EFFECT OF USING RAW MATERIALS RICH IN XANTHOPHYLLS IN LAYER DIETS ON EGG QUALITY

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Abstract

The purpose of the experiment was to produce a new variety of eggs beneficial for consumer health by its qualities improved by layer feeding. We used raw materials rich in xanthophylls, available on forage market, to improve eggs enriched in lutein. We conducted a 5-week trial on two groups (C and E) of Lohmann Brown layers (38 layers/group) aged 27 weeks, housed in enriched cages (2 layers/cages 19 cages/group). The basal diet, same for the two groups, consisted of corn, wheat, soybean meal and sunflower meal and had 17.8% CP and 2800 kcal ME/kg. Additionally, the diet for group E also included 5% alfalfa and 5% corn gluten. The lutein content of the two diets was 4.3 mg/kg CF (C) and 15.63 mg/kg CF lutein (E). Initially and then, every 2 weeks, we collected randomly 18 eggs/group which were measured for the physical parameters and lutein concentration in the yolk. At the end of the experiment, the lutein concentration was significantly ($p \leq 0.05$) higher in group E (15.84±2.39 ppm) compared to group C (3.90±0.27 ppm). Yolk colour was significantly ($p \leq 0.05$) lower in group C (3.361±0.639) compared to group E (5.611±0.728). Egg freshness was higher in group E (33% AA; 61.11% A) compared to group C (25% AA; 44.44% A).

Key words: layers, eggs, compound feeds, lutein, freshness

INTRODUCTION

Presently, macula degeneration (MD) is the main cause of blindness in the developed countries, due to the progressive, degenerative and irreversible changes of the central retina area (macula). The macula is the area for the eye retina which is responsible for the detailed vision. Macula degeneration develops progressively, affecting more than 5% of the people over 65. Unlike the wet form, the dry form of MD is receptive to the administration of nutrients such as vitamins and minerals. The carotenoids are substances that act as antioxidants and immunostimulators [31]. Of the 600 de known carotenoids, lutein and zeaxanthin are the main carotenoids from the retina macula in humans. Different studies have shown that a diet with foods or food supplements containing lutein increase the optic density of the macular pigment [17; 23] and may help improving the eye vision in patients with macula degeneration due to age [22; 27], may help in cataract [9; 39] or in other eye disturbances [32; 34]. Several studies have shown that fruits and vegetables are important sources of lutein and zeaxanthin [7; 21] and their consumption increases the concentration of macular pigment. The egg yolk too, which is a matrix composed on digestible lipids, cholesterol, triglycerides and phospholipids, contains xanthophylls (lutein and zeaxanthin) dispersed within the matrix, next to other liposoluble nutrients, such as the liposoluble vitamins [16]. Fortunately, the lipid matrix of the egg is a food source in which lutein and zeaxanthin have a higher bioavailability than that from vegetal source, due to their association with the lipid matrix of the egg yolk [5; 14; 36; 38]. It was shown that the use of eggs enriched in lutein increase the plasm lutein concentration [4; 10; 38]. Eggs normally contain 0.3-0.5 mg total xenophiles, a little more than half being lutein [3; 11; 37]. Like other nutrients, such as the fatty acids [6] egg composition is sensitive to the manipulation of such components in layer diets. The use of plants as source of carotenoids in layer diets is a natural way of obtaining egg yolk with a pleasant colour and high in carotenoids. This type of plants usually have antioxidant properties too, improve feed efficiency and

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have favourable effects on the poultry because the influence the taste and flavour of the diet, contain several bioactive components, stimulate the development of the animal organism. Worldwide [13; 20; 24; 25; 26; 38], the research on vegetal sources rich in xanthophylls (ear corn, spinach, carrot, corn silage, turnip leaves, basil, savory, marigold flower meal, paprika) intensified, being used as ingredients in layer diets that increase the lutein concentration in the yolk, enhance yolk colour and improve other quality parameters as well. The alfalfa meal, yellow corn, red corn and corn gluten are raw ingredients that can be used layer diets. The alfalfa is usually used in low levels in poultry feeding because its high content of fibre and low energy level [29]. But alfalfa is used in layer diets both for yolk pigmentation and for skin pigmentation [3; 8; 19].

