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Lutein and zeaxanthin content in corn imported from three countries of the American continent and in corn cultivated in Colombian territory

[*Conteúdo de luteína e zeaxantina em milho importado de três países do continente americano e em milho cultivado em território colombiano*]

Y.J. *Aguillón-Páez*, G.J. *Díaz* .

Laboratorio de Toxicología, Facultad de Medicina Veterinaria y de Zootecnia, Universidad Nacional de Colombia, Bogotá, 111321, Colombia

ABSTRACT

Lutein and zeaxanthin are the major xanthophyll pigments found in corn kernels. These pigments provide the orange-red color of the broiler chicken skin and of the egg yolk. Therefore, knowing the corn xanthophyll content is important for the poultry feed producer. The objective of this study was to determine the lutein and the zeaxanthin content in corn cultivated in Colombia and in corn imported to Colombia from the United States, Argentina, and Brazil. Large differences in total lutein plus zeaxanthin content were found among the corn samples analyzed, with the highest mean level found in Colombian corn (2,758µg/100g), followed by Argentina (1,861µg/100g), United States (1,041µg/100g) and Brazil (947µg/100g). Large differences in lutein plus zeaxanthin content were also found among different corn hybrids cultivated in Colombia. Differences among geographical regions might be due to differences in UV-B radiation or in the light hours received by the crop during its growth. The differences among different corn hybrids might probably be due to genetic differences. Corn growers might be interested in cultivating hybrids higher in lutein and zeaxanthin as these pigments are very important in poultry production and human eye health.

Keywords: corn, *Zea mays*, Carotenoids, Xanthophylls, animal feed

RESUMO

Luteína e zeaxantina são os principais pigmentos xantofilas encontrados nos grãos de milho. Em aves, esses pigmentos naturais conferem a cor vermelho-alaranjada típica da pele do frango de corte e da gema do ovo. Assim, é importante conhecer o teor de xantofilas do milho utilizado nas dietas de aves. O objetivo deste estudo foi determinar o teor de luteína e zeaxantina em milho cultivado na Colômbia e em milho importado para a Colômbia dos Estados Unidos, da Argentina e do Brasil. Foram encontradas diferenças significativas no teor de luteína total mais zeaxantina entre as amostras de milho analisadas, com o nível médio mais elevado no milho colombiano (2.758µg/100g), seguido pelo milho argentino (1.861µg/100g), pelo milho dos Estados Unidos (1.041µg/100g) e pelo milho brasileiro (947µg/100g). Grandes diferenças no teor de luteína e zeaxantina também foram encontradas entre variados híbridos de milho cultivados na Colômbia. As diferenças entre as regiões geográficas podem ser devido a diferenças na radiação UV-B ou nas horas de luz recebida pela cultura durante seu crescimento. As diferenças entre os diversos híbridos de milho provavelmente podem ser devido a diferenças genéticas. Os produtores de milho podem estar interessados em cultivar híbridos mais ricos em luteína e zeaxantina, pois esses pigmentos são muito importantes na produção de aves e na saúde ocular humana.

Palavras-chave: milho, *Zea mays*, carotenóides, xantofilas, alimentação animal

Corresponding autor: yaguillon@unal.edu.co
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INTRODUCTION

Corn (*Zea mays*) is a major crop in many countries where it is grown not only as human food but also as animal feed (Ranum *et al*., 2014). Annual corn production in Colombia is very low (about 920,000 tons per year), and far from the demand of approximately 7 million tonnes per year (Histórico…, 2022a). A high demand and low local production results in the need for animal feed producers to import corn, mostly from the United States. In 2021, about 3.9 million tons arrived in Colombia from the United States, about 1.0 million tons from Argentina and 703,000 tons from Brazil (Histórico…, 2022b).

Corn is not only a source of carbohydrates, protein, and fat, but it also contains carotenoids, a family of hydrophobic compounds with a variety of vital roles in all photosynthetic organisms (Serna-Saldivar, 2018; Wise and Hoober, 2007). Carotenoids contribute to the photosynthetic assembly through light harvesting (by absorbing a broader arrange of wavelengths in the blue region of the visible spectrum than chlorophyll) and transferring the energy to chlorophyll; further, carotenoids provide protection from excess light via energy dissipation and free radical detoxification, limiting potential damage to membranes (Wise and Hoober, 2007).

