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Effect of Natural and Chemical Colorant Supplementation on Performance, Egg-Quality Characteristics, Yolk Fatty-Acid Profile, and Blood Constituents in Laying Hens

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Abstract: Natural and chemical colorants are attracting a lot of attention as sustainable feed additives due to their effect on food color and because presentation matters to consumers. Color also is a major sensory factor that helps consumers determine food quality and its possible health benefits. For example, highly colored egg yolks can only come from healthy laying hens fed a well-balanced, nutrient-rich diet. Consumers associate an intense yolk color with healthy, nutrient-rich food. There is a growing market request for eggs with rich yolk coloring. The purpose of the current study was to evaluate the impact of natural (paprika) and chemical (carmoisine) colorant supplementation on the performance, egg-quality characteristics, yolk fatty-acid profile, and blood constituents in laying hens. A total of 240 Bovans laying hens were randomly distributed in a completely randomized design in four treatments according to four experimental diets: a control diet (without supplementation of any colorants), a control diet supplemented with 4 kg/ton paprika, a control diet supplemented with 150 g carmoisine/ton, and a control diet supplemented with a combination of 4 kg/ton paprika plus 150 g carmoisine/ton, fed from 42 to 54 weeks of age. Each treatment consisted of 10 replications with six hens. Dietary inclusion of paprika colorant improved ($p < 0.05$) the feed-conversion ratio and egg-production rate, while final body weight, weight change, feed intake, and egg weight and mass were not affected. The degrees of egg yolk and white were increased ($p < 0.05$) by the dietary supplements. Yolk color and shell thickness were enhanced ($p < 0.05$) by adding paprika and/or carmoisine colorants. Supplementation of the paprika colorant alone or in combination with carmoisine increased ($p < 0.05$) linolenic acid, oleic acid, and vitamin E concentrations in egg yolks. In contrast, egg yolk palmitic acid and liver malondialdehyde contents were decreased ($p < 0.05$). Paprika and carmoisine colorants and their combination improved ($p < 0.05$) blood lipid profile in treated hens. We concluded that the dietary supplementation of natural (paprika) colorants has an influential role in improving egg yolk color, production performance, and egg yolk fatty-acid profile in laying hens.

Keywords: natural colorants; egg quality; yolk color; fatty acids; laying hens

1. Introduction

As the world's population grows, demand for eggs will continue to rise. To meet this demand sustainably will be a big challenge because of the high cost of yellow corn in different countries, which will push farmers to use white corn in layer diets, which will affect yolk color. In addition, poultry nutritionists have been working for decades on sustainability in higher egg production with high quality. Using these colorants is an innovative solution for reducing diet costs and improving production and quality, which will lead to sustainable production and improving the environment. Nowadays, eggs, as simultaneously available, accessible, and nutritious foods, are one of the complete food sources in human nutrition, and have many applications in the food industry. In addition to containing an ideal combination of amino acids and a high biological value of protein for humans, eggs contain essential fatty acids, vitamins, and minerals that the body needs. Eggs are an excellent source of nutrients and have antibacterial, antiviral, anticancer, and many other mediators that play a role in the immune system and therefore are very important for human health and disease prevention [1]. Egg appearance characteristics, such as shape, transparency, and color, influence egg consumers' choices [2]. In general, different factors can be considered to determine the quality of eggs, but the three essential characteristics of shell, white, and yolk, which can be changed in terms of quantity, can be the most critical factors in determining egg quality [3].

Egg yolk is one of the most important indicators of consumer evaluation of chicken eggs' internal quality. The desired color intensity of egg yolks varies in different countries. Most consumers in the world prefer eggs with an orange yolk (whose yolk color index is more than 10 on a Roche fan scale) [4]. These eggs are more expensive on the market and are popularly known as golden yolk eggs [5]. The primary natural colorants that affect egg yolks' color are xanthophyll or carotenoids [6]. Egg yolk color ranges from pale yellow to deep orange, depending on yolk pigmentation, which is mostly based on the amount and type of carotenoids present in the feed [7,8]. Various factors affect yolk colors, such as quantity, quality, variety of xanthophylls, bird strain, individual differences between birds, cage breeding, diseases, stress, dietary fat, antioxidants, and the feedstuffs used in diet formulation [9,10]. Although birds cannot synthesize carotenoids in their body and receive all their carotenoids from feed [10], carotenoids can be easily picked up by poultry from their diet, released into the small intestine after enzymatic degradation, and absorbed [11]. Consequently, free carotenoid emulsification occurs to form portomicrons (oil droplets) transmitted to the liver. In the hepatocytes, portomicrons are integrated into very-low-density lipoprotein (VLDL) molecules and transmitted to the yolk [12,13].

