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Carotenoid content and deposition efficiency in yolks of laying hens fed with dent corn hybrids differing in grain hardness and processing

Dora Zurak,[*] Zlatko Svečnjak,[*] Veronika Gunjević,[*] Goran Kiš,[*] Zlatko Janječić,[*] Vasil Pirgozliev,[†] Darko Grbeša,[*] and Kristina Kljak[*,1]

[*]*University of Zagreb Faculty of Agriculture, Zagreb 10000, Croatia; and* [†]*National Institute of Poultry Husbandry, Harper Adams University, Newport TF10 8NB, United Kingdom*

ABSTRACT Yolk carotenoid profile reflects the hen diet when corn grain is the only source of carotenoids, but corn origin and processing may affect carotenoid utilization. In the present study, 2 commercial dent corn hybrids differing in grain hardness (soft- and hard-type) were dried at low (40°C) and high (85°C) temperature and ground through a 5- and 9-mm sieve to investigate their effects on carotenoid bioavailability in laying hens. With 3 hens per cage, 168 Lohmann Brown laying hens were allocated to 8 dietary treatments (2 hybrids × 2 drying temperatures × 2 grinding sieves) in a completely randomized design (8 treatments × 7 cages). The trial lasted 8 wk, during which eggs were collected for analysis every 3 d until carotenoid content stabilized, and then once a week until the end of the experiment. The carotenoid profile of the experimental diets and yolks was analyzed using an HPLC method and deposition efficiency was calculated based on carotenoid contents, yolk weight, egg production and diet intake. The deposition efficiency for lutein, zeaxanthin, α- and β-cryptoxanthin, and β-carotene averaged 27.37, 18.67, 6.29, 3,32, and 0.94%, respectively. As expected, the tested hybrids highly affected the carotenoid content in egg yolk due to their differences in carotenoid profile. Interestingly, hard- and soft-type hybrids differed in the deposition efficiency for all individual carotenoids but not for the total carotenoids. High grain drying temperature tended to increase the bioavailability of lutein and zeaxanthin in both hybrids. For the hard-type hybrid, the content of β-carotene in egg yolk was higher when grains were dried at a high temperature, while the opposite response was found in the soft-type hybrid. The effect of grinding sieve size was important for the zeaxanthin bioavailability in the soft-type hybrid only. In conclusion, our findings showed that corn hybrid had a primary influence on the carotenoid content in the yolks of laying hens, but grain processing may change the bioavailability of carotenoids.

Key words: corn hybrid, grain hardness, carotenoids, egg yolk, laying hen

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INTRODUCTION

Carotenoids are a group of lipid-soluble pigments that are synthesized in plants, fungi, bacteria and algae. In contrast, animals and humans ingest carotenoids through their diet, where they provide various health benefits. The common functions of carotenoids are antioxidant effects, anti-inflammatory activity and enhanced immune function. Through these functions, they have a positive effect on cognitive function and cardiovascular health and may contribute to the reduction of some types of cancer in humans (Eggersdorfer and Wyss, 2018). In addition, certain carotenoids such as β-carotene protect the skin from oxidative damage, improve fertility and convert to retinol, an essential component of the visual pigment rhodopsin, or retinoic acid, which regulates gene expression (Eggersdorfer and Wyss, 2018; Blaner, 2020). Others, such as lutein and zeaxanthin, play an important role in protecting the macula and the outer segments of the retina from oxidative stress. In animals, they not only improve immune defense and fertility, but are also important for signaling (Blount, 2004) and for the quality of their products, such as meat and eggs in poultry (Nabi et al., 2020).

Among the cereals most commonly used in laying hen diet, yellow corn is considered a suitable alternative source of carotenoids. Although corn genotypes differ in grain carotenoid content (Zurak et al., 2021), their inclusion in laying hen diets has shown promising potential for improving both carotenoid content and yolk pigmentation (Kljak et al., 2012; 2021). However, previous

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[1]Corresponding author: kkljak@agr.hr

studies reported a wide range of results among the genotypes studied (Liu et al., 2012; Moreno et al., 2020; Ortiz et al., 2021; 2022), suggesting that other dietary factors such as grain processing may affect carotenoid bioavailability. Priyadarshani (2017) indicated that the structure of the feed matrix and the way it is processed are the first steps that precede the utilization of carotenoids. Thus, thermal and mechanical processing of corn grains with different proportions of floury and vitreous endosperm may lead to different changes in physical and chemical properties (Kirleis and Stroshine, 1990; Odjo et al., 2015), potentially affecting the bioavailability of carotenoids.

After physiological maturity, corn grains are dried either in the field or, more commonly, in hot-air dryers at 50 to 130°C until the moisture content reaches about 12 to 15%, and then used as animal feed (Odjo et al., 2015). Although carotenoids are sensitive to heat treatment (Rodriguez-Amaya, 2016). Burt et al. (2010) found no greater loss of carotenoids at 90°C than at 25°C in the corn genotypes studied. However, increasing the drying temperature has different effects on the physical properties of corn genotypes with different endosperm hardness (Kirleis and Stroshine, 1990) and leads to changes in the structure and functionality of starch granules, proteins and lipids (Gehring et al., 2013; Malumba et al., 2014; Odjo et al., 2015). This has been shown to affect nutrient utilization in poultry (Huart et al., 2018; Córdova-Noboa et al., 2020; 2021a) and could therefore likely affect the bioavailability of carotenoids from corn.

On the other hand, reducing particle size through grinding is a common practice in diet production as it aims to disrupt the seed coat and expose the endosperm matrix. However, grinding seems to have minimal effect on carotenoid content and composition, as all parts of the grains and not only some of their fractions are usually used in the laying hen diets (Mugode et al., 2014; Blandino et al., 2017). Grinding size affects nutrient digestibility and digestive efficiency (Safaa et al., 2009; Bozkurt et al., 2019; Ege et al., 2019), and it has been demonstrated that grinding grains with different endosperm hardness and drying at different temperatures results in different particle fragmentation (Singh and Ravindran, 2019; Córdova-Noboa et al., 2021b), highlighting their importance for corn utilization and their potential impact on carotenoid bioavailability in laying hens.

