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Effects of Supplementation of *Eurca* Seeds as Nutraceutical Feed Additive on Productivity, Antioxidant Activity, and Yolk Cholesterol Level of Laying Hens

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ABSTRACT

Eruca sativa (*ES*) seeds are annual herbs belonging to the *Brassicaceae* family, widely grown in Mediterranean countries, such as Egypt, Italy, and Greece. The *ES* is rich in macronutrient components and phytochemical content, exhibiting potent antioxidant properties and functional properties for vital processes such as digestion and absorption of nutrients. Therefore, this research was conducted to evaluate the effects of dietary *ES* supplementation on laying performance**,** some blood parameters, and egg yolk cholesterol. A total of 300 Silver Sabahia strain hens, aged 26 weeks, were randomly distributed among four groups of five replicates, each replicate consisting of 15 hens. Chickens in group 1 served as a control and were fed the basal diet. Those in groups 2, 3, and 4 were fed basal diet supplemented with 1, 2, and 3% *ES*s, respectively. Productive performance traits, egg quality traits, hematological parameters, blood parameters, and yolk cholesterol profiles were performed throughout the study. The study lasted for 13 weeks (until week 39 of chickens' age). Results indicated that 3% *ES* supplementation had higher results on egg mass (35.68%), egg production (21.13%), and improved feed conversion ratio by 30.37%, compared to all groups. Furthermore, *ES*s supplementation positively affects the shell thickness and yolk color score compared to the control. Compared to the control, the highest significant blood hemoglobin and lymphocytes were recorded in the groups supplemented with 2% and 3% of *ES*s. The *ES* inclusion at a higher level (3%) in the diet of laying hens led to significantly enhanced serum high-density lipoprotein and total antioxidant capacity, while reducing cholesterol, low-density lipoprotein, and malondialdehyde levels, compared to the control diet. Serum calcium, tri-iodothyronine, and alkaline phosphatase levels increased significantly in response to 3% *ES* treatment, while liver enzymes decreased significantly compared to the control diet. Notably, the addition of 2% and 3% *ES*s to the hens' ration resulted in reduced egg cholesterol content, which is desirable for consumers seeking healthier dietary choices. Finally, adding 3% *ES*s to hens' diet improves productive performance, egg quality traits, hematological parameters, blood parameters, and yolk cholesterol profile.

Keywords: Blood parameters, Egg production, *Eruca* seed, Nutraceutical additive, Yolk cholesterol

INTRODUCTION

Domestic Egyptian chickens appear to have great genetic diversity and can survive harsh environmental conditions. One of the major problems in the Egyptian local breeders is the high conversion rate and low egg production as their egg production curve ends rapidly (Khalil, 2020; El-Saadany et al., 2022a; Farag et al., 2022). Moreover, table eggs from local Egyptian chickens are very popular among consumers, in Egypt but egg yolks are high in cholesterol, which may cause health problems (Deif Allah et al., 2020). Several attempts have been made to improve egg production, feed conversion rate, egg quality, and yolk cholesterol, and one of these ways was to manipulate the diet by using natural products (El-Saadany et al. 2022b,c). Nutraceuticals are biologically active substances found in natural products. They can be added

to poultry diets for nutrition and health benefits (El-Sabrout et al., 2023).

Eruca sativa (*ES*) seeds are one of the annual herbs of the *Brassicaceae* family. It is easily grown in Mediterranean countries like Egypt, Italy, and Greece. *Eurica sativa* seeds are of great importance for human and animal health. They contain various nutritional and therapeutic properties, such as antibacterial, anti-carcinogenic, antifungal, and antioxidant properties (Kim et al., 2004). These seeds are a good source of essential oils, proteins, and phytochemicals, such as flavonoids and glycosinolates (Barillari et al., 2005; Bell and Wagstaff, 2014), which is incorporated in poultry biological functions for promoting health and productivity. Additionally, it is rich in minerals like Ca, Zn, Cu, Fe, I, K, and other elements (ELSadek, 2014). It also contains carotenoids, vitamins C, E, and K, most types of vitamin B groups, and volatile oils. For all these benefits, Egyptian farmers tend to grow *ES*s to provide a cheap source of phytochemicals and antioxidant products to consumers (El-Gengaihi et al., 2004; Barillari et al., 2005).