Oxycarotenoids can be present in free, monoester, or diester forms in nature, which affects their absorption and metabolism in birds [9]. However, it has been reported that consumption of diets with xanthophyll content less than five parts per million leads to the production of pale egg yolks (less than 0 on the Roche fan scale) [14]. On the other hand, the overuse of some synthetic pigments, such as cantazantine, in poultry diets, has been restricted in some countries, as they may damage the human eye due to crystal formation in the retina. Therefore, in the European Union, cantazantine and other synthetic supplementations are classified as potentially harmful substances in humans [15,16]. Additionally, in recent years, due to increasing attention to the importance of food health and public concern about the use of synthetic additives, interest in replacing natural carotenoid sources such as red pepper, bell pepper, parsley, citrus pulp, and paprika, which contain significant amounts of xanthophyll, has been increased [9,10]. In addition to yolk color, yolk lipid stability during storage against oxidation is another important indicator of egg quality. Natural xanthophylls such as lutein and xanthine also have antioxidant properties in chicken diets [17,18]. Since eggs are a good choice for a person's daily food basket, the use of large amounts of natural oxycarotenoids in layer diets can cause the transfer of sufficient quantities of oxycarotenoids to the body, which has several beneficial effects on consumer health, in addition to creating a suitable and customer-friendly color in egg yolks [19].

For these reasons, commercial layer diets are typically enriched with natural or synthetic pigments, such as yellow or red xanthophyll, at high levels to make eggs more attractive to customers and more desirable for the food industry [20]. The use of synthetic colorants in layer diets has gradually shifted to natural ones because of their disadvantages, including being more expensive and less safe [21–23]. Carmoisine A is of the azo class of synthetic colorants, and gives human foods a red color. As a typical azo colorant, because of the sulfonic group, effluent containing carmoisine A is also challenging to treat in environmental systems, as it is polar and soluble in water [24]. However, its use in food was finally prohibited because of the presence of beta-naphthylamine, a known carcinogen [25,26]. Contrarily, natural pigments are cost-efficient, consistent, and can improve human health [27]. However, very few studies are available concerning the effect of using natural colorants in laying hens' diets on the quantitative and qualitative egg properties, particularly the yolk.

Therefore, our research aims to evaluate the effects of natural (paprika) and/or chemical (carmoisine) colorants on performance, egg quality, yolk fatty-acid profile, and blood constituents of laying hens that would result in sustainable production of high-quality eggs for the markets.

2. Materials and Methods

2.1. Ethical Statement

This work has been conducted following recommendations from the Local Experimental Animal Care Committee on Ethics, University of Kafrelsheikh, Egypt (Number 4/2016EC).

2.2. Birds and Experimental Diets

In an open-sided shed, 240 42-week-old Bovans brown hens, with an egg production rate of 81%, were housed in laying cages under a 16 h/8 h light/dark cycle under summer conditions and the same management protocols (temperature, moisture, ventilation). Birds were randomly allocated into 4 groups, with 10 replicates of 6 hens each. The first group was fed the basal diet and considered as a control; the second group was fed a control diet supplemented with 4 kg/ton paprika; the third group received the basal diet supplemented with 150 g carmoisine/ton feed, and the fourth group was fed a control diet supplemented with 4 kg/ton paprika plus 150 g carmoisine/ton feed. The paprika and carmoisine were provided by Ragab El-Attar Co, Egypt. The composition of the experimental diet, which was formulated to comply with the recommendations of NRC [28], is presented in Table 1.

2.3. Laying Performance

At the beginning (42 weeks of age) and the end (54 weeks of age) of the trial, the birds were weighed individually with a ZIEIS Digital Bird Scale (A63SS-NMP, 0.05 ounce accuracy, 5000 gram capacity). Eggs were hoarded every day. The laying rate was calculated as hen-day (% hens-day), applying the following equation (number of daily eggs produced per treatment/number of birds accessible in the treatment on that day $\times$ 100). Each egg weight was assessed and then utilized for all experimental times to evaluate the mean egg weight. The total egg mass was determined by laying rate multiplied by the weights of the eggs. As the hens were fed by an ad libitum system, the feed amount was added according to the catalog, and after seven days, the remaining feed was measured, and then the intake of feed was calculated on a cage basis (one hen). Daily feed consumption per hen for all days during the trial was determined. The FCR (kg of feed/kg of eggs) was assessed by utilizing egg production, egg weight, and feed intake.

Table 1. Ingredients and specific nutrients of the diet.