The xanthophylls lutein and zeaxanthin are the major carotenoids in yellow corn and they are also the major pigments present in the *macula lutea* of the human eye, where they provide protection against harmful radiations and free radical formation (Bernstein *et al*., 2016). Corn carotenoids are synthesized from isopentenyl diphosphate (IPP) and its isomer dimethylallyl phosphate (DMAPP) (Fig. 1), through reactions catalyzed in the plastids, chloroplasts or chromoplasts (Ordoñez and Rodriguez, 2013). Four IPP molecules and one DMAPP molecule produce geranylgeranyl pyrophosphate, and the condensation of two of these molecules result in phytoene, the first uncolored carotenoid this is a limiting step that is regulated by external stimuli like temperature, drought, and light exposure (Cazzonelli and Pogson, 2010). Phytoene undergoes a series of reactions to form lycopene (the first coloured carotenoid), which in turn can take two different biochemical routes by which either end of the molecule suffers cyclization to form either δ-carotene or γ-carotene (Sajilata *et al.,* 2008). Lutein is synthesized from δ-carotene, through the intermediate metabolites α-carotene, and zeinoxanthin, whereas zeaxanthin is produced from γ-carotene, through the metabolites β-carotene, and β-cryptoxanthin (Sajilata *et al.,* 2008). In photosynthetic tissues and germinating seedlings, the synthesis of carotenoids and chlorophylls and their subsequent binding to pigment-binding proteins must be precisely balanced to meet the appropriate photosynthetic demands on a daily and seasonal basis (Wise and Hoober, 2007). These carotenoids exist in plants as the all-*trans* geometric form (Updike and Schwartz, 2003). Lutein and zeaxanthin content in corn is very significant in poultry feeds because they contribute to the pigmentation of the skin in broiler chicks and the egg yolk in laying hens (Castañeda *et al.,* 2005).

Achieving proper pigmentation of poultry products is very important in several countries due to the need to meet consumer preferences. Since the synthesis and accumulation of lutein and zeaxanthin is under the regulation of several factors, it is conceivable to expect differences in the content of these carotenoids in different corn crops. The purpose of this study was to determine and quantitate the lutein and zeaxanthin content in samples of corn imported to Colombia from temperate zones (USA, Argentina, and Brazil) and in corn cultivated in Colombia (a tropical country). Potential differences in corn carotenoid content from different varieties and different countries can be very significant for countries like Colombia, in which the poultry feed industry depends almost entirely on imported corn.