Previous studies showed that feeding layers with *ES* increased egg quality by improving eggshell thickness and the density of the yolk color (Al haj et al., 2019). Also, the addition of *ES*s in broiler feed improved immunity and serum oxidation systems, which led to increases in the broilers' productivity, and modulations of intestine histomorphology characteristics under the various types of stresses (Shani, 2019; Al-Shammari and Batkowska, 2021). Shani (2019) stated that dietary broilers with ESs at 2.5 g/Kg had a protective effect against any oxidative stress induced. Moreover, Abou El-Maaty et al. (2021) demonstrated that applying *ES*s to broilers' diets increased serum total antioxidant, follicle-stimulating hormone, and thyroid hormones while decreasing the concerning activities of liver enzymes. Similarly, Abdul-Majeed and Taha (2019) found that adding *Eruca* seeds to the quail diet as a rich omega 3 and 6 sources improved the lipid profile and egg production. Although some aimed to decrease egg yolk cholesterol by adding natural substances, such as sumac and ginger (Gurbuz et al., 2017), green tea extract (Huang et al., 2019), and grape seeds (Sun et al., 2018) to layer diets, there is no information about the impact of *ES*s supplementation on egg yolk cholesterol. Therefore, the current research was carried out to study the impact of *ES*s inclusion into Egyptian layer diets on their physiological state to improve productive performance and reduce egg yolk cholesterol.

MATERIALS AND METHODS

Ethical approval

The current study was performed according to the guidelines of the Departmental Committee of Animal and Poultry Production, and the pronouncement of the Ministry of Agriculture in Egypt on animal ethics and welfare (Decree No. 27 (1967) that generally enforces the humane treatment of animals.

Experimental design

A total of 300 layer hens at 26 weeks old from the Silver Sabahia strain (Egyptian local strain), with average body weight (1751 ± 95 g), were used in this trial. The experiment was conducted during the spring period of 2022, lasting for 13 weeks. Chickens for the study were obtained from two sources, namely El-Sabahia Poultry Research Station in Alexandria, and the Animal Production Research Institute within the Agricultural Research Centre in Alexandria, Egypt. Chickens were vaccinated according to the following program. The chicks received vaccinations against Newcastle disease, Gumboro, and Infectious Bronchitis during the first day. In week 3, they were vaccinated against Gumboro. At week 6, they received vaccinations against Newcastle and Infectious Bronchitis. Week 7 included vaccinations against foul typhoid. In week 14, the chickens were once again vaccinated against Newcastle and Infectious Bronchitis. Subsequently, vaccinations against Newcastle were administered every 4 weeks. All hens, which were from the same hatching batch and had similar body weights, were randomly divided into four groups, each consisting of 15 birds, resulting in a total of 5 replicates per group. These hens were individually housed in cages measuring 30 * 50 cm. The lighting was controlled artificially, with 16 hours of light per day, and the average temperature was maintained at 25℃, with an average humidity rate of 74%. Throughout the experimental period, which spanned from weeks 26 to 39 of age, feed and water were made available to the hens *ad libitum*.

Regarding the dietary treatments, the control group was provided with the basal diet, while the second group had the basal diet supplemented with 1% *ES*s/kg of diet. In the third group, the basal diet was supplemented with 2% *ES*s/kg of diet, and the fourth group had the basal diet supplemented with 3% *ES*s/kg of diet.

The *ES*s (with no preparation) were purchased from the General Company for Agricultural Agencies in Damanhor, Egypt. The ingredients and chemical

composition of the basal diet were prepared according to (NRC, 1994), and the ingredients are presented in Table 1. The methods used for calculated chemical analysis of the basal diet were according to the Association of Official Analytical Chemists (AOAC, 2000).

