EFFECT OF DIFFERENT SOURCES OF LUTEIN IN LAYER DIETS ON EGG QUALITY

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Abstract

The lipid matrix of the egg is a food source in which lutein and zeaxanthin have a higher bioavailability than from the vegetal sources. Within this context, we conducted a 6-week trial on 19,500 TETRA layers, which aimed to enhance the lutein concentration in the yolk by feeding natural (corn gluten and alfalfa) and commercial sources of lutein to the layers. The layers were assigned to 3 groups (C, E1 and E2) and reared in modernized production halls. The basal diet was similar for all three groups (17.96% CP and 2724.31 kcal ME /kg). Compared to the diet for group C, the diet formulation for group E1 also included 5% alfalfa hay and 5% corn gluten. The diet formulation for group E2 included 25% commercial lutein powder (10%). Every day during the first week and every 2 days, afterwards, eggs were sampled and assayed for lutein concentration in the fresh yolk. Lutein concentration in the yolk increased during the experiment only in the yolk of the eggs from the experimental groups: 2.09 times (E1) and 2.89 times (E2). The higher lutein concentration in the yolk from the experimental groups compared to groups C was obvious from the second day of experiment: 12,777ppm (C), 17,636 ppm (E1) and 21,487 ppm (E2). No increase was determined in group C. There was a very high correlation (R^2=0.9226) between lutein concentration in the compound feed and its concentration in the yolk.

Key words: layers, yolk, lutein, sources

INTRODUCTION

Lutein is known as the eye protective nutrient, because it deposits specifically in tissues (inverse relation between the dietary supply of lutein and the eye disturbances). The dietary lutein supplements increases its concentration in the eyes and improves the vision of the patients having eye disturbances [1; 2; 20]. Lutein bioavailability and metabolism are determined by the characteristics of the source and by the interactions with the other dietary nutrients [24]. Besides vegetables and fruits [17; 21] the egg yolk is a natural source of xanthophylls (lutein + zeaxanthin) dispersed in its matrix composed of digestible lipids [26]. The lipid matrix of the egg yolk is a dietary source of lutein and zeaxanthin with a higher bioavailability than that of the vegetables, as shown by many studies [16].

To enrich the egg yolk in xanthophylls, the diets for layers use both vegetal ingredients rich in xanthophylls, and synthetic products. These artificial products, most times sold as lutein, have a short shelf life because the carotenoids are unstable molecules. Their stability is drastically affected by light, humidity and temperature. The carotenoid pigments that can build up in the hen eggs depend largely on the dietary carotenoids, while the total amount of carotenoids in poultry organ ism depends on the amount of ingested and absorbed carotenoids. The lutein concentration in the egg also depends on the type of feeds given

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to the layers and on the rearing conditions [11; 22]. The yolks produced by layers reared on the floor have two times higher concentration of carotenoids than the layers reared in battery cages [11]. Alfalfa is one of dietary ingredients rich in xanthophylls [19]. It has a moderate content of protein [7; 14] but the amino acids profile is balanced and it is rich in vitamins [9; 13; 23]. Recent studies have proven that alfalfa can also have antioxidant properties [8; 10; 27] and that it may reduce the cholesterol concentration in the meat and egg yolk [8; 15].

Layer diets rich in corn and corn derivatives may contain about 10 mg lutein and about 5 mg zeaxanthin, while in the diet treated with dyestuffs, the concentration may reach 20-30 mg lutein kg^{-1} and 8-12 mg zeaxanthin kg^{-1}. Such dietary levels may produce in the egg yolk concentrations of 13-25 mg de lutein kg^{-1} and 8-10 mg zeaxanthin kg^{-1} [18].

In order to respond the demand of consumers for functional eggs, we conducted an industrial-scale trial to enrich the egg yolk in lutein by using conventional and natural sources of lutein (corn gluten, alfalfa) and a commercial source of lutein.

MATERIAL AND METHOD

The trial was conducted for 6 weeks on a stock of 19,500 TETRA layers (58 weeks) assigned to 3 groups (C, E1 and E2). The layers were reared on the floor in experimental houses, under controlled environmental conditions. The diet formulations for the three groups (Table 1) had the same basal structure with 17.96% CP and 2724.31 kcal ME/kg. Compared to the diet formulation for group C, the diet formulation for group E1 included 5% alfalfa and 5% corn gluten. The diet formulation for group E2 included additionally 25 g% of a commercial lutein product (10% lutein).

