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Carotenoid Absorption in Chicken Intestine

R. Gómez, A. Alonso and M. Martín

Departamento de Bioquímica
Facultad de Ciencias
Bilbao (España)

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The powdered flowers of marigold *(Tagetes erecta)* are used as a cheap source of carotenoids in avicultura. Lutein (3,3'-dyhydroxi-α-carotene) constitutes up to 85 to 90 % of marigold carotenoids. In the plant, lutein is found esterified to palmitic or estearic acid. In chicken, carotenoid is hydrolized in the first portion of the small intestine, and absorbed as free lutein. After the absorption, lutein is not re-esterified in the different chicken tissues.

Carotenoids are used as dietary additives in aviculture, in order to obtain yellow-coloured fat and eggs (1). They are frequently, administered in the form of marigold *(Tagetes erecta)* petal powders. In this powdered preparation, lutein (3,3'-dyhydroxi-α-carotene) constitutes up to 85 to 90 % of the total carotenoid content (4). In the plant, most of lutein is found esterified to one or two fatty acids (2).

The object of this paper is to describe the chemical modifications, absorption and fate of the carotenoid in the digestive tract of chicken.

The chemical hydrolysis of the diesterified lutein was also studied for comparison with the hydrolysis as performed under physiological conditions.

Materials and Methods

Hubbard chickens *(Gallus domesticus)*, 2-3 weeks old, were used throughout this work. In order to avoid interferences from previously absorbed carotenoids, the animals were fed for two weeks on a «bleaching diet», consisting of fish and wheat flours in equal parts. This diet is but slightly coloured, and the corresponding pigments do not interfere in any way with lutein in the chromatographic analysis. Foeces were analyzed daily for their content in carotenoid pigments: foeces (approx. 1 g) were weighed and treated with 5 ml of acetone, with shaking. Hexane (2 ml) was added, and the mixture shaken. Finally, the extract was washed with 5 ml distilled water and left until phase separation occurred. The hexane layer, which contained the pigment, was then carefully removed, and diluted to 10 ml with hexane. Absorption spectra were recorded in the visible range with the aid of a Beckman dual-beam grating spectrophotometer. For quantitative purposes, absorbance was read at 475 nm.

After two weeks of feeding on the bleaching diet, the chickens were fed on the same mixture supplemented with 5 % marigold petal powder and sacrified after 4 hours. The digestive tract was quickly excised and divided into the following fragments: crop, gizzard, intestine, caecum, and rectum. In each fragment, the tissue was separated from the partially digested food. The different tissues were homogenized in acetone with a Waring blendor. Carotenoids were extracted from tissues and ingesta as described for foeces.

The extracted carotenoids were separated into free, mono and diesterified lutein by TLC with silicagel «G», with ethyl ether-hexane (7:3), as described previously (2). The carotenoids were then eluted from the silicagel following the extraction procedure described above, and quantified by measuring absorbance at 475 nm.

The chemical hydrolisis of the carotenoids was carried out as described by QUACKENBUSH (3, 4) with methanolic KOH.

Results and Discussion

Figure 1 shows the decrease in carotenoid content of chicken foeces, following the bleaching treatment. A corresponding loss of pigmentation was simultaneously observed in peaks and legs. *Post-mortem* observations at this stage also revealed pale yellow fat, instead of the usual intense yellow colour. Remarkable changes could also be observed in the absorption spectra of the hexane-acetone extract of foeces along the bleaching treatment. According to these, most of the original pigmentation could be atributed to lutein, where at the end of the treatment the spectra did not correspond to any of the marigold pigments.