Table 1. Ingredient and chemical composition (g/kg) of the experimental diet for laying hens through 26-39 weeks of age

Ingredients	(%)
Corn	66.33
Soybean meal (48% CP)	24.2
Limestone	7.5
Dicalcium phosphate	1.32
Vit+Min Premix[1]	0.25
NaCl	0.25
DL-methionine	0.15
Total	100
Chemical composition	
Metabolizable energy (kcal/kg)	2700
Dry matter (%)	90.73
Crude protein (%)	16.97
Crude fat (%)	2.45
Crude fiber (%)	3.96
Ash (%)	6.37
Nitorgen free extract (%)	60.98

[1]Vit+Min mixture provides per kilogram of diet: vitamin A, 12000 IU; vitamin E, 10 IU; menadione, 3 mg; Vit. D_3, 2200 ICU; riboflavin, 10 mg; Ca pantothenate, 10 mg; nicotinic acid, 20 mg; choline chloride, 500 mg; vitamin B_{12}, 10 µg; vitamin B_6, 1.5 mg; vitamin B_1, 2.2 mg; folic acid, 1 mg; biotin, 50 µg. Trace mineral (milligrams per kilogram of diet): Mn, 55; Zn, 50; Fe, 30; Cu, 10; Se, 0.10; Antioxidant, 3 mg. CP: Crude protein

Production and egg quality traits

The body weight was determined at the age of 26 weeks, and the hens were weighed again at the end of the experiment at the age of 39 weeks. Egg weight was recorded as eggs were weighed individually to the nearest 0.01 g for each replicate, and the average was calculated. Egg mass was calculated by multiplying egg numbers by the average egg weight. The egg production was recorded according to the following equation [(number of eggs / Period) * 100] from 26 to 39 weeks of age. Feed consumption by gram was evaluated for each chicken per day. The feed conversion ratio was calculated as the amount of consumed feed required for producing a unit of egg mass. Fifteen eggs from each group once every four weeks (at 30, 34, and 38 weeks) were randomly taken from the same days of production. Eggs were collected to determine egg quality traits. Shell, albumen, and yolk percent were determined by dividing each previous item by the egg weight and multiplying by 100. The shell thickness was measured by a micrometer to the nearest 0.01 mm. The yolk color intensity was calculated based on the standard color of the yolk using a Roche yolk color fan with a score range of 1-15 from light yellow to dark yellow (Vuilleumier, 1969).

Hematological and biochemical parameters

At 39 weeks of age, 40 blood samples from the experiment (ten blood samples from each group) were randomly taken from the branchial wing vein in a tube with an anticoagulant (EDTA) and without anticoagulant for biochemical parameters. 40 Blood (10 from each group) samples were used to determine blood morphology, including hemoglobin (Hb), red blood cells (RBC'S), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscle hemoglobin (MCH), mean corpuscle hemoglobin concentration (MCHC), white blood cells (WBC'S), and their fractions (percentage of lymphocytes and heterophils). The RBCs were counted on an acridine orange (AO) bright-line hemocytometer using a light microscope at 400x magnification, while the WBCs were counted on an AO bright-line hemocytometer using a light microscope at 100x magnification after diluting blood samples 20 times with a diluting fluid (1% acetic acid solution with a little of Leishman's stain), and their fractions (lymphocytes and heterophils) were determined according to Altan et al. (2003). The Hb was determined by the cyanomethemoglobin method as cited by Coles (1986), while wintrobe hematocrit tubes were used for determining the PCV as a percentage. Serum samples were obtained by centrifuging the blood at 3000 rpm for 20 min, and it was stored at −20 °C for biochemical analysis. Serum total protein, glucose, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), calcium, phosphorus, alkaline phosphatase (ALP), total antioxidant capacity (TAC) and malondialdehyde (MDA) were measured using commercial kits (Diamond Diagnostics Chemical Company, Egypt) following the manufacturer's instructions by a spectrophotometer (SELECTA®UV-2005 SPAIN). The value of serum total tri-iodothyronine (T3) was tested using the radioimmunoassay technique according to (Hollander and Shenkman, 1974) by chemical commercial kits (Diamond Diagnostics Chemical Company, Egypt).

Yolk's cholesterol profile

Yolks were carefully separated without albumen for determination of yolk cholesterol (Allain et al., 1974), high-density lipoprotein (HDL, Lopez-Virella, 1977), and low-density lipoprotein (LDL, Wieland and Seidel, 1983) using commercial Kits (Diamond Diagnostics Chemical Company, Egypt).

