

Enriching Egg Yolk with Carotenoids & Phenols

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Abstract—Dried tomato peel (DTP) was tested in vivo (n=10) in 42 week-old laying hens at rates of 0, 40, 70, 100 and 130g/kg DM feed. Laying hens were fed in group 120 g DM/day/animal for 26 days. After 21 days, feed intake was not affected after DTP incorporation (97% of the offered feed in the five groups). Laying rate was not significantly different after DTP incorporation at 4 and 10% from the control group. Egg yolk resulting from DTP-enriched diets, contained lower amounts of cholesterol (14 to 17mg/g) and triglyceride (188mg/g) compared to the control group (22 and 241 mg/g, respectively) ($P<0.0001$). After DTP-enriched diets, content in total phenol was 2.0 to 3.6-fold higher, β -carotene 1.7 to 2.7-fold higher, and lycopene increased between 26.5 and 42.8 μ g/g compared to the control ($P<0.0001$). The optimal incorporation rate was 7% DTP.

Keywords—Carotenoid, dried tomato peel, lycopene, laying hens, phenols.

I. INTRODUCTION

TOMATO consumption is traditionally linked to the Mediterranean diet, associated with low risk of coronary diseases and cancer [1]. Lycopene is the main carotenoid in tomato and tomato products, which are considered as its important dietary source in the human diet [2]. In tomato, lycopene (all *trans* form) is attached to its membranes [3]. It has a structure that consists of a long chain of conjugated double bonds, which makes lycopene highly apolar. Whereas, lycopene has no provitamin A properties, the long conjugated double chain renders its antioxidative activity of importance to human health [4]. Lycopene can be extracted in oil and organic solvent, and is better absorbed when ingested in a lipid matrix. However heating can transform the *trans* double bonds to *cis*, resulting in more absorbed form of it. In addition to cooking, *trans* double bonds can be converted to *cis* in vivo to a certain extent [3]. Tomato by-products, and especially peel, have a great interest in human and animal nutrition because they are excellent sources of natural antioxidants largely in the form of carotenoids and phenols [5].

Egg constitutes a good source of high biological value proteins, with balanced contents of vitamins and minerals [6]. Despite their nutritional value, egg consumption has decreased, in many developed countries, due to negative consumer perception of high dietary supply in cholesterol

especially in yolk egg [6]. Although several studies suggest that there is no direct link between egg consumption and levels of blood cholesterol [7], controversy about different response to dietary cholesterol among individuals remains [8].

Cholesterol content in egg is largely influenced by genetic factors, and dietary manipulation, especially with some pharmaceuticals [8]. Also, in addition to reducing egg cholesterol content, some phytonutrients have increased overall antioxidant status, immunoglobulin and carotenoid contents [9]. Elkin explained in details the mechanism by which dietary nutrients and/phytochemicals can greatly reduce cholesterol content in egg. Additionally, many studies have shown that bioactive compounds introduced into the poultry feed can be transferred from hen to egg yolk [9]. In animal models, some natural flavonoids have shown a variety of biological and pharmacological activities, including inhibition of reductase 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) and acyl-CoA: enzymes of the cholesterol acyl transferase (ACAT) [10]. Enriching egg with additional phytonutrients might enhance further their nutraceutical value and acceptability by consumers.

Egg yolk colour remains a criterion of choice for consumers [11]. Egg yolk colour is determined by content and profile of carotenoids, where lutein and zeaxanthin are by far the most studied carotenoids for their preventive role against macular degeneration related to age [12].

The objective of our study was to determine the extent to which egg yolk will be enriched with carotenoids and phenols through sun dried tomato peel (DTP) feeding. Our hypothesis was that lycopene would compete with other fat soluble compounds for deposit into the egg yolk increasing its antioxidant activity and reducing cholesterol content. Also we looked at the possible passage of phenolic acids to egg yolk.