Most commercial corn hybrids used in laying hen diets are of the dent type, but they differ in the ratio between hard and soft endosperm, which affects the physicochemical properties of the grain, processing behavior and nutrient utilization (Kirleis and Stroshine, 1990; Safaa et al., 2009; Kljak et al., 2018; Bozkurt et al., 2019). A recent study by Saenz et al. (2021) found higher levels of total carotenoids and β-branch carotenoids (zeaxanthin, β-cryptoxanthin and β-carotene) in grains from hard endosperm genotypes, while soft endosperm genotypes tended to have higher levels of α-branch carotenoids (lutein, α-cryptoxanthin and α-carotene). However, little information is available on the effects of grain hardness and processing on the bioavailability of carotenoids in corn hybrids. Therefore, the aim of this study was to investigate the carotenoid content and deposition efficiency in the yolks of laying hens fed 2 dent corn hybrids differing in grain hardness, drying temperature and grinding sieve size.

MATERIALS AND METHODS

The animal experiment was conducted in accordance with the Croatian directives (Animal Protection Act, OG 102/17, and Regulation on the Protection of Animals Used for Scientific Purposes, NN 55/13; NN 39/17), which correspond to the European guidelines for the care and use of animals used for scientific purposes. The animal procedures used in this study were approved by the Ethics Committee for the protection of animals used in scientific research within the Ministry of Agriculture of Republic of Croatia (EP 349/2022).

Corn Hybrids, Grain Processing Management and Treatment diets

The corn hybrids used in the study were selected on the basis of previous research. Firstly, a total of 103 hybrids were evaluated for their physical-chemical properties, carotenoid profile and in vitro bioaccessibility (Zurak et al., 2021; Zurak et al., 2024), and 15 hybrids were selected for the trial with hens. Based on the results of the trial with laying hens (D. Zurak, unpublished data), 2 hybrids were selected for the current study. Hybrids selected for this research were characterized by a relatively similar total carotenoid content but different profiles. In addition, these hybrids differed in grain hardness determined using the method described by Pomeranz et al. (1986). Samples of 20 g were ground at 3,600 rpm using a hammer mill (Polymix PX-MFC 90D, Kinematica, Segrate, Italy) equipped with a 2 mm sieve. The time required to grind 17 mL of grits, the height of the grits in the grinding column, and the ratio of coarse (>0.7 mm) to fine particles (<0.5 mm) were measured. The total mass obtained by grinding 20 g of the sample was sieved through 0.7 and 0.5 mm sieves (AS 200 Basic, Retsch, Haan, Germany) to determine the coarse-to-fine ratio. A harder hybrid has a longer time to grind 17 mL of grits, a lower height of the grits, and higher coarse-to-fine ratio (Figure 1). According to the grain hardness of the 2 hybrids, the hybrid with softer grains was labelled as the soft-type, while the hybrid with harder grains was labelled the as the harder-type.

The tested corn hybrids were grown on the same test field in central Croatia near Zagreb in the 2022 growing season. Each hybrid was planted on a 70 m wide and 50 m long plot under the same agro-climatic conditions, following the recommendations of seed companies for seeding density and using an intensive production system (Svečnjak et al., 2004). At harvest, corn cobs were collected from 5 locations (representing 5 replicates) across the plot for hardness analysis and then crop was mechanically harvested. Then, the grain of both hybrids

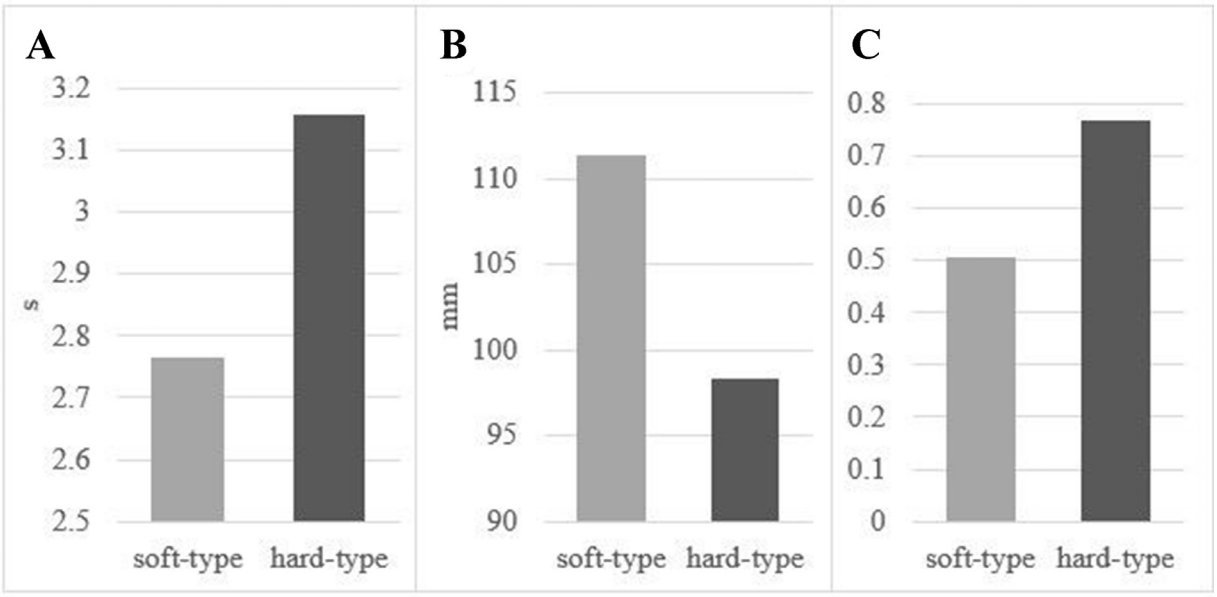

Figure 1. Difference between the tested hybrids in the hardness parameters according to the method described by Pomeranz et al. (1986). (A) time required to grind 17 mL of grits, (B) height of the grits in the grinding column, (C) ratio of coarse (> 0.7 mm) to fine particles (< 0.5 mm).

was divided into 2 lots. The first batch was dried at a low temperature (40°C) and the second at a standard (high) temperature (85°C) until the moisture content reached about 12%. After drying, the corn grains of both hybrids were divided into 3 more batches and packed in storage bags until laying hen diets were produced.