Statistical analysis

This study used an entirely statistical randomization design. All results were subjected to standard statistical one-way analysis of variance (ANOVA) in the Statistical Package for the Social Sciences, SPSS, 2008, version 17. Duncan's multiple range test was implemented to evaluate whether the means of the variables differed significantly

or not (Duncan, 1955). Means were considered statistically significant at $p \leq 0.05$.

RESULTS

Productive and egg-quality traits

Table 2 shows the influence of *ES*s supplementation on the productivity of laying hens. As can be seen, there was an improvement in egg production (21.13%), egg mass (35.68%), and feed conversion ratio (30.37%) by adding 3% *ES*, compared to other experimental groups ($p \leq 0.05$). Table 3 illustrates the influence of *ES*s on the quality of eggs. Results indicated that Eggshell, albumin, and yolk percentage were not affected by *ES*s supplementation, while the administration of *ES*s in laying diets significantly improved shell thickness and yolk color score ($p \leq 0.05$).

Table 2. Productive performance of laying hens as affected by the supplementation of *Eruca* seeds through 26-39 weeks of age

Traits \ *Eruca* seeds levels	Control	1%	2%	3%	SEM	P value
Initial bW (g)	1741.00	1753.11	1762.33	1750.44	95.55	0.008
Final bW (g)	1905.33	1777.66	1866.78	1810.33	99.11	0.313
Egg Weight (g)	52.45^{d}	55.64^{a}	54.29^{c}	54.84^{b}	0	0.000
Egg Mass (g/h/d)	29.01^{b}	29.02^{b}	29.60^{b}	39.36^{a}	1.34	0.000
Egg Production (%)	57.13^{b}	53.74^{b}	53.86^{b}	69.20^{a}	1.58	0.000
Feed Intake (g/h/d)	120.54^{a}	118.68^{a}	108.33^{b}	113.71^{ab}	2.96	0.026
Feed Conversion ratio (g feed/g egg)	4.28^{a}	4.27^{a}	3.71^{a}	2.98^{b}	0.22	0.000

^{abc} Means in the same row having different superscripts are significantly different ($p \leq 0.05$). SEM: Standard error of the means, BW: Body weight

Table 3. Egg quality traits of laying hens as affected by the supplementation of *Eruca* seeds through 26-39 weeks of age

Traits \ *Eruca* seeds levels	Control	1%	2%	3%	SEM	P value
Shell weight (%)	10.11	9.63	10.70	10.59	0.29	0.138
Albumin weight (%)	57.60	58.71	56.78	56.19	0.69	0.063
Yolk weight (%)	32.29	31.67	32.52	33.22	0.66	0.457
Shell thickness (mm)	0.32^{c}	0.34^{b}	0.35^{b}	0.38^{a}	0.01	0.000
Yolk color score	5.83^{c}	6.79^{b}	7.31^{b}	8.19^{a}	0.27	0.000

^{abc} Means in the same row having different superscripts are significantly different ($p \leq 0.05$). SEM: Standard error of the mean

Hematological parameters

Table 4 presents the effects of *ES*s on the hematological traits of laying hens' diets. The results revealed that the supplementation of *ES*s could significantly improve the Hb content at different levels of *ES*, compared to the control group ($p \leq 0.05$). Similar trend was observed on MCHC and Lymphocytes as they significantly improved by *ES*s addition at different levels compared to the control ($p \leq 0.05$).

Biochemical parameters

Table 5 summarises the impact of *ES*s on laying diets' biochemical parameters. Results showed that cholesterol profile, liver enzymes, antioxidant parameters and Ca significantly improved in the treated groups with *ES*s, compared to the control group ($p \leq 0.05$). However, total protein, glucose, and phosphorous were not affected by the tested material ($p \geq 0.05$).

Yolk cholesterol profile

Table 6 indicates the impact of *ES*s on the cholesterol profile of yolk. It was found that administration of *ES*s at different levels significantly decreased yolk cholesterol and LDL ($p \leq 0.05$) and significantly increased the HDL, compared to the control group ($p \leq 0.05$).