Samples of feed ingredients were analysed prior to compound feeds manufacture. Three batches of compound feeds were manufactured, and a sample from each batch was analysed. The chemical methods used to determine the chemical composition (dry matter, crude protein, ether extractives, crude fibre, ash), the Ca and P from the samples of feed ingredients and compound feeds, were in accordance with the provisions of Regulation (CE) no. 152 /2009.

Table 1 Diet formulations

Ingredients	C	E1	E2
	%	%	%
Corn,%	46.97	55.53	55.48
Gluten,%	-	5.00	5.00
Alfalfa (pellets),%	-	5.00	5.00
Lutein,%	-	-	*0.025*
Sorghum,%	10.00	-	-
Soybean meal,%	23.04	14.28	14.29
Sunflower meal,%	4.32	5.00	5.00
Vegetal oil,%	3.50	3.28	3.29
Lysine,%	0.02	0.16	0.16
Methionine,%	0.18	0.16	0.16
Tryptophan,%	0.04	0.04	0.04
Ca carbonate,%	9.67	9.52	9.52
phosphate,%	1.06	0.85	0.85
Salt,%	0.23	0.23	0.23
Na bicarbonate,%	0.16	0.16	0.16
Mycofix,%	0.10	0.10	0.10
Biotronic Top3,%	0.20	0.20	0.20
Yellow dye,%	0.0025		
Red dye,%	0.001		
Premix A5	0.50	0.50	0.50
TOTAL	**100.00**	**100.00**	**100.00**
Calculated (theoretical calculation)			
ME, kcal/kg	2870.14	2870.00	2870.00
CP, %	16.70	16.70	16.70
Lysine, %	0.85	0.85	0.85
Methionine, %	0.45	0.45	0.45
Cystine, %	0.46	0.44	0.44
Met.+cist., %	0.74	0.74	0.74
Threonine, %	0.63	0.57	0.57
Tryptophan, %	0.21	0.21	0.21
EE, %	5.42	4.94	4.96
Fiber,%	3.90	5.08	5.08
Ca, %	3.88	3.90	3.90
P, %	0.59	0.63	0.63

Premix (A5 – 0.5%) = (2,000,000 IU/kg vit. A; 500,000 IU/kg vit.D3; 5000 UI/kg vit.E; 600 mg/kg Vit.K; 200 mg/kg Vit.B1; 800 mg/kg Vit.B2; 1600 mg/kg pantothenic acid; 6000 mg/kg nicotinic acid; 600 mg/kg Vitamin B6; 4 mg/kg vitamin B7; 100 mg/kg vitamin B9; 3000 mcg/kg vitamin B12; 100 mg/kg folic acid; 5000 mcg Biotine, 80000 mg/kg choline, 60000 FTU/kg fitasa; 20000 U Endo 1,3 (4)-β-glucanase; 14000 U Endo 1,4 β-xylanase; 2000 mg/kg BHT; 5000 mg/kg iron; 1000 mg/kg copper; 12000 mg/kg zinc; 20000 mg/kg manganese; 10 mg/kg cobalt; 100 mg/kg iodine; 40mg/kg selenium; Calcium carrier = 26%

Lutein and zeaxanthin from the samples of feed ingredients and compound feeds, were determined by high performance liquid chromatography at 445 nm wave length, method developed and validated by the Laboratory of Chemistry and Nutrition Physiology within IBNA Balotesti, using an HPLC series 200 fitted with UV/VIS detector

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(Perkin Elmer, SUA), column Nucleodur C18 ec, with silicagel, size 250 × 4.6 mm, particle size 5 µm, with reversed phase (RP-HPLC) (Macherey-Nagel, Germany), rotavapour HS-2005V (Hahnshin Scientific, Korea).

Egg samples were collected throughout the experimental period. The physical parameters were measured on eggs harvested at the beginning of the experiment (58 weeks) and at the end of the experiment (64 weeks). The eggs were analysed for yolk colour intensity (Egg Analyser TM), egg weight and yolk weight. Sixty eggs/group were collected daily during the first week, and then every 2 days, in order to determine the bioavailability of the dietary lutein. The lutein and zeaxanthin concentration in the yolk samples were determined according to the method described above, adapted for egg samples.