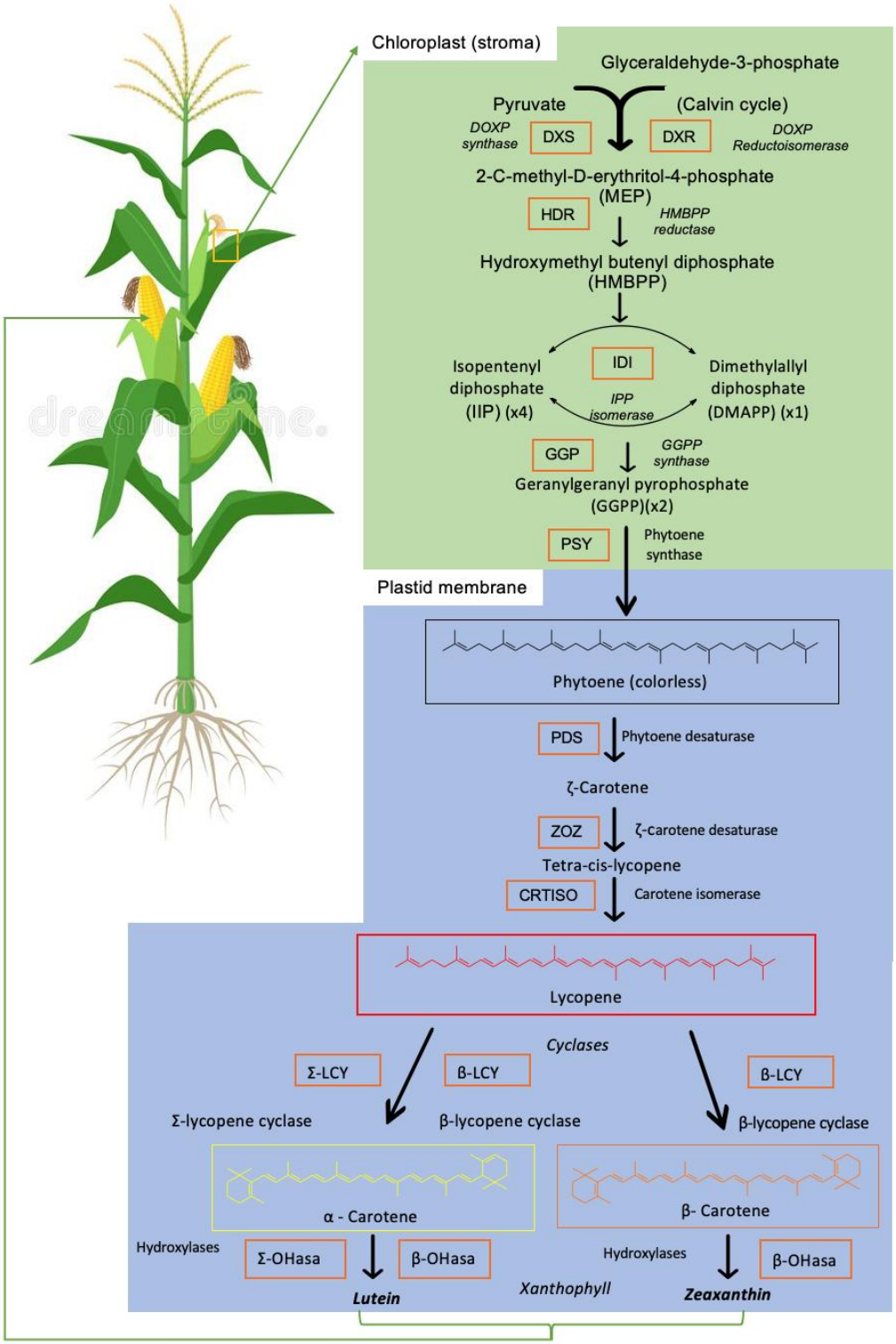

Figure 1. Biosynthesis of the xanthophylls lutein and zeaxanthin in corn. The orange squares correspond to the enzymes involved in the reactions. DOXP: 1-deoxy-D-xylulose-5-phosphate.

MATERIALS AND METHODS

Sampling was carried out by the "Federación Nacional de Cultivadores de Cereales y Leguminosas" (FENALCE). Samples of imported whole kernel corn were collected at the three major Colombian sea ports (Cartagena, Santa Marta, and Buenaventura) over a one-year period. Samples were taken from a minimum of ten sampling points, with each subsample of 500g, for a total of 5kg. Colombian whole kernel corn samples were collected in the same way in the agricultural regions of Valle del Cauca, Meta, and Tolima. The samples of corn cultivated in Colombia corresponded to corn that had been harvested between two and three months prior to sampling and processing. Samples were packed in paper bags to prevent moisture condensation and fungal degradation, and they were sent to the laboratory immediately after collection. Information about storage times, harvest dates or specific corn hybrid types was not possible to obtain for the imported samples. The total number of samples collected and analyzed were 32 from Colombia, 32 from the United States (USA), 8 from Argentina and 5 from Brazil. Unfortunately, it was not possible to obtain a higher number of samples from Brazil and Argentina since the Colombian feed industry relies mostly on corn imported from the USA (due to a free-trade agreement signed between the two countries) rather than from other countries.

In a separate sampling group, 63 samples of corn cultivated in Colombia were analyzed to investigate possible differences in xanthophyll content among different corn hybrids. These samples corresponded to the hybrids Pioneer P30F35 (n=16), Advanta ADV 9293 (n=15), Dekalb DK-7088 (n=9), Pioneer P30F35 VYHR (n=7), Semillas Valle SV 3245 (n=6), Fenalce FNC 8134 (n=6), and DOW 810 (n=4). Unfortunately, it was not possible to obtain the same number of samples for each hybrid because some hybrids are more common among corn growers than others.

The carotenoid extraction procedure was based on a previously published methodology (Perry *et al.*, 2009), with minor modifications. Table 1 summarizes the sample preparation procedure.

Table 1. Sample preparation for the determination of lutein and zeaxanthin in corn

1. Weigh 1 g of finely ground corn in a 10 mL borosilicate culture tube with a Teflon-lined screw cap and add 5 mL of methanol. Homogenize in vortex stirrer. Leave at 4°C overnight.
2. Remove from the refrigerator and centrifuge at room temperature at 1400 x g (3000 rpm on a Hitachi centrifuge Model 05P-21) for 10 minutes; transfer the supernatant (methanol) to a 25 mL graduated flask.
3. Extract the pellet again with 5 ml tetrahydrofuran (THF), shake in vortex for 30 seconds, centrifuge at 1400 x g for 10 minutes and combine the supernatant with the first extraction.
4. Repeat step 3 twice more.
5. Combine all supernatants in the 25 mL graduated flask and take to volume (25 mL) with THF.
6. Transfer 300µL of the dilute extract into a 2.0mL silanized autosampler vial and add 10µL of a 1mg/mL ascorbic acid solution.
7. Add 1190µL of mobile phase A for a final volume of 1.5 mL and homogenize in vortex.
8. Inject 10 µL into the liquid chromatograph.