Ingredient	g/kg
Corn	605
Soybean meal, 46%	238
Gluten meal, 62%	32
Soybean oil	18
Dicalcium phosphate	20
DL-Methionine, 99%	2.1
Threonine, 99%	0.5
Limestone	72
NaCl	3
Vitamin mineral premix [1]	4
Sodium bicarbonate	2.4
Potassium carbonate	3
Total	1000
Calculated nutrient levels [2]	
Crude protein, %	17.49
ME, Kcal/kg diet	2851
Calcium, %	3.26
Total phosphorus, %	0.71
Available phosphorus, %	0.46
Ether extract, %	4.46
Fiber, %	2.80
Lysine, %	0.88
Methionine, %	0.49

[1] Vitamin mineral premix (units/kg diet): 10,000 IU vitamin A; 3500 IU vitamin D3; 35 IU vitamin E; 1.5 mg menadione; 5 mg riboflavin; 8 mg pantothenic acid; 0.012 mg vitamin B12; 1.5 mg pyridoxine; 1.5 mg thiamine; 0.5 mg folic acid; 30 mg niacin; 0.06 mg biotin; 0.8 mg iodine; 10 mg copper; 80 mg iron; 0.3 mg selenium; 80 mg manganese; 80 mg zinc. [2] Calculated according to Council (1994) for brown Bovans laying hens.

2.4. Egg Quality

Egg-quality parameters, involving egg length, egg width, egg shape, shell thickening, high albumin, high yolk, yolk width, yolk index, and yolk color score, were measured at the beginning of the experiment (42 weeks of age) and at the end of the experiment (54 weeks of age). From each test, 30 eggs laid between 08:00 and 12:00 h were arbitrarily selected. A digital egg scale was used to individually weigh eggs (accurate to 1/10th of a gram, 100 g maximum capacity). The egg-quality estimation and the egg weight were performedusing individual eggs. The eggs were broken on the plate measurement stand for EQM, and the albumen and yolk heights were determined. The yolk color's strength was measured and reported using the Roche yolk-color fan process [4]. Eggshell thickness was determined using the thickness mean values taken at 3 locations on the egg (air cell, equator, and sharp end) utilizing a micrometer caliper (Mitutoyo, 0.01 to 20 mm, Tokyo, Japan).

2.5. Egg Yolk Fatty-Acid Profiles

At 54 weeks of age, 40 eggs were collected per procedure to measure yolk fatty-acid content, including linolenic acid, oleic acid, palmitic acid, total cholesterol, vitamin E, and calcium concentration. A Shimadzu GC-4 CM gas chromatograph (PFE), equipped with a flame ionization detector (FID), was used to study previous fatty acids. A normal methyl ester mixture was analyzed before the samples were taken under similar conditions. The retention times of the unknown methyl ester samples were compared to that of the standard. The concentrations of methyl esters were determined using the methods of Radwan et al. [29], and Saleh et al. [30] in the triangulation process.

For determination of vitamin E in the egg yolks, pooled samples (40 samples, 10/treatment) were homogenized in a 0.054 mol/L dibasic sodium phosphate buffer amended to 7 pH with HCl. After being mixed with absolute ethanol and hexane, the upper layer of α-T was evaporated and dissolved in ethanol before evaluation by HPLC3 (UV detector fixed at 290 nm). Egg yolk total cholesterol was measured through the extraction of fat from the egg yolk with a chloroform and methanol admixture (2:1 vol:vol), as per methods according to Surai [31].

2.6. Blood Constituents

For blood, 40 samples, 10/treatment were collected from a brachial vein into heparinized tubes and centrifuged at 3000× *g* for 20 min to separate sera samples, and stored at −20 °C until biochemical analysis. Serum concentrations of total lipid, total cholesterol, triglyceride, high- and low-density lipoproteins (HDL and LDL), total protein, albumin, and glucose were analyzed using a commercial colorimetric kit (Egyptian Company for Biotechnology, Cairo, Egypt; and Wako Chemicals, Richmond, VA, USA) [32]. Liver malondialdehyde (MDA) content was determined using a commercial colorimetric kit (Liquizyme MDA; Egyptian Biotechnology Company, Cairo, Egypt). The absorbance was monitored using a spectrophotometer (Unico UV-2000; SpectraLab Scientific Inc., Alexandria, VA, USA) at a wavelength of 545 nm [33].

2.7. Data Analysis

Using SPSS statistical software version 26 (IBM Corp., Armonk, NY, USA), the obtained data were analyzed according to a completely randomized design. The significance of all differences in means was checked using Tukey's multiple comparison test based on $p < 0.05$.

3. Results

3.1. Laying Growth Performance

The effects of natural (paprika) and/or chemical (carmoisine) colorants on the performance of laying hens are showed in Table 2. Neither paprika nor carmoisine colorants affected the final body weight (FBW), weight gain (BWG), FI, EM, or egg weight of laying hens during the experimental period. On the other hand, dietary supplements of natural colorants improved ($p < 0.05$) FCR and egg production rate compared with other treatments.

Table 2. Effect of natural (paprika) and chemical (carmoisine) colorants on growth performance in laying hens.

