Enriching Egg Yolk with Carotenoids & Phenols

Amar Benakmoum, Rosa Larid, and Sofiane Zidani

Abstract—Dried tomato peel (DTP) was tested in vivo (n=10) in 2-week-old laying hens at rates of 0, 40, 70, 100 and 130 g/kg DM feed. After 21 days, feed intake was not affected after DTP incorporation at 4 and 10% from the control group. Egg yolks resulting from DTP-enriched diets, contained lower amounts of cholesterol (14 to 17 mg/g) and triglyceride (188 mg/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 μg/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 bonds can be converted to trans form) is attached to its membranes [3]. It

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 ground 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.

| 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*        | 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). 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.

\[
\text{Total carotenoids (µg/g)} = \frac{(\text{Abs}_{450} \times F_d \times 10^6 \times V)}{(3450 \times 100 \times W)}
\]

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 (0.22µm 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 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 at 540nm, using a spectrophotometer (Hitachi U-2000, Japan). 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 used to reach a total of 120 g/animal/day.

\[
\text{β-carotene (µg/mL)} = 4.624 \times A_{450} - 3.091 \times A_{503}
\]

\[
\text{Lycopene (µg/mL)} = 3.965 \times A_{450} - 0.806 \times A_{503}
\]

Colour of freeze-dried egg yolk was measured in the L*, a*, b* system (refractance chromometer, CM- 2025 Minolta,
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 μg/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 TDP at 4 and 10% from the control group. When adding TDP 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.

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 TDP 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.

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].
were converted to evidence about why 25% of is higher than that of lycopene. Also there is no scientific reason or the mechanism by which lycopene in egg yolk [33], [34]. However, no report indicates that feed containing tomato by-products can transfer up to 5.8% of 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 lycopene 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 that obtained in other experiments using dried tomato pomace [35].

![Graph showing β-carotene and lycopene contents](image)

$$\text{Fig. 2 } \beta\text{-carotene (upper) and lycopene (lower) contents of freeze-dried egg yolk after DTP addition if hens fed, measured by UV-Vis spectrophotometer.}$$

### Table III

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

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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References


© Table gives average values from triplicate measurements ± standard deviation.