The purpose of our experimental study was to obtain a new variety of eggs, enriched in xanthophylls, with positive impact on consumer health, using in layer diets raw ingredients rich in xanthophylls, available on the market for feeds.

MATERIAL AND METHOD

Poultry and experimental house: The experiment runs for 5 weeks on 76 Lohmann Brown layers, aged 27 weeks. The layers were weighed individually, in the beginning of the experiment, and were assigned to two homogenous groups according to their weight (38 layers/group): 1.675±0.11 kg LW, group C, and 1.668±0.12 kg LW, group E. The layers were housed in digestibility cages (2 layers/cage; 19 cages/group), which allowed the daily recording of the ingesta and excreta, which helped determining the average daily feed intake (g CF/layer/day) and the feed conversion ratio (g CF/kg egg). The layers had free access to the feed and water. The light regimen was provided with bulbs and it was in agreement with Lohmann Brown hybrid guidebook (16 h per day), with 21.94±1.96^{0}C temperature and 56.83±6.38 % humidity in the house. Throughout the experiment we monitored the laying intensity and egg weight. Initially, then every 2 weeks, we collected randomly 18 eggs/group.

Diet formulations: The diets used conventional feed ingredients for layers: corn, soybean meal, sunflower meal, oil. The raw materials were assayed by the Laboratory of Chemistry and Nutrition Physiology within IBNA Balotesti, and the results were used to develop the compound feeds formulations for each individual group (Table 1). Only one batch of CF was manufactured, in the pilot station of IBNA Balotesti, the bags being labelled for each group individually and stored under special conditions of humidity and temperature.

Table 1 Formulation of the experimental diets

Ingredients	Control diet	Experimental diet
	%	%
Corn	39.96	39.64
Wheat	15.00	14.67
Soybean meal	21.17	13.44
Sunflower meal	8.73	6
Oil	3.20	2.5
Gluten	-	*5*
Alfalfa	-	*5*
Carrot flakes	-	*2*
Lysine	0.03	0.21
Methionine	0.18	0.14
Calcium carbonate	8.95	8.48
Monocalcium phosphate	1.32	1.46
Salt	0.36	0.36
Mycotoxin inhibitor	0.10	0.10
Premix	1	1
Total raw materials	100.00	100.00
Calculated values		
E.M, kcal/kg	2800	2800
Crude protein, %	17.80	17.80
Crude fat, %	4.92	4.27
Crude fibre, %	4.41	4.77
Calcium , %	3.90	3.90
Phosphorus total, %	0.68	0.65
Sodium, %	0.17	0.17
Chlorine, %	0.28	0.30
Lysine, %	0.86	0.84
Methionine, %	0.47	0.46
Met+cis , %	0.88	0.76
Threonine,%	0.64	0.47
Tryptophan, %	0.20	0.16

Premix IBNA (A5) = (1,350,000 IU/kg vit. A; 300,000 IU/kg vit. D3; 2700 IU/kg vit. E; 200 mg/kg Vit. K; 200 mg/kg vit. B1; 480 mg/kg vit. B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 300 mg/kg vitamin B6; 4 mg/kg vitamin B7; 100 mg/kg vitamin B9; 1.8 mg/kg vitamin B12; 2500 mg/kg vitamin C; 7190 mg/kg manganese; 6000 mg/kg iron; 600 mg/kg copper; 6000 mg/kg zinc; 50 mg/kg cobalt; 114 mg/kg iodine; 18 mg/kg selenium; 6000 mg/kg

After CF manufacture, 500 g CF were collected from each bag and assayed chemically.