Dietary treatments were formulated according to the National Research Council (1994) and adapted to Lohmann Brown laying hens according to the management guide (Lohmann Breeders GmbH, 2022) for nutrient supply to laying hens in the initial phase of egg production (19 to approximately 50 wk of age). To reduce differences in nutrient composition, the basal mixture contained all the ingredients for laying hen diets from the same batch, except for corn grains, which were the only pigment sources in the experimental diets. Immediately prior to the start of the feeding trial, 3 batches of both hybrids were transported to a feed mill near Zagreb, Croatia, and ground through sieves of 5 and 9 mm. Then, the grains of each commercial hybrid (combination of drying at 40°C and 85°C and grinding at a sieve size of 5 and 9 mm) were mixed with the basal mixture at the same proportion (60%; Table 1), resulting in 8 experimental diets. The analyzed nutrient content of experimental diets is shown in Table 2. Experimental diets were sieved using a sieve shaker (AS 200, Retsch, Haan, Germany) on the following set of sieves: 2000 μm, 1250 μm, 630 μm, 300 μm, 160 μm, and pan. A 100 g sample in duplicate was sieved for 10 min at an amplitude of 80%. The proportion of each fraction (>2,000 μm, 2,000−1,250 μm, 1,250 −630 μm, 630−300 μm, 300−160 μm, and <160 μm) was calculated from weight retained on the lower sieve, and the particle size distribution of experimental diets is shown in Figure 2.

Hens, Housing and Experimental Design

A total of 168 Lohmann Brown laying hens at 18 wk of age were obtained from a commercial hatchery. Upon arrival at the experimental poultry house of the University of Zagreb Faculty of Agriculture, the hens were weighed and randomly allocated in groups of 3 in 56 enriched cages (Council Directive 1999/74/EC). The cages were divided into 4 freestanding laying batteries and provided 1,269 cm^2 per hen, a feeder on the outside of the cage front (minimum 12 cm/bird), nipple-type drinkers (2 drinkers per cage), a perch at least 15 cm long per hen, and a claw shortening device. Eggs laid by the hens were collected in a wired egg cradle placed parallel to and below the feeder. The experimental house had a fully automated temperature control system with temperature sensors placed throughout the house to monitor conditions and adjust ventilation accordingly.

Table 1. Ingredient composition of the experimental diets.

Ingredient	Content (%)
Corn hybrid	60
Soybean meal	26.2
Sunflower oil	3.0
Calcium carbonate	8.8
Monocalcium phosphate	1.2
Sodium chloride	0.4
DL methionine	0.15
Vitamin premix[1]	0.12
TRT Poultry Pack[2]	0.13

[1]The vitamin premix provided per kg of diet: vitamin A 10,000 IU, vitamin D3 2,500 IU, vitamin E 200 IU, vitamin K3 3 mg, vitamin B1 1 mg, vitamin B2 45 mg, vitamin B3 30 mg, vitamin B5 10 mg, vitamin B6 3 mg, vitamin B7 50 mg, vitamin B9 0.5 mg, vitamin B12 25 mg, choline 400 mg, antioxidant (BHA, EQ) 50 mg.

[2]TRT Poultry Pack (Alltech Ireland Ltd., Dunboyne, Ireland) provided per kg of diet: I 1 mg, Fe 5 mg, Cu 5 mg, Mn 30 mg, Zn 30 mg, Se 0.2 mg.

Table 2. Analyzed nutrient content of experimental diets.

Dent hybrid	Grain drying temperature	Grinding sieve	Moisture	Ash	Crude protein	Crude fat	Crude fiber	Neutral detergent fiber	Starch
			%						
Soft-type	40°C	5 mm	10.7	12.7	17.2	5.4	2.4	8.3	39.8
		9 mm	10.4	12.8	17.8	5.6	2.3	7.6	40.9
	85°C	5 mm	9.7	12.4	17.3	6.0	2.3	7.3	39.2
		9 mm	9.6	13.0	17.5	5.7	2.4	7.9	39.2
Hard-type	40°C	5 mm	10.3	12.7	17.5	5.6	2.4	7.8	38.6
		9 mm	10.3	13.0	17.3	5.9	2.6	7.9	41.0
	85°C	5 mm	10.1	12.4	17.3	5.1	2.4	7.9	41.7
		9 mm	9.4	13.0	17.2	6.3	2.2	8.4	38.6

Throughout the experimental period, the ambient temperature was 18±2°C and the light period consisted of 16 h of light per day. Diet and water were provided ad libitum to the hens.

Prior to the feeding trial, a 4-wk depletion period began during which all hens were fed a white corn-based diet without added pigment, which had the same ingredient composition as the experimental diets (Table 1). After depletion, cages were assigned to one of 8 dietary treatments in a completely randomized block design with 7 replicates per dietary treatment, resulting in a 2 × 2 × 2 factorial arrangements. The experimental period lasted 8 wk and was divided into a period of stabilization of carotenoid content in the yolks (3 wk) and a sampling period (5 wk), during which the number and weight of the eggs were recorded daily, and the diet intake was recorded weekly. Based on obtained results, ADFI, egg production, egg weight, daily egg mass and FCR were calculated, and results are showed in Suppl. Table S1. Laying hens were additionally weighed at the beginning (22 wk of age) and at the end (30 wk of age) of the 56-d trial period.

Sample Collection

From the beginning of the feeding trial, 7 eggs per treatment (one egg per cage) were collected every 3 d to determine the total carotenoid content in yolks and the stabilization of the carotenoid content. The carotenoid content stabilized by the end of wk 3, after which 21 eggs per treatment (3 eggs per cage) were collected once per week until the end of the trial. Eggs were analyzed in the shortest possible time and stored at 4°C if necessary. The collected eggs were cracked immediately before analysis and the yolks were separated from the whites and dried on a paper napkin. After the yolks were separated, their weight was recorded. To determine the stabilization of carotenoid content, each yolk was analyzed individually. During the sampling period, 3 yolks from each cage were combined for carotenoid analysis, resulting in 7 samples per treatment each week. The same combined yoks were used to determine the Commission Internationale de l'Eclairage (**CIE**) color scale for the lightness (L*), redness (a*), and yellowness (b*) using a colorimeter (model CR-410, Minolta Co. Ltd., Osaka, Japan), and the obtained results are presented in Supplementary Table S2.

Diet samples were collected at the beginning of the experimental period. The samples for each experimental diet consisted of subsamples from 5 bags and were stored at -20°C until the carotenoid analysis. Prior to analysis, diet samples were ground in a laboratory mill (Cyclotec 1093, Foss Tocator, Hoganas, Sweden) using a 0.3 mm sieve. All samples were analyzed for dry matter content (DM), which was determined by drying 3 g of each sample for 4 h at 103°C according to the method ISO 6496:1999.