Table 4. Hematological parameters of laying hens as affected by the supplementation of *Eruca* seeds through 26-39 weeks of age

Traits \ *Eruca* seeds levels	Control	1%	2%	3%	SEM	P value
Hb (g/dL)	10.17^{b}	10.32^{ab}	10.60^{a}	10.67^{a}	0.12	0.028
RBC (10^6/mm^3)	2.28	2.37	2.54	2.45	0.08	0.262
PCV (%)	31.51	31.95	32.80	33.00	0.36	0.028
MCV (fI)	138.08	135.02	130.28	137.12	4.06	0.709
MCH (pg)	44.56	43.60	42.10	44.32	1.31	0.719
MCHC (g/dL)	32.28^{b}	32.29^{ab}	32.32^{a}	32.32^{a}	0.11	0.021
WBC (10^3/mm^3)	13.47	13.63	13.67	13.93	0.15	0.218
Heterophils (%)	23.00	23.33	23.67	24.00	0.70	0.801
Lymphocytes (%)	41.67^{c}	43.33^{bc}	45.00^{ab}	47.33^{a}	0.80	0.001
H/L ratio	0.55	0.54	0.52	0.51	0.02	0.392

^{abc} Means in the same row having different superscripts are significantly different ($p \leq 0.05$). SEM: Standard error of the mean, Hb: Hemoglobin concentration, RBC: Red blood cell, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscle hemoglobin, MCHC: Mean corpuscle hemoglobin concentration, WBC: White blood cell

Table 5. Blood parameters of laying hens as affected by the supplementation of *Eruca* seeds through 26-39 weeks of age

Traits \ *Eruca* seeds levels	Control	1%	2%	3%	SEM	P value
Total Protein (g/dl)	6.07	6.03	6.33	5.33	0.28	0.184
Glucose (mg/dl)	175.00	176.67	168.33	157.00	10.84	0.315
Cholesterol (mg/dl)	178.00^{a}	168.00^{b}	156.33^{c}	154.67^{c}	1.85	0.000
HDL (mg/dl)	29.23d	30.53^{c}	35.57^{b}	39.00^{a}	0.41	0.000
LDL (mg/dl)	92.67^{a}	89.00^{b}	84.67^{c}	78.67^{d}	1.20	0.000
AST (U/L)	220.50^{a}	133.50^{b}	213.50^{a}	146.00^{b}	8.44	0.000
ALT (U/L)	43.07^{a}	42.60^{ab}	42.03^{b}	41.73^{b}	0.30	0.037
Calcium (mg/dl)	17.90^{b}	18.30^{b}	18.78^{ab}	19.77^{a}	0.30	0.017
Phosphorus (mg/dl)	5.86	6.18	6.04	6.25	0.09	0.075
T3 (ng/dl)	2.03^{bc}	2.18^{ab}	1.99^{c}	2.19^{a}	0.05	0.022
T4 (ng/dl)	4.72	5.26	4.95	5.16	0.11	0.061
Alkaline Phosphatase (ng/dl)	453.57^{a}	450.50^{a}	422.67^{b}	408.50^{b}	4.07	0.000
TAC (mmol/ml)	1.79^{b}	1.96^{c}	2.26^{b}	2.43^{a}	0.04	0.000
MDA (Mmol/ml)	2.62^{a}	2.10^{b}	1.92^{c}	1.72^{d}	0.06	0.000

^{abc} Means in the same row having different superscripts are significantly different ($p \leq 0.05$). SEM: Standard error of the mean, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, T3: Triiodothyronine, T4: Thyroxin, TAC: Total antioxidant capacity, MDA: Malondialdehyde

Table 6. Yolk Cholesterol profile in eggs as affected by the supplementation of *Eruca* seeds

Traits \ *Eruca* seeds levels	Control	1%	2%	3%	SEM	P- value
Cholesterol Egg (g/g yolk)	14.99^{b}	15.68^{a}	12.61^{c}	12.57^{c}	0.18	0.000
HDL (mg/g yolk)	4.28^{c}	4.85^{b}	5.01^{b}	5.53^{a}	0.15	0.000
LDL (mg/g yolk)	9.91^{a}	9.57^{a}	6.79^{b}	6.56^{b}	0.11	0.000