Statistical analysis: The analytical data were compared performing analysis of variance (ANOVA), using STATVIEW for WINDOWS (SAS, version 6.0). The differences between mean values within the groups were considered significant at $P<0.05$. Results were expressed as the mean of replications ± SD for all measurements.

RESULTS AND DISCUSSIONS

Table 2 shows the results of the chemical determinations on samples of feed ingredients. Corn is the main ingredient of the basal diet (Table 1). It has a considerable concentration of xanthophylls, but corn gluten (170.22 ppm/kg) and alfalfa (65.86 ppm/kg) have the highest concentrations of xanthophylls. Alfalfa is used in limited amounts in poultry diets due to its high level of fibre [6].

The determinations made on compound feeds samples (Table 3) show that their chemical composition is not significantly different, all three diet formulations being balanced in terms of energy and protein concentration. As expected, the use of feed ingredients rich in xanthophylls (gluten and alfalfa) increased significantly ($P\leq0.5$) xanthophylls level in the compound feeds for groups E1 and E2.

Table 2 Chemical composition and metabolisable energy content of the feed ingredients

Item	ME Kcal/kg	CP %	EE %	Fibre %	Ash %	Ca %	P %	Lutein + zeaxanthin, ppm
Corn flour	3200	7.5	4.79	4.71	1.89	0.02	0.44	12.282
Corn gluten	3800	52.51	0.82	0.27	4.36	0.03	0.97	170.22
Sunflower meal	1650	35.15	1.15	17.33	6.7	0.34	1.23	nd*
Alfalfa - pellets	1670	16.05	1.06	27.43	9.15	1.15	0.32	65.86
Soybean meal	2450	47.65	0.8	4.46	6.61	0.32	0.71	0.677
Sorghum grains	3180	7.24	2.59	2.31	1.21	0.02	0.32	0.101

ME (metabolisable energy); CP (crude protein); EE (ether extractives); Ca (calcium); P (phosphorus); *not traceable

Table 3 Chemical composition and metabolisable energy content of the compound feeds

Item	CF for C	CF for E1	CF for E2
ME, kcal/kg	2862.64±20.59	2897.49±18.68	2899.09±18.34
CP, %	16.54±1.12	16.86±1.04	17.40±0.50
EE, %	5.33±0.43	5.73±1.55	5.47±1.20
Fibre, %	5.13±0.44	5.21±0.36	4.94±0.43
Ash, %	15.02±1.87	13.40±1.51	12.14±1.25
Ca, %	3.88±0.81	3.90±0.64	3.90±0.87
P, %	0.69±0.22	0.59±0.11	0.57±0.06
Lutein + zeaxanthin, ppm	6.41±0.17	18.99±0.41	43.88±2.64

a = significant differences ($P\leq0.05$) compared to C; b = significant differences ($P\leq0.05$) compared to E1; c = significant differences ($P\leq0.05$) compared to E2;

Figure 1 shows overlapped chromatograms of the two xanthophylls in compound feeds samples. Thus, compared to group C, the concentration of lutein + zeaxanthin determined in group E1 was significantly ($P\leq0.05$) higher, 2.96 times, and even much higher in E2, 6.85 times. Xanthophylls concentration was significantly different between the experimental groups too, being 2.31 times higher in E2 (supplemental synthetic lutein) compared to E1.

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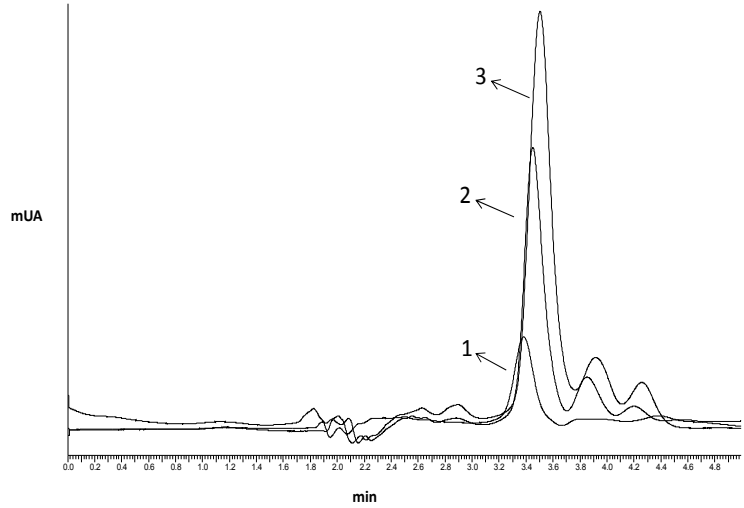