Carotenoid Analysis

Experimental Diets: Carotenoids from the experimental diets were extracted and quantified according to the procedure described by Kurilich and Juvik (1999), using β-apo-carotenal as an internal standard. Each sample was analyzed in triplicate, and the mean value was taken as the result. The grinned samples of 600 mg were homogenized with 6 mL of ethanol containing 0.1% butylated hydroxytoluene (**BHT**) and then precipitated in a water bath at 85°C (5 min) before saponification with 120 μL 80% of potassium hydroxide for 10 min. All samples were vortexed once during saponification. Upon removal, the test tubes were cooled in an ice bath with the addition of deionized water (3 mL). Then, 3 mL of hexane was added to each sample, and they were vortexed and centrifuged at 2,200 × *g* for 5 min (Centric 322A, Tehtnica, Železniki, Slovenia). The upper hexane layer was then pipetted into a separate tube and the extraction procedure was repeated until the upper hexane layer was colorless (about 4 extractions). The collected supernatants were evaporated using rotary vacuum concentrator (RVC 2-25CD plus, Martin Christ, Osterode am Harz, Germany) and dissolved in 200 μL acetonitrile:dichloromethane:methanol (45:20:35, v/v/v) containing 0.1% BHT.

Lutein, zeaxanthin, α- and β-cryptoxanthin, and β-carotene in the extracts were quantified according to the reversed-phase HPLC method described by Kurilich and Juvik (1999). Carotenoids were separated and quantified using a SpectraSystem HPLC instrument (Thermo Separation Products, Inc., Waltham, MA) equipped with a quaternary gradient pump, an autosampler and a UV-vis detector. Compounds were separated on 2 sequentially connected C18 reversed-phase columns:

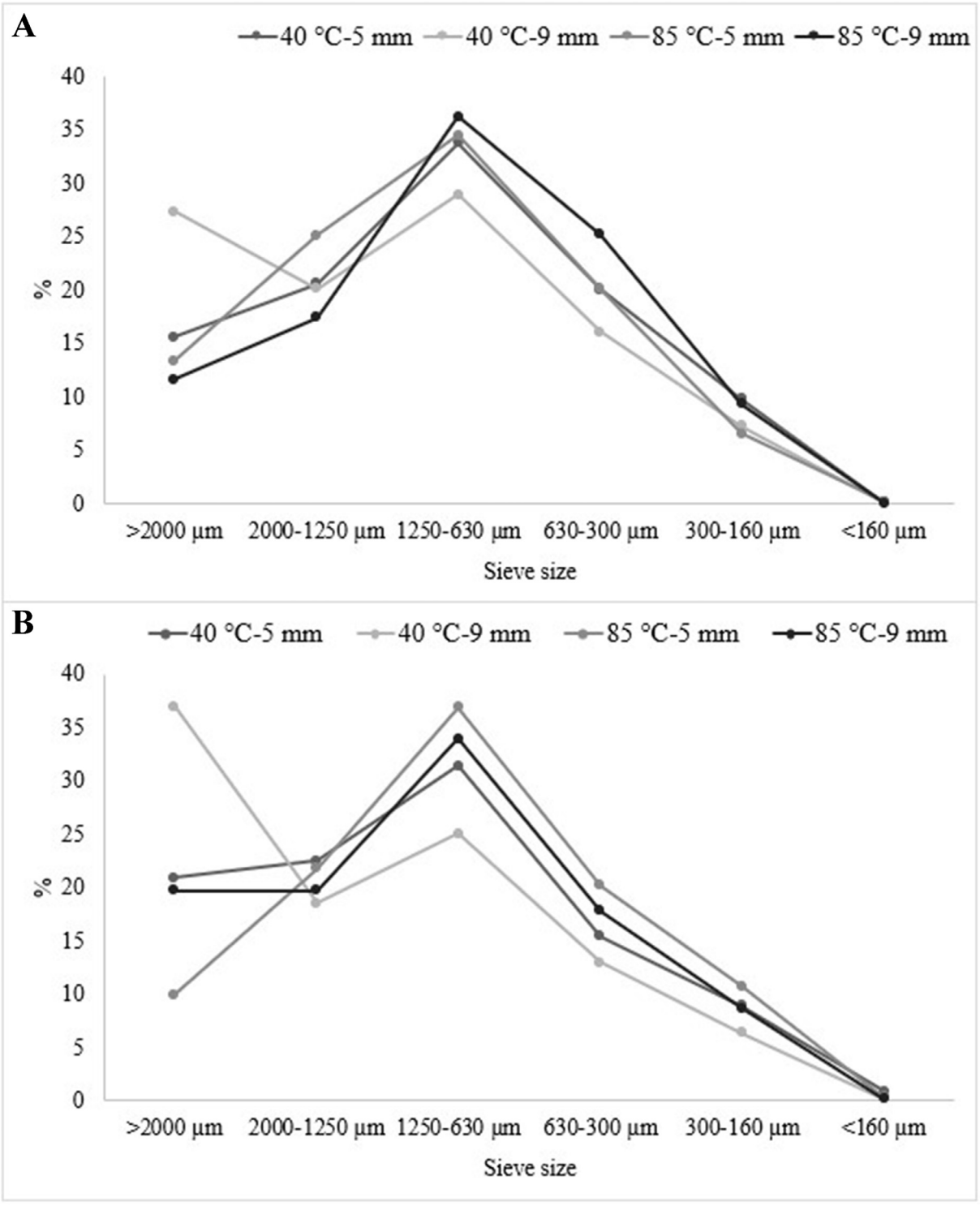

Figure 2. Particle size distribution of experimental diets based on 2 corn hybrids differing in grain hardness (A − soft-type, B − hard type), grain drying temperature (40°C or 85°C) and grinding sieve size (5 or 9 mm).

Vydac 201TP54 column (5 μm, 4.6 × 150 mm; Hichrom, Reading, UK), followed by a Zorbax RX-C18 column (5 μm, 4.6 × 150 mm; Agilent Technologies, Santa Clara, CA). The separation columns were protected by a Supelguard Discovery C18 guard column (5 μm, 4 × 20 mm; Supelco, Bellefonte, PA). The mobile phase consisted of acetonitrile:methanol:dichloromethane (75:25:5, v/v/v) containing 0.1% BHT and 0.05% triethylamine. An aliquot of 30 μL was injected, and the flow rate was 1.8 mL/min. The separations were performed at room temperature, and carotenoids were monitored at 450 nm.