Collected samples and assays

a. Samples of raw ingredients and feeds: The following physical and chemical methods were used to characterize the raw ingredients and the compound feeds samples:

- *Lutein and zeaxanthin* were determined by the method developed and validated by the Laboratory of Chemistry and Nutrition Physiology within IBNA Balotesti. The principle of the method involves extraction with organic solvent (acetone), re-extraction with petrol ether and saponification with methanolic solution of potassium hydroxide. Lutein and zeaxanthin were determined by high performance liquid chromatography at 445 nm wave length, using an HPLC series 200 fitted with UV/VIS detector (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).
- *Dry matter (DM); crude protein (CP); ether extractives (EE); crude fibre (CF); ash (ash)* were determined in accordance with the provisions of Regulation *(CE) nr. 152 /2009.*

b. Egg samples: Initially, then every 2 weeks, we collected randomly 18 eggs/group. At each collection, the whole eggs were measured for the physical parameters of the eggs. Thereafter, 6 samples (3 eggs/sample) of yolk and eggshell were formed. Measurements were performed during the experiment to determine *egg weight and the weight of the egg components*: egg white, yolk, eggshell (Kerm scales, precision 0.001), *colour intensity*, on La Roche colour fan (1-15) measured with an Egg Analyzer TM; *egg freshness*, measured by the value of the Haugh indicator and points of fresh ness evaluation (Egg Analyzer TM); *eggshell thickness* (Egg Shell Thickness Gauge); *eggshell breaking strength* (Egg Force Reader);

The samples of yolk, dried at 65^0C were assayed for dry matter (DM) using the gravimetric method, for crude protein (CP) using the Kjeldahl method, the ether extractives (EE) by extraction in organic solvents, ash (ash) using the gravimetric method. The lutein and zeaxanthin concentration in the yolk samples were determined as shown above.

Data interpretation: All determinations were performed in triplicate. Analysis of variance (ANOVA) and Fisher's least square difference (LSD) tests were applied to compare means at 5% significance level using the statistical data analysis software StatView for MS Windows (Statistical Analysis System, Version 6.0). Results were expressed as the mean of replications ± SD for all measurements.

RESULTS AND DISCUSSIONS

The feed ingredients rich in xanthophylls used for the experimental group were analysed for their content of feeding elements (Table 2). The chemical analyses showed that alfalfa is rich in protein (14.54%) but that it also has a lot of fibre (34.14%). Similar results were reported by [29] for protein (17.5%) and fibre (24.1%) but low in metabolisable energy. According to [40], the chemical composition of alfalfa varies with the time and stage of harvesting (17.2 - 21.7% CP) and fibre depending on the stage of harvesting.

Table 2 Chemical analysis of the plants used for the experimental group (average values/group)

Item	DM, %	OM, %	CP, %	EE, %	CF, %	Ash, %	NFE, %	Lutein + zeaxanthin, ppm
Alfalfa- pellets	90.16	83.3	14.54	0.87	34.14	6.86	33.75	58.54
Corn gluten	93.73	89.37	52.51	0.82	0.27	4.36	35.77	170.22
Carrot flakes	93.03	89.98	4.18	0.43	4.89	3.05	80.48	5.472

Regarding the concentration of xanthophylls (lutein + zeaxanthin), Table 2 shows that the corn gluten has the highest concentration of them. These values are lower than those reported by [41] who studied the effects of the enzymatic treatment on lutein and zeaxanthin extraction from corn gluten, reporting 113.5 mg lutein /100g DM

and 140.1 mg zeaxanthin /100 g DM. Other researchers, [28] determined lutein and zeaxanthin from commercial corn gluten and reported a concentration of 14.5 mg/100g. These authors analysed the yellow corn obtained concentrations of 2.197 mg lutein /100 g and .57 mg zeaxanthin /100 g. According to [33], corn gluten can contain up to 30 mg xanthophylls/100 g.

The compound feeds samples were analysed chemically to determine their feeding value. Table 3 shows that the two formulations were balanced in terms of energy and protein content, providing the nutrients required by the age and category of experimental animals according to Lohmann Brown Management Guide (2007). The concentration of xanthophylls (lutein + zeaxanthin) was significantly (P≤0.05) higher in the compound feed E compared to the compound feed C: 3.63 times higher, due to the use of vegetal ingredients (Table 1) rich in xanthophylls (Table 2).