The time course for the chemical hydrolysis of esterified lutein was studied at 25°. Hydrolysis was complete in about 1 h. 50 % hydrolysis was reached in about

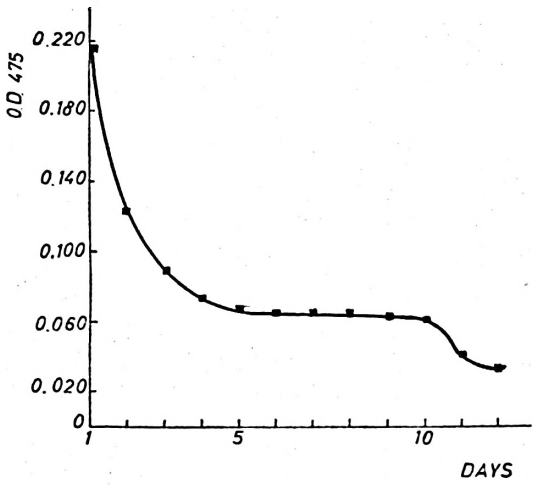

Fig. 1. *Decrease in carotenoid content of chicken foeces, as O.D. at 475 nm, along the bleaching treatment.*

15 min. A transient monoesterified species was formed but readily hydrolized.

Hydrolisis of marigold lutein in the digestive tract of chicken is shown in figure 2. The graph corresponds to mean values of 20 experiments. Apparently, hydrolysis took place in the first portion of the intestine. In the following segments, a relative increase in diesterified form

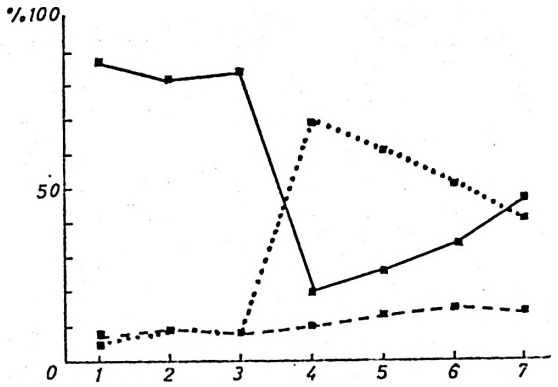

Fig. 2. *Hydrolysis of marigold carotenoids in the different segments of the chicken digestive tract.*

1. Diet. 2. Crop. 3. Gizzard. 4. Intestine (1st. portion). 5. Intestine (2nd. portion). 6. Intestine (3rd. portion). 7. Caecum. —— diesterified lutein; ---- monoesterified lutein; .... free lutein.

could be seen, which was attributed to the absorption of the free lutein. The free carotenoid could be recovered as such in the intestinal wall or in the liver of marigold treated chickens. The pigment content of the intestinal wall (1st portion) before and after the ingestion of marigold powders, expressed as $\mu g/g$ of organ, was respectively of 1.2 ± 0.08 and 4.4 ± 0.8.

In a separate series of experiments, bleached chickens were fed on marigold flour and allowed to live for several days, so that their fat was again coloured with lutein. The carotenoids were extracted and analyzed by thin-layer chromatography. Non-esterified lutein was the only pigment to be found in all cases. The same was true for lutein extracted from intestinal wall, liver and blood.

The above experiments could be interpreted in terms of enzymatic hydrolysis of diesterified lutein taking place in the first portion of the small intestine. Hydrolysis would be followed by intestinal absorption and distribution of the pigment. The pigment would not be re-esterified after the absorption.

These results should not be extrapolated to other speceis. Carotenoid absorption seems to be rather peculiar in the different species. Several attemps were carried out in our laboratory in order to induce carotenoid absorption in albino Wistar rats. All of them proved unsuccessful.

Resumen

La harina de flor de clavelón *(Tagetes erecta)* constituye una fuente económica de carotenoides, utilizada en avicultura. El 85-90 % de los carotenoides del clavelón son derivados de la luteína (3,3'-dihidroxi-α-caroteno). En la planta, la mayor parte de la luteína se presenta esterificada a los ácidos palmítico o esteárico. En el pollo, el carotenoide es hidrolizado en la primera porción del intestino delgado, y absorbido como luteína no esterificada. Después de la absorción, la luteína no se reesterifica en los tejidos del pollo.

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