Carotenoids [lutein (purity 99%), zeaxanthin (purity 99%), α- and β- cryptoxanthin (purity of both 99%), and β-carotene (purity 98%)] were identified by comparing their retention times and quantified by external standardization with calibration curves using commercially available standards (Extrasynthese, Genay, France; r2

≥ 0.99 for all carotenoids). The total carotenoid content was calculated by summing the contents of the individual carotenoids.

Egg Yolks During the Stabilization Period (From d 0 to d 21 of the Trial Period): The spectrophotometric method described by Surai et al. (2001) was used to determine the stabilization of carotenoid content in egg yolks. The yolk samples (200−500 mg) were homogenized with 2 mL of a 1:1 (v/v) mixture of 5% sodium chloride solution and ethanol, followed by adding 3 mL of hexane and further homogenization for 3 min. After centrifugation (5 min, 2,200 × *g*), the extract was collected in a 10 mL volumetric flask, and the extraction was repeated until the upper layer was colorless. The combined extracts were then diluted to volume with hexane and their spectrum was measured between 400 and 500 nm (Helios γ, Thermo Electron Corporation, UK). The absorbance at the maximum was used and the total carotenoid content was calculated as β-carotene equivalents (μg/g) using the β-carotene calibration curve with concentrations between 0.2 and 2.5 mg/L.

Egg Yolks During the Sampling Period (From d 21 to d 56 of the Trial Period): Quantification of carotenoids from egg yolks collected from d 21 to the end of the experiment was performed using the reversed-phase HPLC method described previously (Section Experimental Diets) following the extraction described in Section Egg yolks according to Surai et al. (2001) with some differences; 200 mg of the combined egg yolk sample was taken for analysis. After the extraction procedure, the combined hexane extracts were evaporated using a rotary vacuum concentrator (RVC 2-25CD plus, Martin Christ) and reconstituted in 300 μL acetonitrile: dichloromethane:methanol (45:20:35, v/v/v) containing 0.1% BHT.

Carotenoid Deposition Efficiency

The carotenoid deposition efficiency for each cage within the dietary treatment and for each week of sampling period was calculated using the following equation (Karadas et al., 2006):

$$\text{Carotenoid deposition efficiency (\%)} = \frac{\text{Carotenoid production by egg}}{\text{Carotenoid consumption by diet}} \times 100$$

where carotenoid production by eggs and consumption by diet were calculated using the following equations:

$$\text{Carotenoid production by egg} = \text{yolk weight (g)} \times \text{yolk carotenoid content } (\mu\text{g/g}) \times \text{egg production}$$

$$\text{Carotenoid consumption by diet} = \text{diet intake (g/d/hen)} \times \text{diet carotenoid content } (\mu\text{g/g})$$

based on the data obtained in the hen trial and after sample analysis.

Statistical Analysis

Statistical analyses of the obtained results were performed using SAS statistical software (version 9.4; SAS Institute Inc., Cary, NC). The dietary experiment was conducted as a completely randomized design with a factorial arrangement of 2 hybrids, 2 grinding sieve sizes and 2 grain drying temperatures with 8 dietary treatments. Each dietary treatment had 7 cages with 3 hens. Differences between the dietary treatments were subjected to an analysis of variance using the MIXED procedure, with corn hybrid, grain drying temperature and grinding sieve size and as fixed effects using repeated measurements ANOVA, with results obtained from the 4th wk and until the end (8th wk) of the dietary experiment. Mean values were defined by the least squares means statement and compared using the PDIFF option; letter groups were determined using the PDMIX macro procedure. Tukey multiple comparisons test at $P = 0.05$ was used for means of dietary treatments.

RESULTS

Carotenoid Content in Experimental Diets

The carotenoid profile of the 8 experimental diets is shown in Table 3. In general, the total carotenoid content ranged from 20.09 to 22.73 μg/g DM, with zeaxanthin and lutein being the predominant carotenoids (on average 10.55 and 6.19 μg/g DM). Nevertheless, the lutein content in the experimental diets consisting of a

Table 3. Average content (μg/g DM) of total and individual carotenoids in experimental diets.

Dent hybrid	Grain drying temperature	Grinding sieve	Lutein	Zeaxanthin	α-cryptoxanthin	β-cryptoxanthin	β-carotene	Total carotenoids
Soft-type	40°C	5 mm	8.37	9.75	1.19	2.31	0.87	22.49
		9 mm	8.06	9.31	1.30	2.52	0.86	22.05
	85°C	5 mm	7.92	8.91	1.33	2.71	0.86	21.73
		9 mm	8.58	9.58	1.33	2.42	0.83	22.73
Hard-type	40°C	5 mm	4.31	12.15	0.45	3.19	0.97	21.08
		9 mm	4.11	11.70	0.59	3.31	1.12	20.83
	85°C	5 mm	4.10	11.54	0.45	3.17	1.21	20.47
		9 mm	4.04	11.42	0.45	3.22	0.96	20.09

soft-type hybrid was almost twice as high as in the diets based on a hybrid of higher hardness, which in turn had an approximately 1.3-fold higher zeaxanthin content. With a range of 2.31 to 3.31 μg/g DM, β-cryptoxanthin was the following carotenoid in all experimental diets. The content of α-cryptoxanthin was higher in experimental diets consisting of hybrid with lower grain hardness, whereas those containing harder grain hybrid had a higher content of β-carotene. Overall, the experimental data based on the same hybrid had a similar total carotenoid content but a different carotenoid profile.

Effect of Grain Processing on the Carotenoid Content of Egg Yolk

On average, zeaxanthin was the carotenoid with the highest content in the egg yolks of hens fed 8 dietary treatments in the present study (on average 18.28 μg/g). Lutein was on average 20% lower than zeaxanthin, and these 2 carotenoids were predominant. β-cryptoxanthin was detected at a 2-fold higher content than α-cryptoxanthin (on average 0.835 vs. 0.462 μg/g), while β-carotene was below 0.1 μg/g in the egg yolks of most dietary treatments. The yolk of hens fed soft- and hard-hybrid-based diets contained 2.18- and 3.02-fold higher lutein content than the diets themselves, respectively, although the lutein content of hard-hybrid-based diets was twice as high. In contrast, the zeaxanthin content in the yolk was 1.73 times higher than in the diets, but with no differences between the hybrids tested (Tables 3 and 4A).