II. MATERIALS AND METHODS

A. Feeding Experiment

Tomato peel provided by a local farm (Algiers, Algeria). Tomato was manually peeled; peel was sun dried the whole day avoiding exposure from 11:00 am to 15:00 pm. Average temperature recorded was 39°C. Dried tomato peel was grinded and stored at room temperature until feeding experiment started. Laying hens from the breed Lohmann Brown-Classic were used in this experiment. They are characterized by their red shell eggs, and very appreciated for their high performance and the quality of their eggs. Fifty laying hens (41 weeks of age) were divided into five groups of 10 hens each and housed in battery cages. Laying hens were assigned to receive one of the five different diets during 26

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days. A standard diet produced locally and composed of corn, soy and wheat bran was used as a control (Table I). The other four diets were obtained by supplementing standard diet with dried tomato peel. Four rations a day were distributed 4 hours apart, to reach a total of 120 g/animal/day.

TABLE I
INGREDIENTS AND CHEMICAL COMPOSITION OF THE EXPERIMENTAL DIETS
(G/KG)

Diet	Control	4% DTP	7% DTP	10% DTP	13% DTP
Corn	540	540	540	540	540
Soy	230	200	215	185	175
Wheat bran	125	115	80	70	50
DTP	0	40	70	100	130
Lime	80	80	80	80	80
Phosphorous	15	15	15	15	15
Vitamin-mineral mix ^a	10	10	10	10	10

Water was provided ad libitum, and conventional breeding procedures and management were applied throughout the experiment. The trial was carried out meeting the 'International Guiding Principles for Biomedical Research Involving Animals' as issued by the Council for International Organizations of Medical Sciences.

B. Sampling and Preparation of Egg Yolk Samples for Analysis

Fresh eggs were prepared manually by separating the yolk from the albumen. Egg yolk was later placed on filter paper for few minutes to remove the adhered albumen completely. Egg yolk thus prepared was homogenized at low speed, packed in aluminium boxes and then stored at -18°C for later freeze-drying. Frozen egg yolk was removed from freezer, cut immediately into small cubes (0.5cmx0.5cmx0.5cm), and freeze-dried (Telstar Cryodos, -70°C and 0.1 mbar).

C. Laboratory Analysis

Dried tomato peel was analysed following the methods detailed in [13], for dry matter content (DM), organic matter (OM, NF V 05-113, 1972), pH (NF V 05-108, 1970) and acidity (NF V 05-101, 1974). Total phenols content was analysed in the ethanol extract of DTP following the method described in [14], [15]. Method described in [16] was used for the dosage of total carotenoids in DTP. Briefly, 5g DTP were mixed with 50mL of organic solvent-mix (hexane, acetone, ethanol, 50:25:25 by volume) in a glass Erlenmeyer. The mixture was vortexed at 200 tour/mn for 10mn at room temperature, then centrifuged at 6500 tour/mn for 5mn at -4°C. The supernatant was recovered and diluted by adding 50mL of extraction solvent. This extract was used for measurement at 450nm by UV-Vis spectrophotometer (Hewlett Packard 8453). Total content in carotenoids was expressed as β -carotene equivalent following this formula: Total carotenoids ($\mu\text{g/g}$) = $(\text{Abs}_{450} \times F_d \times 10^6 \times V) / (3450 \times 100 \times W)$

^a Contained per kg: Ca, 138 g; P, 108 g; Mg, 151 mg; Na, 123.5 g; Cu, 119 mg; Mn, 774 g; I, 76mg; vitamin A, 12,500,000 IU; vitamin D3, 300,000 IU; vitamin E, 0.5 g.

where 3450 is the absorption coefficient of lycopene, Abs = absorbance measured at 450nm, F_d = dilution factor, V = volume (mL), W = weight of sample (g), 10^6 = conversion factor.

Egg yolk cholesterol was determined following the method described in [17]. A sample of 3g freeze-dried egg yolk was diluted with 27mL NaCl solution (20g/kg), stirred for 2h, then 1mL of the mixture (egg yolk and NaCl solution) was further diluted with 9mL NaCl solution (20g/kg). A cholesterol reagent kit (Roche Cholesterol Assay) was used for enzymatic determination using cholesterol esterase and cholesterol oxidase. The colour intensity was measured at 540nm, using a spectrophotometer (Hitachi U-2000, Japan). Total cholesterol content in egg yolk (mg/g DM) was calculated based on cholesterol concentration in the diluted mixture and yolk weight. The same extract was used for triglycerides dosage by enzymatic determination using lipoprotein lipase, glycerol kinase, glycerol 3-P-oxydase, amino-4-antipyrine and ATP (Triglyceride kit, GPO-PAP, Biomaghreb). The colour intensity was measured as well at 540nm (spectrophotometer, Hitachi U-2000, Japan).