Item	Diets				*p*-Value
	Control	Paprika	Carmoisine	Paprika + Carmoisine	
Initial body weight (42 wks), g	1908 ± 28	1945 ± 42	1927 ± 33	1940 ± 25	0.205
Final body weight (54 wks), g	1974 ± 22	1987 ± 36	1996 ± 34	1974 ± 22	0.320
Body weight gain, g/84 days	66 ± 15	42 ± 21	68 ± 20	34 ± 13	0.133
Feed intake, g/day	117.2 ± 0.65	114.2 ± 0.18	114.3 ± 0.70	115.1 ± 0.23	0.180
Egg weight, g	59.2 ± 2	56.9 ± 2	57.8 ± 1	56.8 ± 1	0.453
Egg production, %	79.8 ± 1.6 ^{b}	83.7 ± 1.6 ^{a}	81.8 ± 1.6 ^{ab}	81.2 ± 1.6 ^{ab}	0.048
Egg mass, g/hen/day	47.2 ± 3.2	47.6 ± 2.6	47.3 ± 2.4	46.1 ± 1.9	0.142
Feed conversion ratio, g feed/g egg	2.48 ± 0.39 ^{a}	2.39 ± 0.21 ^{b}	2.42 ± 0.14 ^{ab}	2.49 ± 0.08 ^{a}	0.048

Values are shown as means ± their standard error of 60/treatment. $^{a, b}$ Mean values are significantly different when followed by various letters ($p < 0.05$) in the same lines.

3.2. Egg Quality

The effects of natural (paprika) and/or chemical (carmoisine) colorant supplementation on egg-quality characteristics of laying hens from 42 to 54 weeks of age are shown in Table 3. Dietary treatments improved ($p < 0.05$) the albumin and yolk width. Interestingly, dietary addition of paprika and/or carmoisine colorants exhibited significant ($p < 0.01$) improving impacts on yolk color and shell thickness at the end of the experimental period. Treatment with paprika and/or carmoisine reduced ($p < 0.05$) weight and height of egg whites, but did not affect egg weight, egg length, egg width, yolk weight and height, or eggshell weight.

Table 3. Effect of natural (paprika) and chemical (carmoisine) colorants on egg-quality characteristics in laying hens.

Item	Diets				p-Value
	Control	Paprika	Carmoisine	Paprika + Carmoisine	
At the beginning, 42 wks of age					
Egg weight, g	60.32 ± 2.72	59.21 ± 1.62	59.32 ± 2.59	60.44 ± 3.68	0.136
Egg length, cm	5.54 ± 0.05	5.52 ± 0.11	5.47 ± 0.21	5.49 ± 0.15	0.989
Egg width, cm	4.49 ± 0.06	4.34 ± 0.06	4.25 ± 0.07	4.37 ± 0.07	0.127
Eggshell thickness, µm	470 ± 8.37	472 ± 12	468 ± 16.85	456 ± 13.27	0.820
Yolk width, cm	3.80 ± 0.09	3.89 ± 0.09	3.96 ± 0.19	4.02 ± 0.08	0.639
Albumen width, cm	8.41 ± 0.35	11.39 ± 2.23	9.36 ± 0.49	9.54 ± 1.24	0.462
Yolk height, mm	17.95 ± 0.08	18.32 ± 0.43	17.58 ± 0.40	18.90 ± 0.52	0.148
Albumen height, mm	9.14 ± 0.57	7.32 ± 1.08	6.17 ± 0.61	7.70 ± 0.90	0.122
Yolk weight, g	15.25 ± 0.58	15.21 ± 0.37	14.98 ± 0.48	15.11 ± 0.84	0.957
Albumen weight, g	33.84 ± 1.02	32.82 ± 0.86	33.33 ± 1.94	34.17 ± 3.41	0.451
Yolk color, Roche fan	6.22 ± 0.37	6.23 ± 0.37	6.41 ± 0.4	6.61 ± 0.24	0.830
Shell weight, g	11.21 ± 1.58	11.17 ± 0.8	10.99 ± 1.33	11.12 ± 0.71	0.104
At the end, 54 wks. of age					
Egg weight, g	64.23 ± 0.32	64.22 ± 0.97	64.43 ± 1.86	63.90 ± 0.68	0.437
Egg length, cm	5.71 ± 0.09	5.82 ± 0.037	5.62 ± 0.07	5.62 ± 0.09	0.249
Egg width, cm	4.56 ± 0.05	4.40 ± 0.03	4.45 ± 0.06	4.48 ± 0.05	0.163
Eggshell thickness, µm	478 ± 8.14 ^{b}	503 ± 0.66 ^{a}	502 ± 0.87^{a}	497 ± 4.49 ^{a}	0.005
Yolk width, cm	3.99 ± 0.05 ^{b}	4.13 ± 0.04 ^{a}	4.14 ± 0.04 ^{a}	3.96 ± 0.05 ^{b}	0.021
Albumen width, cm	7.46 ± 1.62 ^{b}	10.90 ± 0.44 ^{a}	10.52 ± 0.57 ^{a}	9.30 ± 0.33 ^{a}	0.044
Yolk height, mm	17.69 ± 0.39	17.07 ± 0.37	17.19 ± 0.64	16.55 ± 0.33	0.377
Albumen height, mm	6.34 ± 0.51 ^{a}	5.01 ± 0.25 ^{b}	4.68 ± 0.47 ^{b}	6.69 ± 0.25 ^{a}	0.005
Yolk weight, g	16.12 ± 0.32	17.80 ± 0.49	17.62 ± 0.6	17.41 ± 0.87	0.065
Albumen weight, g	37.01 ± 0.71 ^{a}	34.71 ± 1.44 ^{b}	35.02 ± 1.22 ^{b}	34.91 ± 0.51 ^{b}	0.042
Yolk color, Roche fan	7.81 ± 0 ^{b}	9.40 ± 0.24 ^{a}	8.82 ± 0.2 ^{a}	9.21 ± 0.37 ^{a}	0.005
Shell weight, g	11.12 ± 0.63	11.70 ± 0.71	11.81 ± 2.49	11.62 ± 0.6	0.872