In general, corn hybrid was the main effect influencing the content of all individual carotenoids in egg yolk (Table 4A). Consistent with dietary carotenoid content, yolks from hens fed dietary treatments based on harder corn hybrid had higher content of zeaxanthin (20.23 vs. 16.32 μg/g) and β-cryptoxanthin (0.927 vs. 0.743μg/g) and a lower content of lutein (12.31 vs. 17.92 μg/g), α-cryptoxanthin (0.37 vs. 0.56 μg/g) and β-carotene (0.059 vs. 0.103 μg/g). In addition, grain drying temperature affected the content of all carotenoids, resulting in higher contents in the egg yolks from hens fed diets based on grain dried at 85°C. Grinding sieve size only affected zeaxanthin content ($P = 0.014$), with higher contents in the yolks from hens fed diets prepared by grinding corn grain using the 5-mm sieve. However, a tendency to affect was observed for β-cryptoxanthin and β-carotene ($P = 0.065$ and 0.086, respectively). For total carotenoids, corn hybrid and grain drying temperature affected the content, with higher contents in the yolks from hens fed diets based on the softer grain hybrid (35.65 vs. 33.92 μg/g, $P = 0.002$) and the yolks from hens fed diets based on the grain dried at 85°C (35.56 vs. 34.01 μg/g, $P = 0.006$), whereas grinding sieve size resulted in similar contents (Tables 4A and 4B).

Of the interactions, hybrid × drying temperature and hybrid × grinding sieve were significant for some carotenoids while drying temperature × grinding sieve was significant only for zeaxanthin and total carotenoids. In agreement with the identified interactions, the hybrids differed in the content of β-carotene and β-cryptoxanthin in egg yolk as a function of grain drying temperature. In general, the content of β-carotene decreased with increasing grain drying temperature in the diets with soft-type hybrid, while it increased in those with hard-type hybrid. In diets with grain dried at 40°C, the content of total carotenoids was significantly higher for diets whose grain was ground using a 5-mm sieve than with the 9-mm sieve. For zeaxanthin and total carotenoids, higher contents were detected in egg yolks when grain was ground using a 9-mm sieve in diets based on grain dried at 85°C. A significant hybrid × drying temperature × grinding sieve interaction was found only for the content of α-cryptoxanthin and total carotenoids.

Effect of Grain Processing on the Efficiency of Carotenoid Deposition in Egg Yolk

In contrast to the content in egg yolk, the deposition efficiency of lutein in yolks from hens fed 8 dietary treatments in the present study was lower than that of zeaxanthin (on average 27.38 and 18.67%). A similar relationship as for content was observed for α- and β-cryptoxanthin, and the deposition efficiency of α-cryptoxanthin was higher than that of β-cryptoxanthin (on average 6.29 vs. 3.32%). Consistent with the extremely low content in the egg yolk, the deposition efficiency of

Table 4A. Analysis of variance for the carotenoid content in yolks of laying hens with their means (μg/g) for the main factors.

Source of variation			Lutein	Zeaxanthin	α-cryptoxanthin	β-cryptoxanthin	β-carotene	Total carotenoids
Hybrid (H)			<0.001	<0.001	<0.001	<0.001	<0.001	0.002
Grain drying temperature (DT)			0.036	0.026	<0.001	<0.001	0.017	0.006
Griding sieve (GS)			0.791	0.014	0.145	0.065	0.086	0.185
H × DT			0.054	0.258	0.558	0.019	<0.001	0.162
H × GS			0.904	0.016	0.491	0.588	0.004	0.152
DT × GS			0.059	0.037	0.271	0.223	0.057	0.027
H × DT × GS			0.077	0.094	0.006	0.873	0.784	0.046
Dent hybrid	Grain drying temperature	Grinding sieve						
Soft-type			17.92	16.32	0.56	0.74	0.103	35.65
Hard-type			12.13	20.23	0.37	0.93	0.059	33.92
	40°C		14.83	17.91	0.42	0.78	0.070	34.01
	85°C		15.41	18.64	0.51	0.89	0.092	35.56
		5 mm	15.08	18.68	0.45	0.86	0.073	35.15
		9 mm	15.16	17.87	0.48	0.81	0.089	34.42

Table 4B. Effect of corn hybrid, grain drying temperature and grinding sieve on carotenoid content (μg/g) and composition in yolks of laying hens.

Dent hybrid	Grain drying temperature	Grinding sieve	Lutein	Zeaxanthin	α-cryptoxanthin	β-cryptoxanthin	β-carotene	Total carotenoids
Soft-type	40°C	5 mm	18.39	17.55	0.51abc	0.69	0.058	37.21a
		9 mm	17.41	14.72	0.49bc	0.62	0.081	33.33bc
	85°C	5 mm	17.42	16.68	0.58ab	0.83	0.104	35.63abc
		9 mm	18.48	16.31	0.64a	0.83	0.168	36.44ab
Hard-type	40°C	5 mm	11.71	19.76	0.26d	0.96	0.083	32.79c
		9 mm	11.79	19.61	0.40cd	0.86	0.056	32.73c
	85°C	5 mm	12.80	20.72	0.43c	0.97	0.046	34.99abc
		9 mm	12.94	20.84	0.39cd	0.93	0.050	35.17abc

Means followed by the same letter in the same column do not differ statistically among themselves by Tukey test (P = 0.05). Columns without the letters indicate a nonsignificant hybrid × drying temperature × griding sieve interaction.

β-carotene for dietary treatments was below 2% in all dietary treatments tested.

Similar to the carotenoid content in the yolk, the corn hybrid was the main effect affecting the highest number of carotenoids detected (Table 5A); its effect was not determined only for the deposition efficiency of zeaxanthin and total carotenoids. For the carotenoids with significant effect, the hens fed diets based on hard-type hybrid had higher deposition efficiency of lutein (31.01 vs. 23.72%) and α-cryptoxanthin (8.04 vs. 4.53%) and lower deposition efficiency of β-cryptoxanthin (3.07 vs. 3.57%) and β-carotene (0.62 vs. 1.27%). The remaining 2 main effects had a relatively small influence on the deposition efficiency of carotenoids. Grain drying temperature affected only the deposition efficiency of α- and β-cryptoxanthin and β-carotene, which were lower in egg yolks from hens fed diets based on grain dried at 40°C than at 85°C (5.70 vs. 6.88%, 3.14 vs. 3.51%, and 0.81 vs. 1.07%, respectively). On the other hand, the grinding sieve had no effect on the deposition efficiency of the carotenoids determined in the egg yolks except for zeaxanthin and β-cryptoxanthin, which had higher values in egg yolks from hens fed diets when grain was ground using a 5-mm sieve (Table 5A).