^{abc} Means in the same row having different superscripts are significantly different ($p \leq 0.05$). HDL: High-density lipoprotein. LDL: Low-density lipoprotein

DISCUSSION

There has been some recent inquiry into the potential use of novel natural materials as nutritional supplements in chicken diets. These components must provide nutritious and high-quality meals (El-Sabrout et al., 2023). Chickens may benefit from nutraceuticals by increasing their well-being and the quality of their products (Khalifah et al., 2021; Elazab et al., 2022). Plant seeds, such as pumpkin, garden cress, and grapes are examples of these nutraceuticals, and they have long been utilized in industrial chicken farms to maintain chickens' health and boost productivity (Taaifi et al., 2023).

In this study, authors investigated the effect of *ES*s on the production traits and physiological parameters of laying hens as the *ES*s are a good source of essential oils, proteins, and phytochemicals such as flavonoids and glycosinolates (Bell and Wagstaff, 2014).

The results showed that the addition of 3% *ES*s significantly improved the productive performance of laying hens. This result was confirmed by Jabbar et al. (2015), who found that the inclusion of *ES*s in the feed of quails improved egg weight, egg mass, and egg production. The positive effects of *ES*s on layer productive performance could be correlated to the availability of a large number of nutrients in *ES*s, such as proteins, essential oils, vitamins (A, B, and C), minerals, and glycosides, which can serve as antioxidant properties that keep chickens healthy and improve their productive performance. The *ES*s oil is rich in essential oils which improve sexual hormones by increasing mRNA levels (in sexual hormone-related genes (GnRHR, FSHR, LHR, and StAR mRNA levels, Alagawany et al., 2019). Moreover, Janeczka (2021) found that *ES* acts as a phytoestrogen because their content of 17 B-estradiol (247 picogram/gram dry weight [pg/g D.W]), which is found in flavonoids such as kaempferol, and quercetin (Shani, 2019)**.** This phytoestrogen may be involved in promoting steroid formation, resulting in an improvement in the rate of egg production in Silver Sabahia hens. The current study results indicated that adding 3% *ES*s to the diet significantly reduced feed conversion ratio values. In agreement, Abozid et al. (2014) mentioned that *ES*s contain about 35% oil, and unsaturated fatty acids contain 82.1% of total fatty acids. These essential oils affect the digestive system of chickens by enhancing digestive enzyme secretion and intestinal mucosa (Jamroz et al., 2006; Jang et al., 2007) and reducing the number of pathogens in the alimentary tract (Zeng et al., 2015). Abozid and Ayimba (2014) confirmed that *ES* oil contains high amounts of omega 3 and 6 fatty acids, reducing the feed conversion ratio for layer hens.

The addition of *ES*s improved eggshell thickness (Table 5), which is beneficial for breeders to enhance table egg safety and quality. This is in agreement with Rozan and Boriy (2022), who proved that *ES* contained a high amount of calcium (1223.5 mg /100 g). Moreover, El-Saadany et al. (2022a) confirmed that *ES*s contain quercetin, leading to an increase in eggshell thickness. Englmaierova et al. (2013) reported that the consumer preferred the yolk color to be deep, and the significant increase in yolk color in the present study was probably due to the presence of quercetin, carotenoids and essential oils in *ES*s. According to El-Saadany et al. (2022 a), quercetin administration increases the color of the yolk by producing a yellowish pigment. In addition, Al-haj et al. (2019) attributed the increase in yolk color score to the feeding hens *ES* as a source of essential oils, which elevate yolk lipids content that contains pigments (EL-Saadany et al., 2022 b).