The HPLC method was based on a previously published methodology (Yeum *et al.*, 1996). Lutein and zeaxanthin were separated on a Phenomenex Develosil 5µm RP-Aqueous C30, 140 Å, 250 x 4.6 mm I.D. analytical column, protected by a Phenomenex RP-C18 4 x 3.0 mm I.D. guard column (Phenomenex, Torrance, CA, USA), both kept at 16ºC. The separation was carried out using a gradient of two mobile phases at a flow rate of 1 ml/min, as follows: the starting composition was a mix of 90% mobile phase A (methanol:methyl-tert-butyl ether:1.5% ammonium acetate in water; 83:15:2, v/v/v) and 10% mobile phase B (methanol:methyl-tert-butyl ether:1.0% ammonium acetate in water; 8:90:2, v/v/v); this step was followed by a linear gradient from 10 to 45% B in 5 min, then a linear gradient from 45 to 95% B in 5min, followed by 5 min at 95% B, after which the composition returned to the initial step (10% B) and was equilibrated for 10 min before the following injection. HPLC analyses was conducted on a Shimadzu Prominence system (Shimadzu Scientific Instruments, Columbia, MD, USA) equipped

with a DGU-20A3R degassing unit, two LC-20AD pumps, a SIL-20AC$_{HT}$ autosampler, a CTO-20A column oven, an SPD-20AV visible-ultraviolet spectrophotometric detector, and a CBM-20A bus module, all controlled by "LC Solutions" software. Absorbance was monitored at 445 nm for lutein and 450 nm for zeaxanthin, and the analytes were identified and quantitated by means of external standards of known purity, prepared as described below. Fig. 2 shows a chromatogram of a standard mix of lutein and zeaxanthin and of a corn sample containing both analytes.

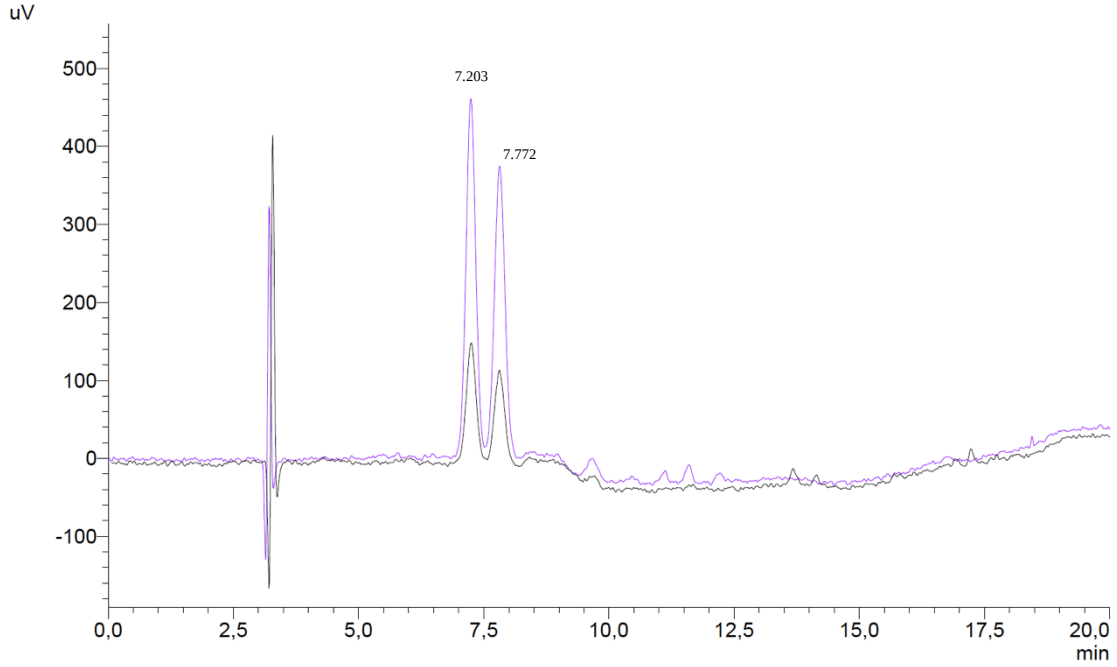

Figure 2. Representative HPLC chromatograms of a lutein and zeaxanthin standard mixture (0.1µg/mL of each compound, violet line) and a corn sample containing 382 and 328µg/100g of lutein and zeaxanthin, respectively (black line). Peak 1 (t_R = 7.203 min) corresponds to lutein (monitored at 445nm) and peak 2 (t_R = 7.772 min) corresponds to zeaxanthin (monitored at 450nm).

Lutein and zeaxanthin are unstable and light sensitive, and all necessary precautions were taken to prevent their degradation prior to the analysis. Lutein and zeaxanthin standards were purchased from Fermentek Ltd. (Jerusalem, Israel). The lutein standard (Lot No. LU002) purity was 96.25%, whereas the zeaxanthin purity was 98.44% (Lot No. ZEA001). Stock standard solutions were prepared by weighing 2mg of each xanthophyll, which were then dissolved in 25 tetrahydrofuran (THF) stabilized with 0.025% 2,6-di-tert-butyl-4-methylphenol (BHT) using a 25mL amber volumetric flask. The stock solutions contained about 80µg/mL of each xanthophyll.