Similar to the content in egg yolk, interactions hybrid × drying temperature, drying temperature × grinding sieve, and drying temperature × grinding sieve were significant for some carotenoids, but to a lesser extent. The hybrid × drying temperature interaction was significant for lutein, zeaxanthin, β-carotene and total carotenoids. The deposition efficiency of major carotenoids and total carotenoids was higher in egg yolks from hens fed soft-type hybrid dried at 40°C than at 85°C (24.89 vs. 22.55% for lutein, 19.73 vs. 18.30% for zeaxanthin, and 18.26 vs. 16.91% for total carotenoids). However, in egg yolks from hens fed hard hybrid, the reverse was observed and deposition efficiency was higher when grain was dried at 85°C (32.42 vs. 29.61% for lutein, 18.81 vs. 17.83% for zeaxanthin and 17.97 vs. 16.91% for total carotenoids). A significant interaction between grain drying temperature and grinding sieve was observed only for α-cryptoxanthin (Table 5A). The deposition efficiency of α-cryptoxanthin increased with increasing grinding sieve size of corn grain in egg yolk from hens fed diets based on grain dried at 40°C, while it decreased in diets based on grain dried at 85°C. Following the pattern of carotenoid content in the yolk, the deposition efficiency of α-cryptoxanthin and total carotenoids in the yolk of hens fed soft hybrid dried at 85°C was more similar depending on the grinding sieve than in the other treatments in the experiment, consistent with the hybrid × drying temperature × grinding sieve interaction. In contrast, lutein had the highest deposition efficiency (34.20%) in the yolks from hens fed diets based on hard-type hybrid when grain was dried at 85°C and ground using the 5-mm sieve (Table 5B).

Table 5A. Analysis of variance for the carotenoid deposition efficiency in yolks of laying hens with their means (%) for the main factors.

Source of variation			Lutein	Zeaxanthin	α-cryptoxanthin	β-cryptoxanthin	β-carotene	Total carotenoids
Hybrid (H)			<0.001	0.201	<0.001	<0.001	<0.001	0.678
Grain drying temperature (DT)			0.777	0.682	0.006	0.010	0.022	0.878
Griding sieve (GS)			0.387	0.005	0.317	0.043	0.144	0.062
H × DT			0.002	0.027	0.132	0.163	<0.001	0.010
H × GS			0.845	0.037	0.285	0.612	0.010	0.252
DT × GS			0.189	0.591	0.003	0.673	0.157	0.384
H × DT × GS			0.019	0.010	<0.001	0.166	0.531	0.010
Dent hybrid	Grain drying temperature	Grinding sieve						
Soft-type			23.72	19.02	4.53	3.57	1.27	17.58
Hard-type			31.01	18.32	8.04	3.07	0.60	17.38
	40°C		27.25	18.78	5.70	3.14	0.81	17.52
	85°C		27.48	18.56	6.88	3.51	1.07	17.44
		5 mm	27.72	19.44	6.07	3.47	0.85	17.94
		9 mm	27.01	17.90	6.50	3.17	1.03	17.02

Table 5B. Effect of corn hybrid, grain drying temperature and grinding sieve on carotenoid deposition efficiency (%) in yolks of laying hens.

Dent hybrid	Grain drying temperature	Grinding sieve	Lutein	Zeaxanthin	α-cryptoxanthin	β-cryptoxanthin	β-carotene	Total carotenoids
Soft-type	40°C	5 mm	25.76cde	21.64a	4.40b	3.54	0.781	19.42a
		9 mm	24.03de	17.83b	4.13b	3.03	1.021	17.10ab
	85°C	5 mm	22.56e	19.08ab	4.72b	3.97	1.286	17.23ab
		9 mm	22.54e	17.52b	4.89b	3.74	1.988	16.59ab
Hard-type	40°C	5 mm	28.38bcd	17.17b	5.29b	2.97	0.812	16.11b
		9 mm	30.84ab	18.48ab	8.96a	3.01	0.610	17.45ab
	85°C	5 mm	34.20a	19.86ab	9.88a	3.40	0.508	19.00ab
		9 mm	30.63abc	17.76b	8.02a	2.92	0.486	16.95ab

Means followed by the same letter in the same column do not differ statistically among themselves by Tukey test ($P = 0.05$). Columns without the letters indicate a nonsignificant hybrid × drying temperature × particle size interaction.

DISCUSSION

The content of individual and total carotenoids was similar in the experimental diets consisting of the same corn hybrid, suggesting that the final dietary content of carotenoids will be similar regardless of the drying temperature and grinding sieve used in the present study. The diets consisting of hybrid with softer grains had higher contents of lutein and α-cryptoxanthin, while those containing hybrid with harder grains had higher contents of zeaxanthin, β-cryptoxanthin and β-carotene. This was consistent with the findings of Saenz et al. (2021), whose results suggest an interaction between grain hardness and the ability to store specific carotenoids. The range of total carotenoid content in the experimental diets was higher than the range reported for diets containing commercial corn hybrids (13.45 −17.13 μg/g DM; Kljak et al., 2021) and higher than the values reported for diets containing commercial yellow corn by Moreno et al. (2020) and Ortiz et al. (2021; 9.2 and 8.8 μg/g DM, respectively).

In the present study, the accumulation of carotenoids in the egg yolks occurred after the 4th d of the experimental period and saturation was reached after the 2nd wk for all experimental diets (data not shown). This was consistent with the duration of the accumulation and saturation phase in previous studies (Moreno et al., 2020; Kljak et al.; 2021; Ortiz et al., 2021) for different corn genotypes. The carotenoid profiles of the yolks reflected the carotenoid composition of the investigated dietary treatments, i.e. corn hybrids used as the basis of the experimental diets (Liu et al., 2012; Moreno et al., 2016; Kljak et al., 2021), with yolk content generally decreasing in the order of lutein > zeaxanthin > β-cryptoxanthin > α-cryptoxanthin > β-carotene for the soft-type hybrid and zeaxanthin > lutein > β-cryptoxanthin > β-carotene > α-cryptoxanthin for hard-type hybrid experimental diets (Table 4A and 4B), and their determined contents were consistent with the results obtained for different corn genotypes (Moreno et al., 2016; Kljak et al., 2021; Ortiz et al., 2021). The 3 times higher content of lutein and the only 1.73 times higher content of zeaxanthin in the yolk compared to the diet are closely related to the findings of Moreno et al. (2016). This suggests that regardless of the processing methods, lutein consistently showed higher deposition efficiency in egg yolk than zeaxanthin in all treatments tested. In contrast, for the lower-content carotenoids, the deposition efficiency of α-cryptoxanthin exceeded that of β-cryptoxanthin and β-carotene (Tables 5A and 5B). These results are consistent with previous studies (Moreno et al., 2016; Kljak et al., 2021; Ortiz et al., 2021) confirming that xanthophylls are preferentially incorporated into the egg yolk of laying hens, ahead of provitamin A carotenoids, with the deposition efficiency decreasing at higher dietary carotenoid levels. In addition, lutein is 5 times more bioavailable than β-carotene in humans (van het Hof et al., 1999), corresponding to a higher deposition efficiency in laying hens (Kljak et al., 2021).