Fig. 1 Overlapped chromatograms of lutein and zeaxanthin concentration in the CF: (1) lutein + zeaxanthin in CF C; (2) lutein + zeaxanthin in CF E1; (3) lutein + zeaxanthin in CF E2

The fresh egg yolk samples, consisting from the eggs harvested daily during the first week and every 2 days thereafter, for 6 weeks, were analysed to determine their concentration of lutein and zeaxanthin (Table 4). Starting with the second day of the trial, the xanthophylls concentration in the egg yolk increased significantly (P≤0.05) in groups E1 and E2 compared to C (Table 4). Significant differences have also been determined between xanthophylls concentration in the two experimental groups, E1 and E2. One can notice the same trend of increasing xanthophylls concentration in the yolk, as it was in the compound feeds, as shown in Figure 1.

Table 4 Lutein concentration in the egg yolk (mg/kg)

Day of collection eggs	Groups		
	Control	E 1	E2
day 1	12.82±0.94 ^{b,c}	17.15±0.55 ^{a,c}	17.16±1.33 ^{a,b}
day 2	12.78±0.54 ^{b,c}	16.85±2.30 ^{a,c}	21.49±1.00 ^{a,b}
day 3	11.88±0.39 ^{b,c}	20.42±0.80 ^{a,c}	28.66±0.79 ^{a,b}
day 4	13.03±1.09 ^{b,c}	24.22±1.94 ^{a,c}	32.13±1.71 ^{a,b}
day 5	12.62±0.61 ^{b,c}	25.81±1.70 ^{a,c}	37.26±2.10 ^{a,b}
day 7	10.05±1.39 ^{b,c}	25.98±3.21 ^{a,c}	39.54±1.58 ^{a,b}
day 10	10.82±1.08 ^{b,c}	34.04±2.65 ^{a,c}	49.35±2.50 ^{a,b}
day 12	10.97±0.60 ^{b,c}	37.34±1.57 ^{a,c}	54.37±1.48 ^{a,b}
day 14	12.68±1.32 ^{b,c}	41.27±1.51 ^{a,c}	54.10±2.90 ^{a,b}
day 17	13.20±0.55 ^{b,c}	42.04±1.63 ^{a,c}	54.80±0.86 ^{a,b}
day 19	12.79±0.58 ^{b,c}	39.46±1.03 ^{a,c}	52.12±2.21 ^{a,b}
day 26	12.44±0.55 ^{b,c}	36.91±1.26 ^{a,c}	48.83±2.50 ^{a,b}
day 28	13.16±0.68 ^{b,c}	35.16±0.73 ^{a,c}	46.98±0.53 ^{a,b}
day 30	13.26±0.26 ^{b,c}	35.33±0.72 ^{a,c}	49.94±0.57 ^{a,b}
day 32	12.77±0.12 ^{b,c}	35.33±1.42 ^{a,c}	51.22±2.71 ^{a,b}
day 35	12.43±0.24 ^{b,c}	35.40±0.23 ^{a,c}	51.69±0.83 ^{a,b}
day 37	12.33±0.16 ^{b,c}	35.89±0.51 ^{a,c}	50.87±0.38 ^{a,b}
Average/period	**12.38±0.84** ^{b,c}	**31.83±8.07** ^{a,c}	**43.70±11.91** ^{a,b}

a = significant differences (P≤0.05) compared to C; b = significant differences (P≤0.05) compared to E1; c = significant differences (P≤0.05) compared to E2

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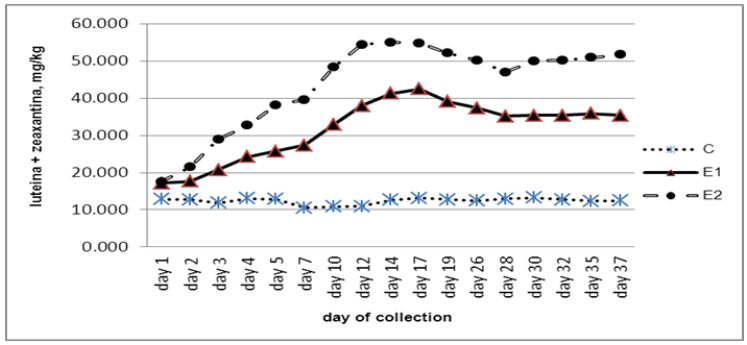