Total phenols analysis, 1g of freeze-dried egg yolk was mixed with 10 ml of methanol/HCl (1 M) mixture (80:20, V/V adjusted to pH = 1.5). The mixture is stirred using a vortex for 2min and then centrifuged at 6000g for 10min at 4°C. Recovered supernatant was evaporated under vacuum using a rotary-evaporator at 35°C, and then the residue was reconstituted with 1ml of methanol. The suspension obtained is filtered using a filter paper (0.22 μm pore size). The extract thus obtained was used for total phenols analysis by the Folin Ciocalteu method [15]. The results were expressed as Gallic Acid Equivalents (mg GAE/100g DM). Extraction and analysis were carried out in triplicate [18].

Total carotenoids extraction and dosage in egg yolk was carried out following the method cited by [19], [20], after slight modification. Briefly, in a beaker 1 to 10g of freeze-dried egg yolk were mixed by adding 100mL of organic solvent mixture (hexane, acetone, ethanol; 50:25:25, V/V/V). The mixture was stirred for 1h30min, then filtered and poured in a separating funnel. The organic phase was washed three times before being separated and filtered through dry sodium sulphate. The filtrate obtained was adjusted to 50mL by addition of hexane. This extract was used for the determination of β -carotene and lycopene concentrations using a UV-Vis spectrophotometer (Perkin Elmer-Lambda 25) at 450 and 503 nm. Concentration of β -carotene and lycopene was calculated using the following formulas [20], where A_{450} and A_{503} represent the absorbance at 450 and 503nm, respectively.

$$\beta\text{-carotene } (\mu\text{g/mL}) = 4.624 \times A_{450} - 3.091 \times A_{503}$$

$$\text{Lycopene } (\mu\text{g/mL}) = 3.965 \times A_{450} - 0.806 \times A_{503}$$

Colour of freeze-dried egg yolk was measured in the L*, a*, b* system (refractance chromameter, CM- 2025 Minolta,

Japan). The measurement was carried out using CIE Lab system, calibrated by the «rose tile» (L^* 44.88, a^* 25.99, b^* 6.67) and light source D-65 [21], [22].

Calculation and statistical analysis: All the measurements were carried out in triplicate. Tables and figures present arithmetic means and standard deviation. Data from the feeding experiment were subjected to analysis of variance using the GLM procedure of SAS (SAS 9.3) with DTP inclusion rate as the main effect. Multiple comparisons among means were made by Turkey's procedure.

III. RESULTS AND DISCUSSION

Sun dried tomato peel was rich in crude protein (13.8 g/100g DM) and contained as secondary metabolites essentially lycopene (112mg/100g DM), β -carotene (29.5 μ g/g DM) and total polyphenols (52.8mg Gallic Acid Equivalent/100g DM).

Feed given to the five groups was isonitrogenous. Feed intake was not affected after DTP incorporation and ranged between 96 and 97% of the offered feed in the five groups. Animal performance as expressed by laying rate was not significantly different after the incorporation of DTP at 4 and 10% from the control group. When adding DTP at 7 and 13%, laying rates decreased to 89 and 91%, respectively, compared to the 1st day of supplementation.

A. Effect of DTP Feeding on Egg Yolk Cholesterol and Triglycerides Contents

Table II gives cholesterol and triglyceride contents in freeze-dried egg yolk after 21 days of feeding hens with increasing amounts of DTP. Compared to the control, a significant decrease in egg yolk cholesterol content resulted with 23%, 38%, 26% and 36%, respectively after inclusion of 4%, 7%, 10% and 13% DTP in laying hens feed ($P < 0.0001$). A maximum decrease was resulted from addition of 7% DTP in the feed.