Table 3 indicated that the improvement of hematological parameters such as hemoglobin and lymphocytes for hens treated with *ES*s could be related to the ability of secondary plant components (phytochemicals) to enhance the digestion and absorption of nutrients and subsequently improve immunity and health (Al-Shammari and Batkowska, 2021). Perhaps the significant increase in blood hemoglobin for hens treated with 2 and 3% of *ES*s is related to the presence of iron and copper in *ES*s, where iron is one of the important factors that enter into the process of producing RBC, while copper increases the absorption of iron from the digestive system to make RBC (Rowely, 1998). Moreover, *ES*s contain vitamins (B12, Niacin, B6, B2, B1, and B1), which have a role in Hb biosynthesis in the body **(**Martinez-Sánchez et al., 2008; Gulfraz et al., 2011). The results of Shani (2019) supported these previous assumptions. The increase in the number of serum lymphocytes in the *ES*s groups (2 and 3% of diet) compared to the control group may have a positive role in raising the immune responses and healthy status of chickens. These findings are consistent with those of El-Saadany et al. (2022c), who discovered that supplementing the diet with photogenic extract of pumpkin and garden cress increased lymphocyte values in local laying hens. In addition, Khalil et al. (2015) found that *ES*s contain vitamins, flavonoids, and glucosinolates, which act as antioxidants for improving growth and immune functions. Moreover, *ES* oil is rich in omega-3

and omega-6 fatty acids that improve lymphocytes in layer chickens, as mentioned by El-Saadany et al. (2022 b).

It was found that *ES* supplementation in laying hens decreased serum cholesterol and LDL, whereas HDL levels were enhanced. The results may be due to the fact that *ES*s have strong antioxidant properties. El-Fadaly et al. (2017) and Jin et al. (2009) demonstrated that glucosinolates in *ES* can inhibit lipid peroxidation. Shani (2019) reported that *ES* is rich in flavonoids like quercetin, which can be associated with the inhibition of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA), the first step enzyme in cholesterol formation (El-Saadany et al. (2022b). Additionally, El-Gengaihi et al. (2004) indicated that *ES*s have β-Sitosterol, which reduces cholesterol absorption from the small intestine. Abozid et al. (2014) reported that *ES*s contained a large amount of omega-3 and omega-6 fatty acids that inhibit lipogenesis. Abou El-Maaty et al. (2021) reported that unsaturated fatty acids in *ES*s, such as linoleic and linolenic acids, can elevate HDL and reduce cholesterol and LDL. Abdul-Majeed and Taha (2019) found that vitamin C and carotenoids in *ES*s could increase thyroid activity, which is one of the important glands for controlling cholesterol and lipid metabolism. In the current study, the obtained serum thyroxin hormone levels (increased in the group fed 3 % *ES*s) supported this assumption.

The AST and ALT activity levels are the most useful indicators for liver function. According to Table 5, adding 3% of *ES*s in the hens ration decreased the activity of AST and ALT enzymes. This could be explained by the presence of more than one active ingredient, such as quercetin, carotenoids, and essential oils in the *ES*s, and the effects are cumulative. The *ES*s are rich in carotenoids, which maintain the body's cell membranes and prevent the release of AST and ALT enzymes into the blood (El-Saadany et al., 2022b). Also, *ES*s are rich in antioxidants, such as Kaempferol, quercetin, and glucosinolates **(**Jin et al., 2009; El-Fadaly et al., 2017**),** which can activate the regeneration of the liver and reduce the activity of AST and ALT. According to Alam et al. (2007), *ES*s have a high content of Sulphur, which activates the liver function and immune system. Abou El-Maaty et al. (2021) reported that *ES*s (as a source of antioxidants) enhanced the health status of broilers by decreasing the serum lipid profile and AST and ALT.

The *ES* dietary supplementation in hens at 2 and 3% decreased serum ALP activity, whereas the highest Ca level was recorded for hens fed a diet with the addition of 3% *ES*s. Al-Daraji and Razuki (2012) demonstrated that *ES*s are rich in vitamin C, which decreases the activity of ALP and calcium in the blood. El-Saadany et al. (2022a) demonstrated that the application of quercetin to laying hens increases calcium levels in their blood. This increase in calcium level is due to increasing calcium absorption from the intestinal epithelium and stimulating the activity of vitamin D receptors, as mentioned by Inoue et al. (2010). Finally, the high blood calcium level recorded in the current study may be due to the high calcium concentration of *ES*s (1223.5 mg /100 g, Rozan and Boriy, 2022).