Values are shown as means ± their standard error of 30/treatment. $^{a, b}$ Mean values are significantly different when followed by various letters (p < 0.05) in the same lines.

3.3. Egg Yolk Fatty-Acid Profiles

The effects of feeding paprika, carmoisine, or their combination on egg yolk fatty-acid profiles are illustrated in Figure 1A–C. Dietary inclusion of paprika colorant alone or accompanied by carmoisine increased (p < 0.05) egg yolk contents of linolenic and oleic fatty acids; however, the palmitic level was reduced (p < 0.05) in all treated groups. Influence of dietary natural (paprika) and/or chemical (carmoisine) colorant supplementation on yolk total lipids, vitamin E, and lipid peroxidation index (MDA) levels are given in Figure 2A–C. Supplementation of paprika with carmoisine colorants in diets decreased the yolk's total lipids, but raised (p < 0.05) the amount of yolk vitamin E and lowered (p < 0.05) the concentration of MDA in laying hens' livers.

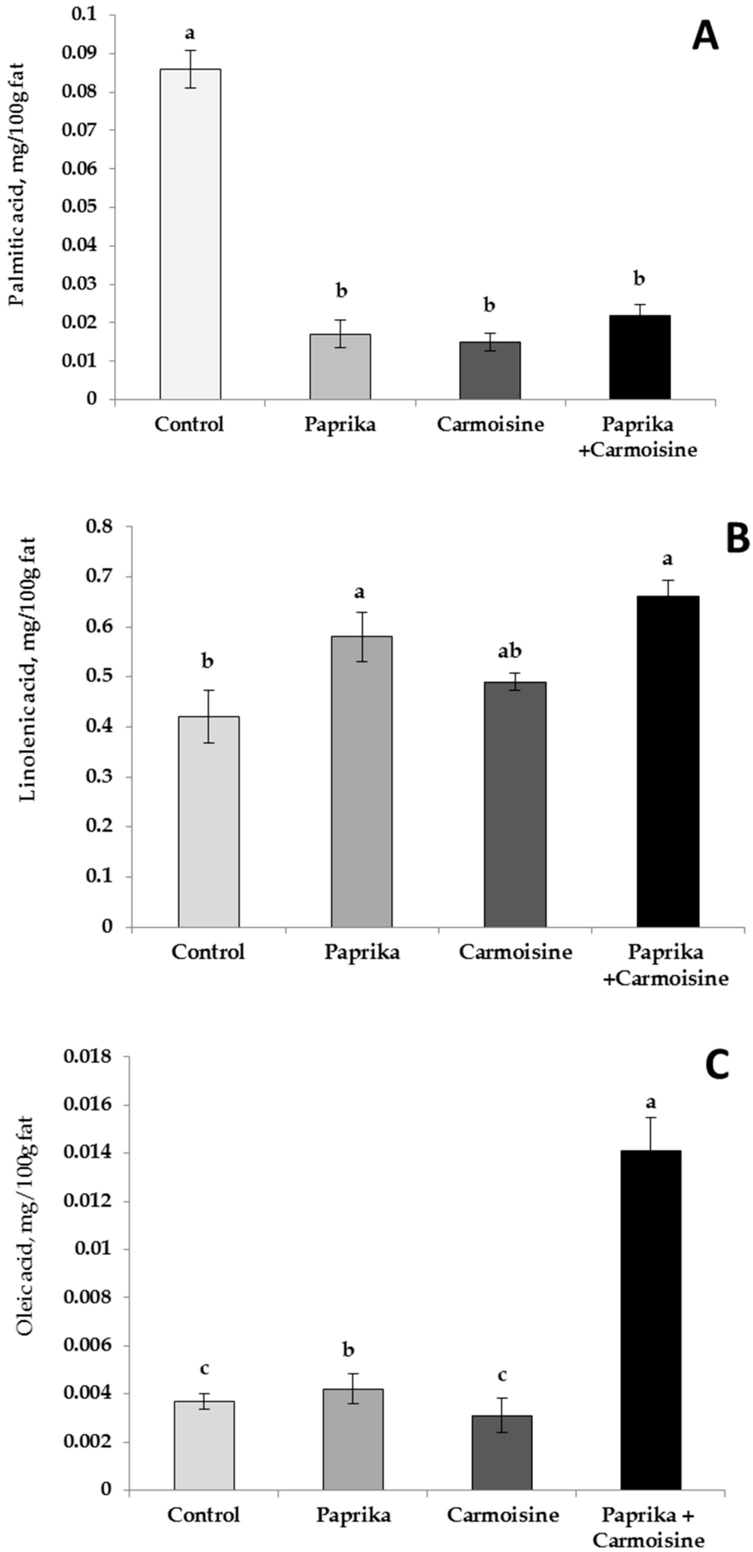

Figure 1. Effect of natural (paprika) and chemical (carmoisine) colorants on palmitic acid (**A**), linolenic acid (**B**), and oleic acid (**C**). Values are defined by a vertical bar; different letters $^{a, b, c}$ mean values were significantly different ($p < 0.05$).

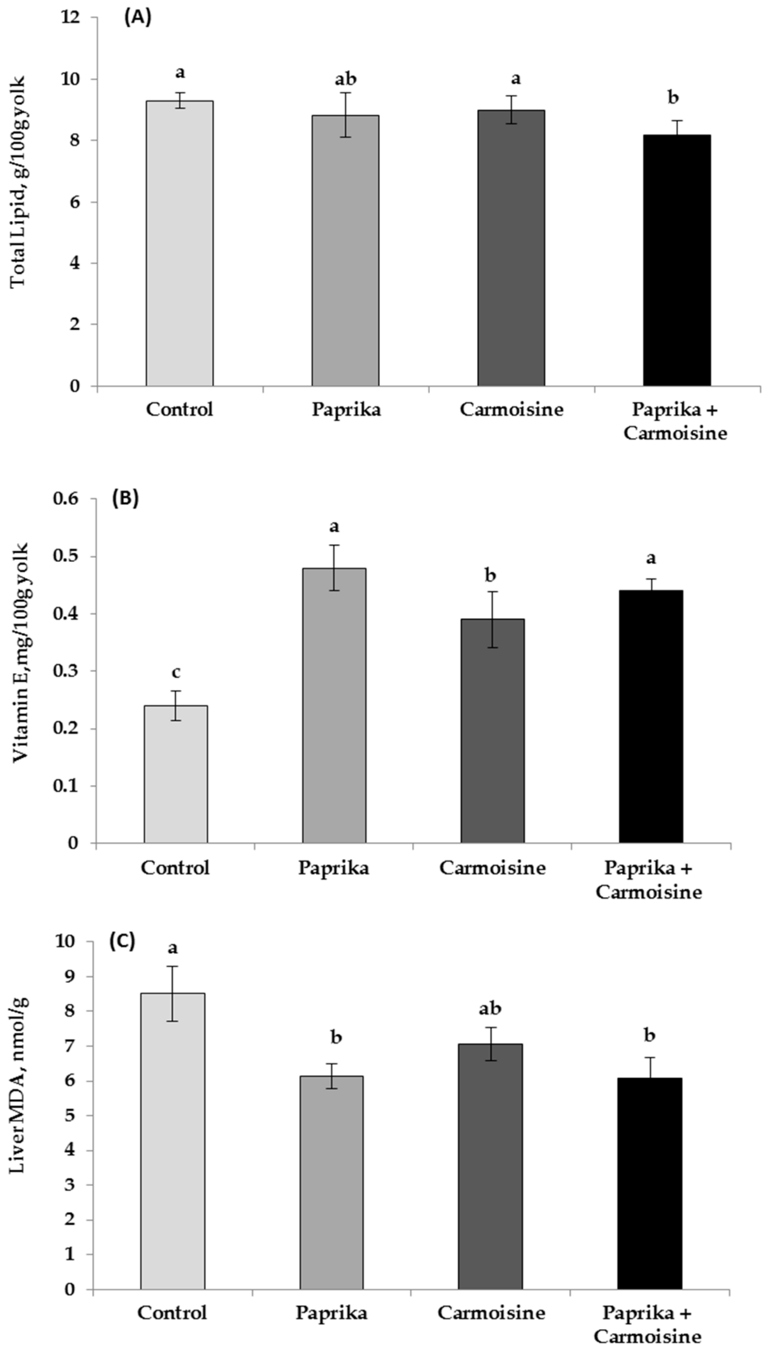

Figure 2. Effect of natural (paprika) and chemical (carmoisine) colorants on total lipids (**A**), vitamin E (**B**), and liver MDA (**C**). Values are defined by a vertical bar; different letters $^{a, b, c}$ mean values were significantly different ($p < 0.05$).