A total of 5.0µg of lutein and of zeaxanthin taken from the stock solution were diluted to 5mL with stabilized THF in 7mL amber vials; the final concentration was 1.0µg/mL for each analyte. The calibration curve was prepared by pipetting 10, 20, 40, 80 or 100 µL of the working solution into 1.5mL autosampler vials, to which 10µL ascorbic acid in methanol were added as an antioxidant and taken to a final volume of 1.0mL using mobile phase A as solvent. The linear regression equations for the lutein and the zeaxanthin calibration curves were as follows: y = 70372x – 135.72 for lutein and y = 71573x – 95.848 for zeaxanthin. In both cases, the linear regressions had r^2 values of 0.99.

The limit of detection (LOD) for lutein and zeaxanthin of the analytical technique was calculated based on the standard deviation of the response (Sy) of the calibration curves and the

slope of the calibration curve (S) at levels approximating the LOD according to the formula: LOD = 3.3(Sy/S) (Shrivastava and Gupta, 2011). The limit of quantitation (LOQ) was calculated as three times the LOD. The calculated LOD and LOQ for lutein and zeaxanthin were 8 and 24ng/mL in vial, respectively, and were identical for both compounds.

Data was analyzed using a non-parametric one-way analysis of variance procedure (Kruskal-Wallis) using Statistix® Version 9 at a significant level of 0.05. Means were separated using the Kruskal-Wallis all-pairwise comparison test.

RESULTS

The average content of lutein and of zeaxanthin in corn samples cultivated in the Northern hemisphere (USA), the Southern hemisphere (Argentina and Brazil) and the tropics (Colombia) are shown in Fig. 3. Corn grown in the Northern and Southern hemispheres had similar lutein/zeaxanthin ratios: 0.9, 1.2, and 1.0, for the USA, Argentina, and Brazil, respectively. However, the lutein to zeaxanthin ratio of the corn cultivated in Colombia was only 0.25, reflecting a higher predominance of zeaxanthin in this corn. Average lutein content was highest in the Argentinian corn (1028µg/100 g), lowest in the Brazilian corn (485µg/100 g) and intermediate for the Colombian (543µg/100g) and USA corn (693µg/100g). Average zeaxanthin content was significantly higher in Colombian corn (P<0.05) compared with corn imported from Argentina, Brazil and the USA. The average zeaxanthin concentration in Colombian corn was 2,215µg/100g, corresponding to 2.6 times then content of the Argentinian corn, 3 times as much compared to the USA corn and 4.8 times higher than the Brazilian corn (Fig. 3).

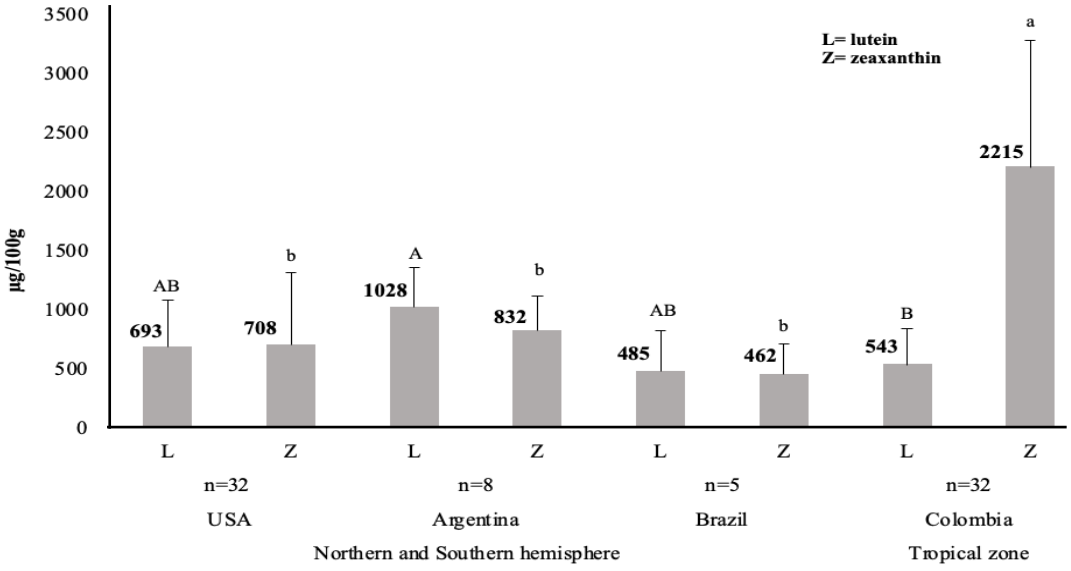

Figure 3. Average±S.D. of the lutein (L) and zeaxanthin (Z) content in corn samples from the USA, Argentina, Brazil, and Colombia. Means with different superscripts differ significantly (P < 0.05).

Regarding the sum of lutein plus zeaxanthin content in corn cultivated in different countries, it was observed that the corn cultivated in Colombia had a significantly higher content (P<0.05) of these two xanthophylls compared to the USA and Brazilian corn (Fig. 4). The average lutein plus zeaxanthin content in Colombian-grown corn was 2758µg/100g, followed by Argentinian corn (1861µg/100g), corn grown in the USA (1041µg/100 g) and Brazilian corn (947µg/100g).