TABLE II

EFFECT OF DTP FEEDING ON EGG YOLK CHOLESTEROL AND TRIGLYCERIDE CONTENT (MG/G DM)^{bc}

Diet	Control	4% DTP	7% DTP	10% DTP	13% DTP
Total cholesterol	21.9 $\pm 0.09^a$	17.0 $\pm 0.04^b$	13.7 $\pm 0.04^c$	16.3 $\pm 0.09^c$	14.2 $\pm 0.10^d$
Total triglycerides	241 $\pm 4.6^a$	228 $\pm 4.4^{ab}$	188 $\pm 2.3^d$	220 $\pm 5.1^{bc}$	208 $\pm 8.3^c$

Several hypotheses were proposed to explain this effect after addition of DTP. DTP contains considerable amount of total phenols (52.8mg Gallic Acid Equivalent/100g DM). A reduction in cholesterol egg yolk content was reported after adding 0.5g/kg hesperidin and naringenin in hens diet [23]. Phenols might inhibit 3-hydroxy-3-methyl-glutaryl-CoA reductase, the main enzyme that regulates the rate of biosynthesis cholesterol in the liver [24]-[26]. Lycopene was

also reported to act as a moderate cholesterol lowering agent [27], [28]. Additionally, lycopene supplementation in hens at a concentration of 200mg/kg live weight increased high density lipoprotein (HDL), while the low and very low density lipoprotein (LDL and VLDL) were reduced [29].

B. Effect of DTP Feeding on Egg Yolk Total Phenols

Total phenol content (mg GAE/100 g DM) of freeze-dried egg yolk after the addition of DTP at different inclusion rate is illustrated in Fig. 1. Inclusion of the different amount of DTP has increased total phenols content in egg yolk with 2, 4.6, 3.5 and 4.1-fold for 3%, 7%, 10% and 13% DTP, respectively, compared to the control group. The results correlated well with the cholesterol reduction, with a maxim total phenols and reduction of cholesterol content after adding 7% DTP to the diet. To the best of our knowledge, there was no literature about total phenols content in egg yolk.

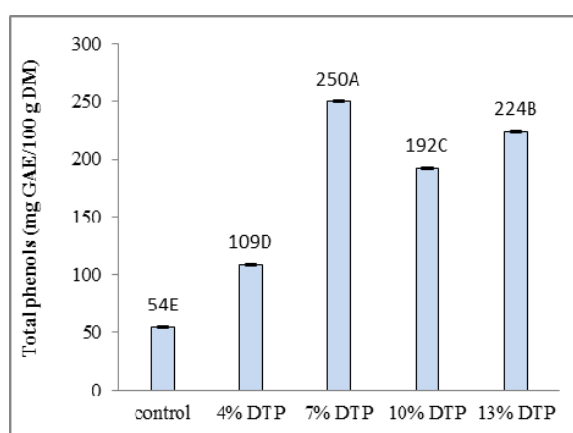


Fig. 1 Total phenol content of freeze-dried egg yolk after DTP addition if hens feed^d

C. Effect of DTP Feeding on Egg Yolk Carotenoids Contents

Incorporation of DTP in laying hen feed increased carotenoid content of egg yolk, especially at inclusion rates of 7% and 13% ($P < 0.0001$) (Fig. 2). This suggests the transfer of carotenoids including lycopene, the main carotenoid in DTP from feed to the egg. After ingestion, carotenoids are absorbed in the small intestine with other fat-soluble nutrients, then transported to the liver with proto-microns, where they are associated with lipoproteins to be transported to the egg yolk [30]. Additionally, in poultry carotenoids act as feather dyes, antioxidants, vitamin A precursors and perform different functions in the endocrine and immune system [31].

^b Table gives average values from triplicate measurements \pm standard deviation.

^c Values in the same line not followed by the same letter are significantly different after Tukey test ($p < 0.05$).

^d Values not followed by the same letter are significantly different after Tukey test ($p < 0.05$).