3.4. Blood Constituents

Table 4 presents the findings concerning the impact of treating layers with natural (paprika) and/or chemical (carmoisine) colorants on serum lipid profiles and protein fractions. Dietary treatments of paprika alone or carmoisine had a hypocholesterolemic impact, as blood concentrations of total cholesterol and triglycerides were reduced. HDL was elevated in the blood of birds treated with paprika or carmoisine. No significant effect of the supplements on LDL, total lipids, total protein, globulin, albumin, and glucose was noticed.

Table 4. Effect of natural (paprika) and chemical (carmoisine) colorants on blood constituents in laying hens.

Item	Diets				*p*-Value
	Control	Paprika	Carmoisine	Paprika + Carmoisine	
Total lipid, mg/dL	4.51 ± 0.25	4.56 ± 0.31	4.43 ± 0.29	4.22 ± 0.40	0.446
Total cholesterol, mg/dL	160 ± 14.62 [a]	148 ± 12.60 [b]	157 ± 5.04 [ab]	159 ± 8.39 [ab]	0.048
Triglyceride, mg/dL	1.57 ± 0.14 [a]	0.99 ± 0.13 [c]	1.41 ± 0.15 [ab]	1.26 ± 0.20 [b]	0.036
HDL, mg/dL	14.5 ± 1.71b [c]	21.5 ± 5.45 [a]	16.0 ± 1.47 [b]	19.2 ± 4.16 [ab]	0.034
LDL, mg/dL	146 ± 6.49	126 ± 9.83	141 ± 4.5	139 ± 5.5	0.081
Total protein, mg/dL	2.83 ± 0.13	2.72 ± 0.20	2.83 ± 0.16	2.85 ± 0.21	0.935
Albumin, mg/dL	1.575 ± 0.19	1.375 ± 0.19	1.812 ± 0.19	1.823 ± 0.25	0.435
Globulin, mg/dL	1.25 ± 0.13	1.33 ± 0.14	1.01 ± 0.26	1.05 ± 0.13	0.514
Glucose, mg/dL	204 ± 12.87	209 ± 3.22	209 ± 6.92	218 ± 5.36	0.688

Values are shown as means ± their standard error of 15/treatment; [a, b, c] mean values are significantly different when followed by various letters ($p < 0.05$) in the same lines.

4. Discussion

This study's primary purpose was to evaluate whether egg-quality characteristics, yolk fatty-acid content, growth performance, and blood constituents of laying hens could be affected by supplementation of natural (paprika) and/or chemical (carmoisine) colorants. In the present study, the supplementation of the natural colorant (paprika) improved the egg-production rate and FCR, while FBW, BWG, FI, EM, and egg weight were not influenced. Other studies on hens fed natural colorants such as paprika or red pepper also noticed insignificant alterations in FBW, BWG, FI [16,34], and egg weight [34–36]. However, previous studies' results were contradictory regarding the effect of natural colorants on laying hens' productivity. In agreement with our results, Abou-Elkhair et al. [35] reported that phytogenic feed additives such as hot red pepper in layer diets improved the egg-production rate, EM and FCR compared to the control.

In contrast, Lokaewmanee et al. [37] found that the addition of red pepper did not affect feed efficiency, EM, or egg production. Rossi et al. [27] also observed that sweet pepper did not affect FI, FCR, EM, or egg production. Moreover, Lokaewmanee et al. [38] noted that the supplementation with paprika extract at a level of 0.1% in layer diets did not affect the FI and egg-production rates. The improvement in the egg-production rate of paprika-treated layers might be attributed to the high amount of carotenoids found in paprika, e.g., capsanthin, capsorubin β-cryptoxanthin, zeaxanthin, antheraxanthin, and β-carotene [39], which represent precursors for vitamin A. Lin et al. [40] reported that supplementation with vitamin A exceeding the NRC recommendations had no effect on hens' laying performance under normal conditions. However, their performance was improved by the addition of vitamin A when reared under heat-stress conditions. The current study was conducted in the summer season; therefore, the birds may have been exposed to moderate heat stress, which might explain the elevation in egg production of layers treated with a natural colorant (paprika).