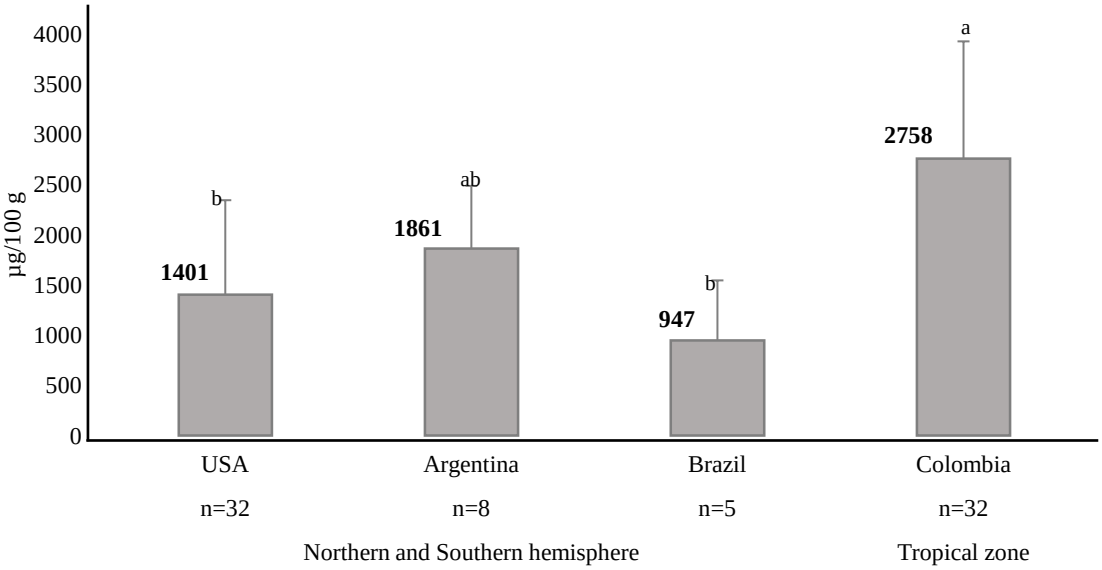

Figure 4. Total lutein plus zeaxanthin content in corn from USA, Argentina, Brazil, and Colombia. Values are means and error bars represent standard deviation. Means with different superscripts differ significantly ($P < 0.05$).

The analysis conducted on the different corn hybrids cultivated in Colombia showed large differences in the individual xanthophyll content, as well as in the sum of lutein plus zeaxanthin. Fig. 5 shows the individual lutein and zeaxanthin content of the seven different hybrids grown in Colombia. Zeaxanthin was the predominant carotenoid in all hybrids, although the ratio zeaxanthin/lutein was very variable. For example, in the Advanta ADV 9293 hybrid this ratio was 6.4, while in the Semillas Valle SV 3245 hybrid it was only 2.0. The zeaxanthin content was highest in the Advanta ADV 9293 hybrid, followed by the Fenalce FNC 8134, Semillas Valle SV 3245, Pioneer P30F35, Pioneer P30F35 VYHR, DOW 810, and Dekalb DK-7088. The only hybrid with zeaxanthin content below 1,000µg/100g was the Dekalb DK-7088 (811µg/100g). Regarding the lutein content, the highest levels were found in the Colombian hybrids (Semillas Valle SV 3245 and Fenalce FNC 8134), with 813 and 787µg/100 g, respectively. However, no significant differences in lutein content were found among any hybrids except for the Colombian hybrids when compared to the Dekalb DK-7088 hybrid. A three-fold difference in average lutein content was found between the highest (813 µg/100 g for the Semillas Valle SV 3245 hybrid) and lowest level (253 µg/100 g for the Dekalb DK-7088 hybrid).

The average ± S.D. lutein plus zeaxanthin content found in the seven corn varieties grown in Colombia is shown in Fig. 6. Significantly higher levels of the sum of these xanthophylls ($P < 0.05$) were found for the Advanta ADV 9293 hybrid when compared to the Pioneer P30F35, Pioneer P30F35 VYHR, DOW 810, and Dekalb DK-7088 hybrids. No significant differences in the average lutein plus zeaxanthin content were found between de Advanta ADV 9293 hybrid (3,427µg/100g), the Fenalce FNC 8134 hybrid (2,916µg/100g) and the Semillas Valle SV 3245 (2,402µg/100g).