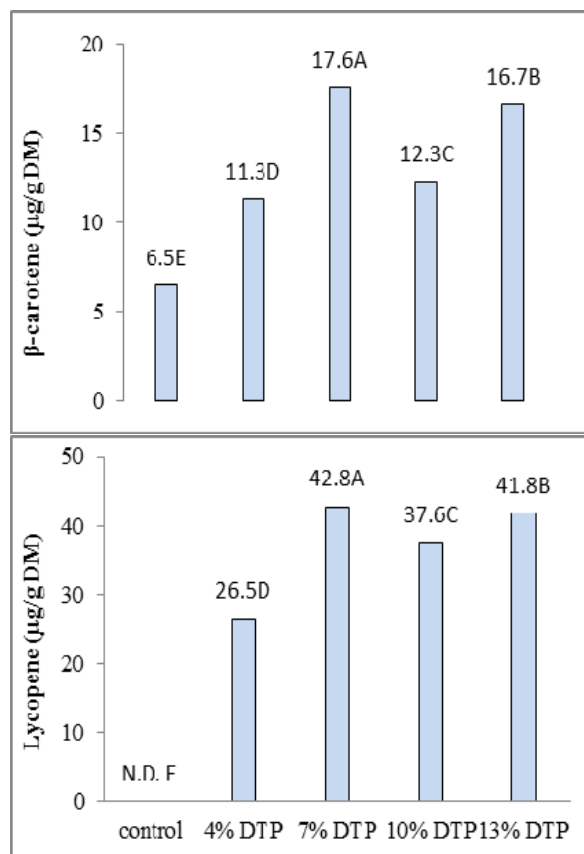


Fig. 2 β -carotene (upper) and lycopene (lower) contents of freeze-dried egg yolk after DTP addition if hens feed, measured by UV-Vis spectrophotometer

In parallel, lycopene enrichment in egg yolk followed the same trend as for β -carotene with a maximum content measured after 7% DTP inclusion ($P < 0.0001$). Lycopene ingested is the sole source of lycopene in the body and in the egg yolk [32], and its recovery in egg yolk is conditioned not only by the ingested amount but also by the transfer rate. Calculated transfer rates from the present study (based on concentration of lycopene in DTP, daily DTP intake and egg yolk lycopene content) varied between 1.86 and 3.48% after 3 to 13% DTP feeding, respectively. Previous results reported a lower rate of 0.1% after including DTP at a rate of 7.5% [33]. Similar results were obtained with Japanese quail fed diet containing 2% DTP [34]. In general, it has been reported that feed containing tomato by-products can transfer up to 5.8% of lycopene in egg yolk [33], [34]. However, no report indicates the reason or the mechanism by which β -carotene transfer rate is higher than that of lycopene. Also there is no scientific evidence about why 25% of β -carotene, and not lycopene, were converted to *cis* isomer [33].

D. Effect of DTP feeding on Egg Yolk Color

Egg yolk colour is attributed to carotenoids mainly xanthophyll, lutein, zeaxanthine and β -cryptoxanthine [22]. Table III summarizes colour index (luminosity L^* , redness a^* and yellow b^*) with colour index of egg yolk after the

different DTP inclusion rates. It is worth to indicate that these differences in colour were perceived by the eye. Results about detailed organoleptic assessment of the resulting eggs will be published separately. However, results presented in Table III, showed clearly the distinction of egg yolk after 7% DTP inclusion with the highest colour index compared to the control group and among the different treatment groups. Additionally, colour index obtained in this study are higher than that obtained in other experiments using dried tomato pomace [35].

TABLE III
EFFECT OF DTP FEEDING ON EGG YOLK COLOUR INDEX^e

Diet	Control	4% DTP	7% DTP	10% DTP	13% DTP
L^*	86.5 ± 0.02	82.5 ± 0.07	76.4 ± 0.04	78.4 ± 0.02	81.1 ± 0.03
a^*	6.53 ± 0.02	10.5 ± 0.03	15.9 ± 0.01	11.9 ± 0.02	12.6 ± 0.02
b^*	43.6 ± 0.02	49.4 ± 0.04	52.6 ± 0.04	50.9 ± 0.02	48.4 ± 0.03
ΔE	97.1 ± 0.02	96.7 ± 0.03	94.2 ± 0.02	94.2 ± 0.02	95.2 ± 0.02
Colour index	8.5 ± 0.02	12.0 ± 0.03	16.8 ± 0.01	13.2 ± 0.02	14.6 ± 0.03

IV. CONCLUSION

Dried tomato peel is an appreciable source of carotenoids, especially lycopene, phenols, and crude protein. DTP supplementation is a simple and convenient strategy to transfer carotenoids and phenols to the egg, adding value to an already a valuable nutritional and functional food. This is all the more true when the supplementation did not affect either feed intake or animal performance. Eggs rich in lycopene, β -carotene, and phenols and low in cholesterol and triglycerides resulted from laying hens fed a diet with dry tomato peel. The optimal incorporation rate was 7% DTP.

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^e Table gives average values from triplicate measurements \pm standard deviation.

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