Fig. 2 Lutein concentration in the egg yolk samples

Figure 2 shows that lutein + zeaxanthin concentration in the eggs harvested throughout the entire experimental period increased significantly (P≤0.05) in groups E1 and E2 compared to group C. Also Figure 2 shows that on days 7, 17 and 28, the lutein + zeaxanthin concentration determined in the fresh yolk samples decreased slightly in the two experimental groups (E1 and E2). This is probably due to the lower dietary lutein concentration because of the fast oxidation of the xanthophylls, because the time span between two batches of compound feeds was of 2 weeks. Similar studies on lutein instability in time due to temperature were performed by [3; 4; 5; 25] conducted a study on the stability of paprika and marigold extracts com pared to the commercial forms of apoester and canthaxanthin, coated and included in vitamin-mineral premixes. They determined a 10-20% reduction of the concentration compared to the theoretical level of dietary carotenoids, going up to 30-50% after one month of storage.

In our study, at the final collection of eggs, the two experimental groups showed a significant (P≤0.05) increase of the lutein and zeaxanthin concentration compared to group C (Fig. 3).

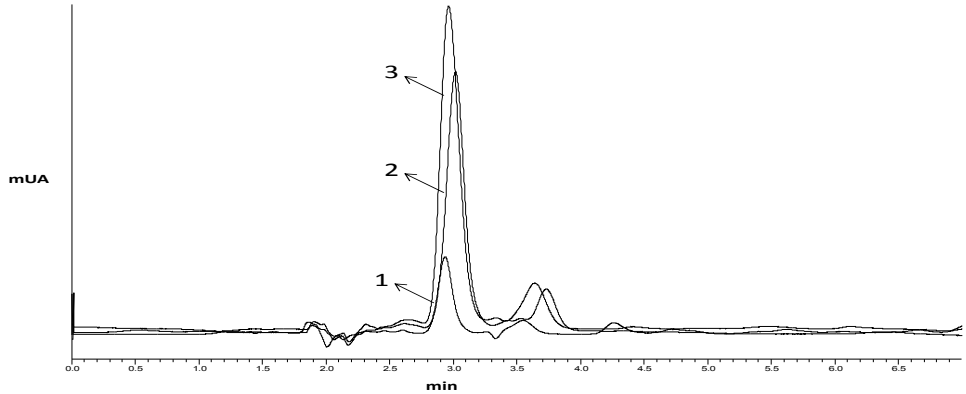

Fig. 3 Overlapped chromatograms of lutein and zeaxanthin concentration in the egg samples collected in the end of the experiment: (1) lutein + zeaxanthin in CF C; (2) lutein + zeaxanthin in CF E1; (3) lutein + zeaxanthin in CF E2

The increase of lutein concentration, during the experimental period, in the yolk of the eggs from group E1 was of 16.11%, and 29.46% in group E2. The lutein concentration in the yolk increased proportionally with the dietary lutein concentration. This observation is supported by the very strong correlation (R^2=0.9226) between the xanthophylls

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concentration in the compound feed and their concentration in the yolk (fig. 4). Similar results have been reported by [12] who used Chlorella in layer diets.

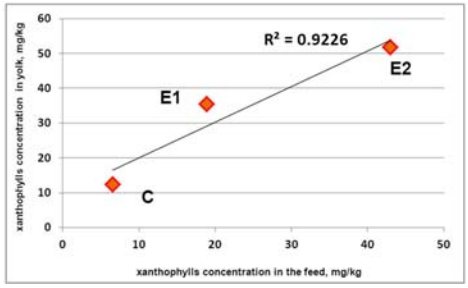

Fig. 4 Correlation between the xanthophylls concentration in the yolk and compound feed

CONCLUSIONS

Starting from the second day of the experiment, xanthophylls concentration in the egg yolk increased significantly ($P \leq 0.05$) in groups E1 and E2 compared to C. Significant differences ($P \leq 0.05$), in favour of group E2, were noticed in the xanthophylls concentration in the yolk obtained from the two experimental groups.

Lutein concentration increased throughout the experiment by 16.11% in group E1, and by 29.46% in group E2.

There has been a very strong correlation (R^2=0.9226) between the xanthophylls concentration in the compound feed and their concentration in the yolk.

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