Table 3 Chemical analysis of the compound feeds (average values/group)

Item	C	E
Real DM, %	93.30	93.46
Crude protein, %	17.46	17.85
Ether extractives, %	5.04	3.69
Fibre, %	5.58	6.07
Ash, %	13.21	11.81
Lutein+zeaxanthin, ppm	4.3	15.63

Table 4 shows significant differences (P≤0.05) between groups E and C in terms of animal performance. The average daily feed intake of group E was 4.72% higher than in group C. Similar results were reported for the feed conversion ratio, while egg weight was significantly (P≤0.05) lower (by 2%) in group E compared to group C. The data are in agreement with the report of [2] in a study on layers, who used alfalfa and reported a higher feed conversion ratio and a higher number of eggs. [13] in and experiment which used 3 varieties of carrots (orange, yellow and purple) in layer diets, reported a significant lower egg weight in all three experimental groups compared to the control group and [29] too, reported a significant decrease of layer performance (feed conversion ration, egg production and egg weight) when they used ß–glucanase and ß-1,4 xylanase to treat the 15% alfalfa used in a 12-week experiment of ISA Brown layers (40 weeks). On the contrary, [18] observed that layer diets supplementation with 1 and 2 ppm spinach lutein and with 1 and 2 ppm lutein from spinach extract influenced significantly layer performance. The effect of the dietary lutein (free, esterified and in both forms) was studied by [35] on Hy-line (W-36) layers aged 31 weeks, assigned according to category and body weight, monitoring the feed intake, egg production, egg weight and weight of egg components. They reported that the tested sources of lutein didn't determine significant differences between the groups in terms of layer performance.

Table 4 Layer performance (average values/group)

Item	C	E
Average daily feed intake, (g/layer/day)	119.74± 4.564[b]	125.39± 3.933[a]
Feed conversion ratio, (kg CF/kg egg)	1.99± 0.098[b]	2.13± 0.094[a]
Average egg weight, (g)	63.62± 1.284[b]	62.39± 1.167[a]
Laying percentage, (%)	95.24± 3.366	96.81± 3.339

a =significant differences (P≤0.05) compared to C; b= significant differences (P≤0.05) compared to E;

No significant differences were determined in terms of the physical parameters of the eggs (Table 5), for any of the egg components (egg white, egg yolk, eggshell). Similar results were reported by [29] and [30] but significant differences were determined for the category of 55-60 g in favour of the groups treated with alfalfa and enzyme. On the other hand, [12] reported different eggshell thickness following the use of dietary alfalfa, while [2] reported higher egg quality by higher albumen, thicker eggshell and a significant decrease of the Haugh units.

Table 5 Physical parameters of quality of the egg (average values /group)

Item	C	E
Egg weight*, (g) of which:	63.20±2.45	63.24±2.82
- white, (g)	40.08±2.67	39.29±2.59
- yolk, (g);	15.17±1.59	15.69±1.30
- shell, (g)	8.35±0.78	8.23±0.43
Eggshell thickness, (mm)	0.37±0.04	0.37±0.02
Eggshell breaking strength, (kgF)	5.03±0.66	4.82±0.84

* weight of the analysed eggs; a, b =significant differences (P≤0.05) compared to C and E, respectively;

The same researchers, [2] reported higher yolk colour intensity in the layers treated with 7% alfalfa. The results of our study confirm the reports of these researchers, the feeding quality of the eggs harvested from group E being higher than the feeding quality of the eggs harvested from group C (Table 6). The feeding ingredients rich in xanthophylls (alfalfa, gluten and the carrot flakes) had a positive effect on yolk colour, significant (P≤0.05) differences being recorded compared to group C: yolk colour intensity was 1.67 times higher in group E compared to group C.

Table 6 Egg freshness parameters (average values/group)

Item	C	E
Yolk colour	3.36±0.64[b]	5.61±0.73[a]
Haugh units	64.88±10.85[b]	69.57±6.41[a]
Egg freshness:		
AA	25.00	33.33
A	44.44	61.11
B	30.56	5.56
Total	100	100

a = significant differences (P≤0.05) compared to C; b = significant differences (P≤0.05) compared to E