Under the present study conditions, yolk color, shell thickness, and albumin and yolk width were improved by the dietary natural (paprika) and/or chemical (carmoisine) supplementation. Numerous researchers agreed with our findings [16,27,34,35,37,38,41]. They revealed that egg-yolk color from hens fed the natural (paprika) colorant was more yellow than that produced from those fed the control diet. This is a significant contribution, particularly considering that egg-yolk color remains an essential criterion for consumer choice [17]. It is well known that the color of yolk is strongly and substantially correlated to the carotenoid content. However, the type and chemical composition of carotenoids present in natural feed additives significantly affect the efficiency of carotenoid transfer to the egg yolk, and consequently their impact on yolk coloration [42,43]. Xanthophylls are the main contributor to egg-yolk pigmentation in chickens. An almost exclusive aggregation characterizes chickens, as β-carotene is practically wholly converted to vitamin A or otherwise metabolized [44]. Several studies have been focused on evaluating the effect of paprika on yolk color more than other characteristics in laying hens; however, when recorded, no alterations in other egg-quality characteristics were noticed after the inclusion of natural (paprika) colorant [16,21,45].

Our results revealed that paprika's dietary supplementation alone or accompanied by carmoisine decreased palmitic acid and high linolenic and oleic acid contents in egg yolks. Paprika content of polyphenols can improve egg and meat quality and improve the oxidative stability of these products through the modification of their fatty-acid (FA) profile, mostly by decreasing contents of saturated FAs (SFAs) and increasing concentrations of polyunsaturated FAs (PUSFAs) [46]. These modifications in the FA profile contribute to consumer health. Unsaturated FAs play a crucial role in animal and human nutrition, as they reduce diseases such as hypertension, diabetes, and coronary artery diseases [47]. The paprika plant is a rich source of essential fatty acids such as linolenic and oleic acids [48]. In paprika, esterification of xanthophylls to fatty acids usually occurs by binding to fatty acids as the ester. However, before their absorption in birds, xanthophylls must be saponified (split), which improves their availability to the animal [49]. In addition, capsanthin found in paprika may exert various functional impacts in the body because of its different structures, since it presents as acylated with fatty acids that make it more polar than β-carotene, and it contains oxygen [50]. Likewise, the increase of linolenic acid content in eggs could be achieved by the inclusion in laying hens' diets of specific sources of it or feed additives enriched with it [51–53]. Omri et al. [54] reported an increase in egg-yolk content of PUSFAs by supplementation of linseeds and fenugreek seeds in laying hens' diets. Furthermore, Shivaji et al. [55] documented that USFAs were increased in egg yolk, while SFAs were decreased, when layers of fenugreek seed, red pepper, and flaxseed were fed. In addition, the unsaturated fatty acids oleic and linolenic were increased by supplementation of paprika and a mixture of paprika and carmoisine colorants, which might be because the pigments in these additives are fat-soluble, and therefore absorbed in the intestine together with lipids. It is known particularly these unsaturated fatty acids are the compounds most susceptible to oxidation, but supplementing these pigments might decreased the oxidation of them [56].

The increase in egg-yolk vitamin E concentration and reduction in liver MDA content by the treatment with the natural colorant could be attributed to the carotenoid content of red pepper paprika (*Capsicum annuum* L.), which has a well-known antioxidant function, such as vitamins C and E and vitamin A precursors [57]. Present results agree with those of Abou-Elkhair et al. [35], who reported that hot red pepper and black cumin helped reduce egg MDA content. Furthermore, it has been reported that the inclusion of red pepper or black cumin in layer diets decreased MDA levels in serum and egg yolk, suggesting their significant role in reducing lipid peroxidation and scavenging free radicals. The antioxidant action of these phytogenic additives could be due to their capsaicin content [58,59].

Dietary supplementation of paprika significantly decreased the total cholesterol and triglyceride and increased HDL in the blood plasma of laying hens. Previous studies confirmed the paprika antioxidant activity; it has hypolipidemic and hypocholesterolemic activities [35,57–59]. The polyphenolic content of paprika can reduce LDL peroxidation and minimize lipid oxidation in plasma and cellular membranes [46]. Moreover, Abou-Elkhair et al. [35] noted that the dietary supplementation of red pepper reduced the serum cholesterol of laying hens, suggesting an elevated oxidative activity. The capsaicinoids in the paprika extract have antioxidant effects and reduce plasmatic cholesterol levels [60]. Niu et al. [21] stated that red pepper is an essential source of natural antioxidants and carotenoids. It has been documented that cholesterol oxidation prevention and, presumably, PUFA auto-oxidation can be achieved by adding sweet red pepper to laying hens' diets.

5. Conclusions

It would seem that in laying hens, the use of natural paprika powder at 4 g/kg feed could be a good way to increase the sustainability of producing high egg quality. Paprika has an essential role in enhancing the color of egg yolks, improving production performance, modifying fatty-acid profiles (by decreasing palmitic acid as source of saturated fatty acids and increasing linolenic acid and oleic acid as sources of unsaturated fatty acids), and decreasing blood total cholesterol and increasing HDL-cholesterol concentrations in hens.

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