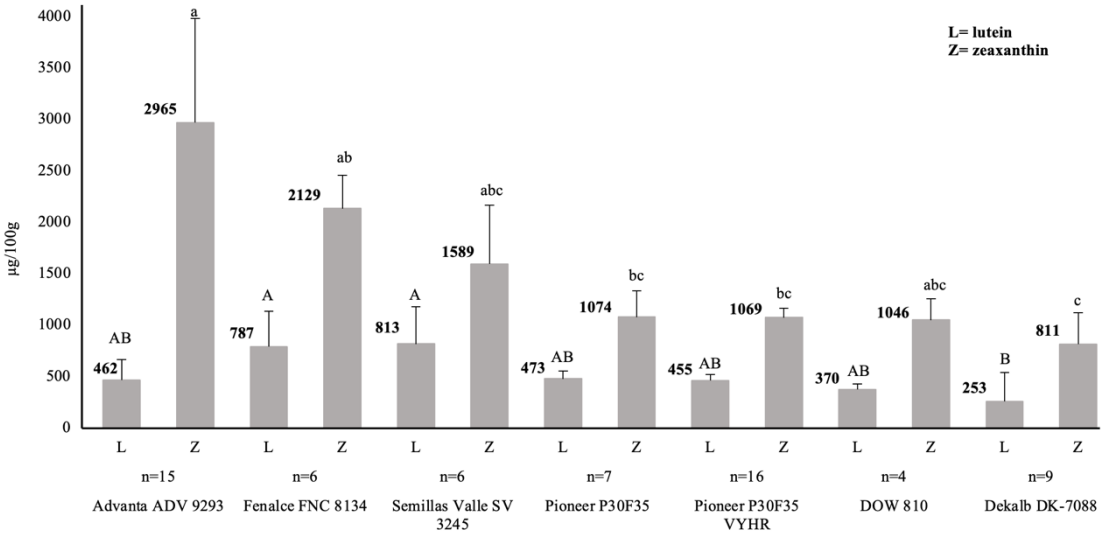

Figure 5. Lutein and zeaxanthin levels in seven different corn hybrids grown in Colombia. Values are means and error bars represent standard deviation. Means with different superscripts differ significantly ($P < 0.05$).

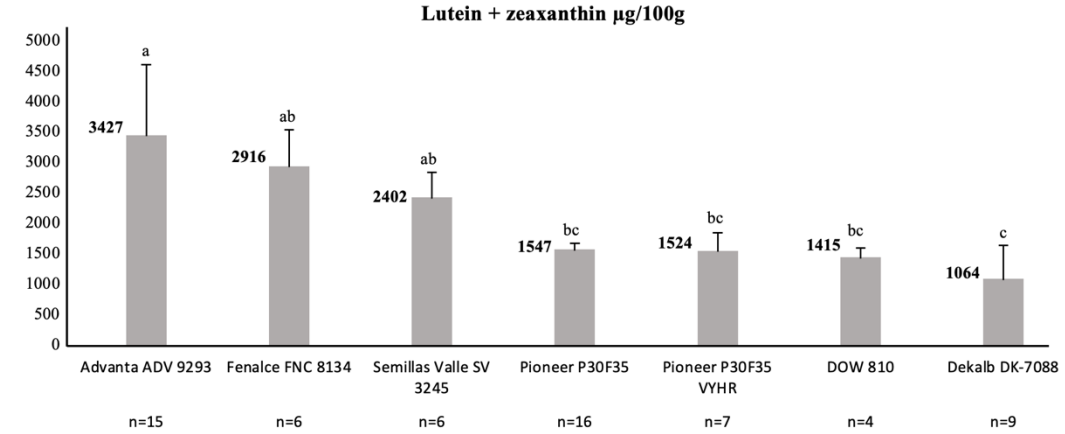

Figure 6. Total xanthophyll content (lutein plus zeaxanthin) in seven different corn hybrids grown in Colombia. Values are means and error bars represent standard deviation. Means with different superscripts differ significantly ($P < 0.05$).

The present study evaluated the lutein, zeaxanthin, and lutein plus zeaxanthin content of corn samples obtained from the USA, Argentina, Brazil, and Colombia. Regarding the lutein plus zeaxanthin content, the results showed lower levels than those reported in previous studies conducted in Brazil and the USA. In one study, lutein plus zeaxanthin content in Brazilian corn cultivars averaged 1,980 µg/100 g (Paes *et al*., 2009), while in another trial the total lutein and zeaxanthin content in USA cultivars was 1,932µg/100g (Moros *et al*., 2002). In the present study the lutein plus zeaxanthin content in Brazilian and USA corn were 1401 and 947µg/100g, respectively. One possible reason for this apparent discrepancy could be the freshness of the grain. It may have been possible that the USA and Brazilian samples analyzed in the present study had been stored for longer periods of time than the ones analyzed in previous studies, and it is known that storage causes a decrease in total xanthophyll content in

corn. In a study conducted in Israel, fresh local yellow corn had a 31.2% decrease in total xanthophyll content when stored for 3 months under good storage conditions and 48.2% when stored under adverse storage conditions (Bartov and Bornstein, 1967). However, this explanation is purely speculative since the storage times and harvest dates were not possible to trace for the imported corn samples. Regarding individual xanthophylls, the average lutein and zeaxanthin content in USA-grown corn were 693 and 708µg/100g, respectively. In a previous study, the contents of *trans*-lutein and *trans*-zeaxanthin in "cooked from frozen corn" were 202 and 202µg/100g, respectively (Perry *et al.*, 2009). The lower carotenoid concentration reported by Perry *et al*. (2009) could have been the result of losses caused by freezing, cooking or both. On the other hand, in another study, the average lutein and zeaxanthin content in raw corn grown in the USA was 1,447 and 523µg/100g, respectively (Moros *et al*., 2002).

