 μg/g on day 7 and 0.16 μg/g on day 14. Therefore assuming a feed intake of 120 g/bird/d and a 15 g daily yolk mass production, the transfer efficiency of lycopene calculated from the tomato extract-supplemented diet to the egg yolk was about 0.13% (w/w). This value is in accord with that reported by KNOBLICH *et al.* (2005). Recently, OLSON *et al.* (2008) ex-

Table 4 - CIELAB parameters (n=10, mean±SD) and lycopene content (n=5, mean±SD) in egg yolks.

		Day 1			Day 7			Day 14		
		C	TE	P	C	TE	P	C	TE	P
L^*		60.92±1.58	60.90±1.58	NS	62.13±2.04	58.05±3.78	< 0.05	63.48±1.46	60.71±1.99	< 0.01
a^*		14.02±1.08	13.98±1.09	NS	14.46±1.31	16.85±0.90	< 0.01	14.45±1.25	16.51±1.32	< 0.01
b^*		34.84±0.85	34.85±0.86	NS	35.38±1.31	34.00±0.94	< 0.05	35.90±1.04	34.55±1.37	< 0.05
C^*		37.57±0.90	37.57±0.90	NS	38.24±1.28	37.96±1.08	NS	36.14±10.03	38.31±1.28	NS
h^*		68.12±1.58	68.12±1.58	NS	67.80±2.00	63.67±1.10	< 0.01	68.09±2.03	64.49±2.11	< 0.01
Lycopene (fresh matter basis)	µg/g	*N.D.	*N.D.		*N.D.	0.12±0.03		*N.D.	0.16±0.06	

C= Control group;
TE = Tomato extract group;
*Limit of detection of yolk lycopene concentration was <20 ng of lycopene/g.

amined the effect of lycopene addition on egg yolk in laying hens, testing at much higher levels; the result was much greater yolk lycopene deposition (0.6-4.5%). The Authors showed that the transfer efficiency of lycopene declined as its dietary intake increased.

CONCLUSIONS

The results of this study confirm that the intensity of egg yolk colour is influenced by a lycopene-supplemented diet. Lycopene is a good natural pigmenting compound, especially for the redness index. During the trial an average of 0.13% lycopene crossed over into the egg yolk; the lycopene transfer occurred even at low dietary concentrations.

The use of tomato extract introduced directly into the diet through a lipid phase (soybean oil) is relevant in terms of hen feedstuff. Compared to tomato peel or tomato supplemented diet (as proposed by KNOBLICH *et al.* 2005), the method described here to obtain a supplemented diet resulted in an increase of lycopene intake by hens, because no fibrous ingredients were used and digestibility was not affected.

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