Nevertheless, the results of the present study showed that the final carotenoid content in the yolks and the deposition efficiency depended on the type of grain processing of the commercial hybrids tested (Tables 4A, 4B, 5A, and 5B). In contrast, the hybrids used for laying hen diets and the applied grain processing conditions had no effect on hen production performance (Supplementary Table S1), suggesting that ADFI and egg production did not contribute to the differences between dietary treatments. Among the 8 experimental diets investigated, the carotenoid content in the yolks differed depending on the grain drying temperature, with better utilization of carotenoids observed in grains dried at 85° C. Considering that carotenoids from corn are predominantly found in the amyloplasts and storage lipids during grain development (Wang et al., 2020), but their exact localization in the mature grain is not clear, it seems that the structural changes in proteins and starch that occur during drying and affect nutrient digestibility (Gehring et al., 2013; Malumba et al., 2014; Odjo et al., 2015) also affect the bioavailability of carotenoids in laying hens. It has been reported that lutein is located in the core of α-zein segments with a triple helix that stabilizes the configuration of this zein (Momany et al., 2006) and that other carotenoids present in corn grains may also have hydrophobic interactions with protein bodies (Larkins et al., 2017). Although higher grain drying temperatures generally lead to a lower protein digestibility in corn (Kaczmarek et al., 2013; Odjo et al., 2018), it is possible that the protein damage caused by higher temperature during grain drying (Odjo et al., 2015) led to changes in the interactions between carotenoids and protein bodies resulting in higher carotenoid bioavailability,

which is consistent with the higher content of all individual and total carotenoids and a higher deposition efficiency of α- and β-cryptoxanthin and β-carotene in the egg yolk of hens fed diets with grain dried at 85°C compared to 40°C in the present study. In addition, the effect of grain drying temperature was hybrid-dependent for the contents of β-cryptoxanthin and β-carotene (Table 4A) and the deposition efficiencies for lutein, zeaxanthin, β-carotene and total carotenoids (Table 5A). In the yolks from hens fed diets based on hard-type hybrid, drying the grain at 85°C resulted in a small increase in content (β-cryptoxanthin) and even a lower content (β-carotene) compared to diets with the grain dried at 40°C. On the other hand, drying the grain at 85°C resulted in a lower deposition efficiency of lutein, zeaxanthin and total carotenoids in the yolks from hens fed diets based on the soft-type hybrid compared to drying the grain at 40°C. The opposite was found for the hard-type hybrid. Odjo et al. (2018) showed that the response of protein digestibility to grain drying temperature depends on the corn genotype and that the protein digestibility of the flint-like genotype was less pronounced due to the limited denaturation of the protein. Consequently, diets based on soft-type hybrid with grains dried at 85°C could have lower deposition efficiency of lutein, zeaxanthin and total carotenoids in the present study due to lower digestibility of the protein and compounds associated with them. Furthermore, the effect of grain drying temperature on the content of zeaxanthin and total carotenoids in egg yolks also depended on the grinding sieve size as higher drying temperature had a greater effect on grains sieved through a larger sieve, that is, with a larger particle size of the diet (Table 4B).

In the present study, the responses to grinding sieve size depended mainly on the interaction with drying temperature and not on the grinding sieve size itself. Although to our knowledge, there is limited data on the effects of grinding sieve size on yolk carotenoid content, Oliveira et al. (2019) reported that hens fed fine and medium corn particles had higher yolk pigmentation than hens fed coarse particles. This is consistent with the results of the present study and suggests that the yolk content and deposition efficiency of some carotenoids, such as zeaxanthin (Table 5A), may increase in laying hens with the reduction in the grain particle size. This suggests that smaller particle sizes increase the surface content with digestive enzymes and improve the bioavailability of carotenoids in laying hens when drying temperatures are applied. In addition, recent studies have shown that reducing particle size increases the apparent digestibility of lipids in laying hens (Ege et al., 2019; Mtei et al., 2019). Because carotenoids share the same digestive fate as lipids, this could lead to their higher bioavailability due to the simulative effect of lipids on the excretion of bile salts, promotion of micelle formation and absorption of carotenoids (Priyadarshani, 2017). Hafeez et al. (2015) reported that the interaction between thermal treatment and particle size affected yolk color, with a higher value found when laying hens were offered mashed diets compared to laying hens that received the feed expanded at 116°C and then ground with a refiner gap of 1.75 mm. The overall results suggest that increasing the grinding sieve size may increase digesta retention time and digestive enzyme secretion (Mtei et al., 2019) and thus improve the bioavailability of carotenoids from diets with corn dried at 85°C compared to diets with grain dried at 40°C. Furthermore, increased bioavailability of carotenoids from grain processed under certain conditions could lead to increased color intensity (Supplementary Table S2).

CONCLUSIONS

The differences in carotenoid profile between the hard- and soft-type corn hybrids had a main effect on the carotenoid content and deposition efficiency. In contrast, grain processing had a relatively small effect on carotenoid content and deposition efficiency. However, the choice of the appropriate drying temperature and grinding size of the grain could increase the deposition efficiency of the carotenoids. Laying hens fed diets with the soft-type hybrid dried at 40°C and ground through the smaller (5-mm) sieve achieved the highest content and deposition efficiency of lutein, zeaxanthin, and total carotenoids in egg yolk. The effect of processing depends on grain hardness, and the grinding sieve size was of lesser importance for the hard-type hybrid, which had the highest bioavailability of the main carotenoids from grain dried at 85°C. Although the content of carotenoids in yolk improved by up to 11% and the deposition efficiency by up to 20%, the results of the present study show that the selection of corn hybrid and suitable processing conditions contribute to a better utilization of the carotenoids from the grain and lead to an increased yolk color intensity, an important egg quality attribute for consumers.

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DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2024.103750.

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