The present study revealed large differences in lutein and zeaxanthin accumulation in corn cultivated in different geographical regions. For instance, corn from Argentina and the USA had higher lutein content than Brazilian or Colombian corn. These particular differences could possibly be attributed to the number of light-hours per day received by the crop during its growth. While in Argentina and the USA the crop usually gets between 14 and 15 light-hours/day, in Colombia it receives only 12 hours/day (World weather, 1995). In a study conducted with pumpkins in the USA, plants that received full sunlight accumulated 20, 92, and 98% more lutein than plants that received 58, 13 and 3% light (Logan *et al*., 1998). Another possible explanation for the difference in corn lutein content found in corn from different countries could be the specific UV radiation received by the plants during their development. The effect of UV-A and UV-B radiation on lutein accumulation was investigated in one study conducted with eight varieties of green leaf lettuce (Caldwell and Britz, 2006). One group of plants received both supplemental UV-B (290–320 nm) and UV-A (320–400 nm) radiation, a second group received only supplemental UV-A, and a third group (greenhouse controls) received no UV supplementation. Growth under supplemental UV-A plus UV-B (but not with only UV-A) increased the lutein levels in all the green leaf lettuce varieties. Since the corn from Argentina had higher average levels of lutein than the corn grown in Colombia or Brazil (1028 vs 543 and 462µg/100g, respectively), it could be possible that the UV-B radiation received by the plants grown in Argentina could have been higher than that received by the plants in Colombia and Brazil. Another possible explanation could be differences in the genetics of the hybrids grown in Argentina vs. the hybrids grown elsewhere. On the other hand, the average zeaxanthin content in Colombian-grown corn was 2.7, 3.1 and 4.8 times higher than the average content in Argentinian, USA, and Brazilian corn, respectively. It would be interesting to investigate whether this effect is due to differences in UV radiation or due to differences in the light-hours/day during the crop growth.

One interesting finding of the present study was the large differences in individual xanthophylls as well as in lutein plus zeaxanthin content among the different corn hybrids cultivated in Colombia (lutein + zeaxanthin content ranged from 1002 to 3427µg/100g). These differences could be due to the specific genetics of each corn variety since several studies conducted with corn germplasm and F1 hybrids have revealed large variations in carotenoid content and composition (Wurtzel, 2004). The genetic control of carotenoid deposition indicates that there is the possibility to improve the content and composition of carotenoids in the corn endosperm if the appropriate genes are selected or introduced. Carotenoids accumulate throughout the corn seed in amyloplasts, mainly in the endosperm and to a lesser extent in the embryo. Accumulation in the endosperm of the developing kernel starts 10-15 days after pollination (DAP) and it can reach a maximum at around 20-25 DAP, depending on the corn variety and environmental conditions (Wurtzel, 2004). It is also possible that different corn hybrids have different carotenoid accumulation patterns due to specific differences in the pathways involved in carotenoid synthesis and accumulation. Regardless of the rationale behind the differences in xanthophyll content among different corn hybrids, the results of the present study are of practical importance for both the poultry industry and the chicken and egg consumers. The cultivation and usage in poultry diets of corn varieties richer in xanthophyll

content could have a two-fold advantage: First it would mean greater pigmentation in the skin of broiler chickens and in the egg yolk of laying hens; second, a greater amount of xanthophylls in the yolk egg and chicken fat results in a healthier food for the consumer, since both lutein and zeaxanthin are key compounds involved in the protection of the eye's *macula densa*.

Since lutein and zeaxanthin are important for both poultry production and human health, it is important to monitor the level of these xanthophylls in corn. From the results of the present study, it can be concluded that different corn hybrids accumulate different amounts of lutein and zeaxanthin, as well as corn grown in different geographical regions. The differences in lutein and zeaxanthin accumulation among different corn hybrids is probably due to specific genetic differences, while the differences between different geographical regions could be due to UV-B radiation or the light hours received by the crop during its growth; this would explain why corn grown in the Northern and Southern hemispheres tend to have a higher lutein content than corn grown in the intertropical zone; on the other hand, corn grown in the tropics tend to have a higher content of zeaxanthin, and it would be interesting to investigate the reason for the much higher zeaxanthin to lutein content found in this corn.

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