According to Table 5, adding 3% of *ES*s to laying chickens ration increased serum T3, which translated into increased metabolism and thus increased egg production. Thyroid hormones are involved in the regulation of anabolic and catabolic pathways of protein, lipid, and carbohydrate metabolism (Lachowicz et al., 2008; 2009). Abd El-Hady et al. (2020) indicated that treating broilers with phytogenic extracts of herbs Such as garden cress increased the T3 hormone and metabolic cycle. Abou El-Maaty et al. (2021) found that broilers fed the *ES*s diet significantly increased T3 and T4. Also, results were confirmed by the finding of Yadav et al. (2016), who found that *ES* meal probably causes improvement in the transport of sodium-iodide and increased absorption of iodide, resulting in increased production of T3 and T4.

The present study showed that treated chickens with a diet supplemented with *ES*s induced the most substantial effect on the antioxidant enzymes compared with the control. The *ES*s are rich in carotenoids, phenolics, glucosinolates, vitamin C, and flavonoids, which have a powerful antioxidant ability (Barillari et al., 2005; Bennett et al., 2006; Keyata et al., 2021). These active components can remove free radicals by potentially improving the TAC and MDA enzymes. Shani (2019) found that adding *ES* to the broiler diet significantly decreased MDA. Moreover, *ES*s contain essential oils that enhance catalase activity, detoxifying hydrogen peroxide and converting lipid hydroperoxides to non-toxic substances (Fki et al., 2005). Furthermore, quercetin in *ES* has antioxidant properties due to the presence of a C-ring, many hydroxyl groups, and conjugated orbitals (Rice-Evans et al., 1997). El-Saadany et al. (2022a) reported that quercetin can reduce MDA production and inhibit cell membrane lipoperoxidation.

Table 6 indicated that groups fed 2 and 3% *ES*s had lower significant concentrations of egg yolk cholesterol by 15.88 and 16.14%, respectively, and lower LDL levels by 31.48 and 33.80%, respectively, compared with the control group. The *ES* groups significantly increased yolk HDL levels by 17.06 and 29.21 % in the 2 and 3% groups,

respectively, compared to the control. This is an indicator of increasing the nutritive value of eggs. Recommendations state that dietary cholesterol should be constrained to less than 300 mg/day **(**Weggemans et al., 2001). As a result of the high cholesterol levels in eggs, many consumers reduce their consumption of eggs (especially among elderly or diabetic individuals) to avoid heart disease. In recent years, a variety of photogenic plants (natural antioxidants) have been widely used as alternative nutritional strategies to reduce cholesterol in eggs as grape, pumpkin, and garden cress seeds (Peipei et al., 2018; El-Saadany et al., 2022c). However, there is no information about the effects of *ES*s administration on yolk cholesterol. The decreased cholesterols content in the egg yolk seen in the present study might be due to the high content of polyunsaturated fatty acids and phytochemicals in ESs. These ingredients may cause multiple effects on laying hens, including reduced absorption or synthesis of cholesterol in the gastrointestinal tract, increased cholesterol excretion in the feces, and inhibition of hepatic cholesterol synthesis (Huang et al., 2019).

CONCLUSION

Adding *Eurca* seeds to the laying hens' diets at a high level of 3% improves production performance, egg quality traits, some hematological parameters, and blood chemical analysis. Also, *Eurca* seeds administration at 2 and 3% levels succeeded in reducing yolk cholesterol levels according to the current demands of consumers. Results in this way allow the producers to use natural feed additives to improve the quality of the final products of eggs. Future studies should be focused on more natural alternative feed additives to improve the health of poultry, products, and consumers.

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Authors' contribution

El-Sahn, El-Barbary, Farag and Khalifah deveoped the idea and designed the study. Iraqi, Farag, and EL-Prollosy collected data. El-Sahn, Iraqi and El-Barbary wrote the paper and performed the statistical analysis. Khalifah drafted the manuscript and approved the final manuscript. All authors checked and confirmed the final analysis data and the last revised manuscript before publication in the journal.

Competing of interests

The authors declared that they have no competing interests.

Availability of data and materials

The data presented in this study are available on request from the corresponding author.

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Ethical consideration

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors before the submission. The final results of the statistical analysis have also been checked and confirmed by all authors.

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