Yolk colour intensity increased significantly (p≤0.05) from 3.361±0.639 (C) to 5.611±0.728 (E), while egg freshness had higher values: 33% AA and 61.11% A in group E compared to 25% AA and 44.44% A, in group C. Figure 1 shows the percent distribution of the yolk colour intensity (cumulated values from the determinations from the first experimental week, 27 weeks, after 2 weeks and at the end of the trial, 32 weeks. In group E, yolk colour intensity was significantly (p≤0.05) higher than in group C. The range of values was between 5 (52.78%) and 7 (13.89%), for group E, and between 2 (8.33% and 4 (44.44%) for group C. Similar results about yolk colour enhancement was reported by [1; 15]

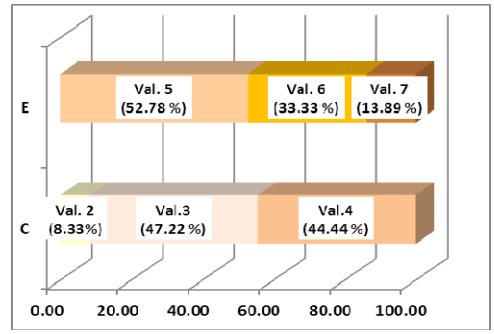

Fig. 1 Percent distribution of the yolk colour intensity

No significant changes were noticed for the chemical composition of the yolk, either, except for the final experimental week in the control group, where the protein concentration was significantly higher com pared to the in initial harvesting, but with no significant difference compared to group E. on the other hand, the concentration of xanthophylls in the fresh samples of yolk, determined at the end of the trial, was significantly (p≤0.05) higher (4.06 times) in group E (15.84±2.39 ppm) compared to group C (3.90±0.27 ppm). Related to the initial harvesting (before the start of the trial), the concentration of lutein increased 1.67 times in group C and 6.79 times in group E, compared to the initial value. The regression coefficient, according to equation: $y1_{(control)} = 0.7802x + 1.842$ was, $R^2 = 0.7146$ for group C, while $R^2 = 0.8373$ according to equation: $y\ 2_{(experimental)} = 6.7524x - 2.6964$ for group E.

Table 7 Chemical composition of the egg yolk (g% fresh yolk)

Item	Start of the experiment	End of the experiment	
	Initial (27 weeks)	C (32 weeks)	E (32 weeks)
DM, %	50.30±1.47	51.19±1.15	50.80±1.07
CP, %	16.77±0.70 [b]	17.77±0.33 [a]	17.34±0.30
EE, %	28.17±0.85	27.19±1.11	27.89±0.84
Ash, %	1.68±0.05	1.52±0.08	1.61±0.06
Lutein+zeaxanthin, ppm	2.34±0.42 [b]	3.90±0.27 [a,c]	15.84±2.39 [b]

a = significant differences (P≤0.05) compared to the initial values; b = significant differences (P≤0.05) compared to C; c = significant differences (P≤0.05) compared to E;

Figure 2 shows that the concentration of lutein increased significantly even after 2 experimental weeks, maintaining at the same level until the end of the experiment.

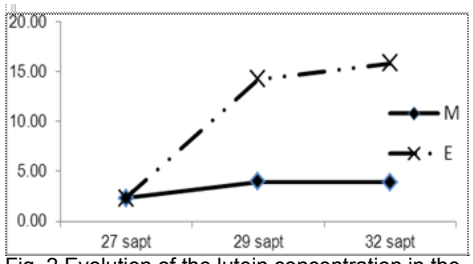

Fig. 2 Evolution of the lutein concentration in the egg yolk

CONCLUSIONS

The use of feed ingredients rich in xanthophylls in the CF formulation for group E, induced a significant ($P \leq 0.05$) increase, throughout the experiment, of the concentration of lutein + zeaxanthin in the yolk of the eggs. At the same time, xanthophylls concentration was 4.06 times higher in the yolks from group E, than in the yolks from group C.

The eggs from group E had a higher level of freshness (33 % AA; 61,11% A) and a higher intensity (1.67 times) of yolk colour intensity, compared to group C.

Except for the laying percentage, which was not different among the two groups, the performance (intake, egg weight) of the layers which received the experimental formulation with 5% alfalfa, 5% gluten, 2% carrot flakes, have been adversely affected.

These feed ingredients are cheap, natural sources of xanthophylls, which can replace successfully the synthetic products used to enhance the colour of the yolks, which are even more expensive.

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