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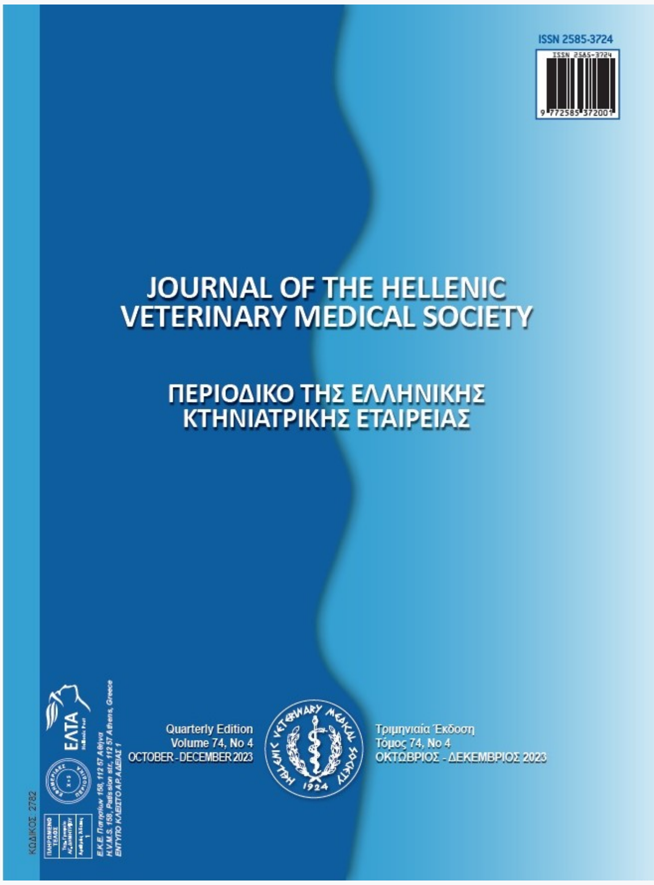

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Research article
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Effects of different levels of iron, iodine and vitamin B_{12} supplementation on egg quality and antioxidant capacity of eggs at different times of storage in laying hens

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ABSTRACT: The experiment was carried out to evaluate the effects of adding different levels of iron salts, iodine and vitamin B12 on yield, quality traits and antioxidant capacity of eggs in laying hens. A total of 320 laying hens (HyLine Variety White, 28wk old) in a 2×2×2 factorial experiment were randomly assigned to 8 groups, and each group consisting of 40 hens (5 replicates of 8 hens each) for a period of approximately 13 weeks in a completely randomized design. Hens in the control group received a basal diet with no supplementation whereas hens in treat 2 received basal diet supplemented with 400 mg Fe/kg as $FeSO_4$, treat 3 (basal diet + 450 mg I/kg as KI), treat 4 (basal diet + 0.1 mg B_{12}vit/kg), treat 5 (basal diet + 400 mg Fe/kg as $FeSO_4$ + 450 mg I/kg as KI), treat 6 (basal diet + 400 mg Fe/kg as $FeSO_4$ + 0.1 mg B_{12}Vit/kg), treat 7 (basal diet + 450 mg I/kg as KI + 0.1 mg B_{12}Vit/kg), treat 8 (basal diet + 400 mg Fe/kg as $FeSO_4$ + 450 mg I/kg as KI + 0.1 mg B_{12}Vit/kg), respectively. The results showed that the use of iodine and vitamin B_{12} increased the shell thickness of eggs ($p<0.05$). The use of iron, iodine and vitamin B12 supplements increased the antioxidant capacity of eggs compared to the control group ($p<0.05$). The highest antioxidant capacity of eggs (0.77) was observed when these three supplements were used together in the diet of laying hens.The length of storage had significant effects on the weight of albumin and yolk and antioxidant content of eggs ($p<0.05$). The highest weight of albumin and antioxidant content was at the beginning of storage, while the highest weight of yolk was obtained in 4 weeks after storage, with increasing of storage time to 8 weeks, all three attributes of albumin weight, yolk weight and antioxidant content of eggs decreased ($p<0.05$).The related effects of interval of the storage and the experimental supplementation, the use of iron, iodine and vitamin B_{12} supplements increased the antioxidant capacity of eggs, so that the highest amount of antioxidants in eggs in 8 weeks after storage and by supplementing the diets with all three additives ($p<0.05$). According to the present experiment, the use of iron, iodine and vitamin B_{12} additives in the diet of laying hens, improves the performance, the content of iron and iodine elements in eggs and maintains the suitable condition of eggs during storage.

***Keywords*:**Antioxidants; Eggs quality; Minerals; Storage time; Vitamins

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INTRODUCTION

Studies show that the efficiency of transferring vitamins and minerals to egg yolk is divided into 4 levels (low, medium, high and very high). The efficiency of transferring vitamins and minerals to egg yolk is around 50% to 80%. Although the antagonistic interactions between some vitamins have been observed, however, normal use of these vitamins does not seem to cause problems in the nutritional performance of laying hens (Gan, 2020).

The use of rations containing high levels of vitamins has increased the vitamin content of ordinary eggs daily recommended intake (DRI) up to 50%. Vitamin B_{12} showed the highest efficiency among all vitamins and when the amount of vitamin B_{12} in the diet of laying hens had increased up to 11 fold its DRI value increased from36 to 139 (Leeson and Caston, 2003). Vitamins K and biotin provide 50% of the DRI (Leeson and Caston, 2003).

Due to antagonism and synergism effects among minerals and vitamins, finding the best and most suitable level of these nutrients seems very important and necessary. In this regard, the interaction between vitamin D3 and calcium can be mentioned as an example. Vitamin D gives the ability to the body to obtain calcium from food sources that passing through the digestive tract instead of taking it from the bones. Also vitamin C helps iron absorption. Furthermore, zinc can be involved in the absorption, transfer, physiological and biochemical efficiency of vitamin B9 (folate). However, nutrients interactions are not always be positive. For example, vitamin C blocks the absorption of copper by the body, or the lowest magnesium deposition causes iron deficiency (Bagheri et al., 2019).The experiments have shown that iodine storage in eggs is mostly done in the yolk (Damaziak et al., 2018). Kaufmann et al. (1998) stated that the consumption of iodine from zero, 0.5, 1, 2, and 5 mg/kg diet cannot improve feed efficiency and egg production.

They also concluded that the amount of iodine in eggs increases significantly by supplementing the diet of laying hens. There is no significant difference in body weight, feed consumption, egg production, egg shell index, egg thickness and yolk index with consumption of zero, 3, 6, 12 and 24 mg/kg iodinein diet of laying hens that were fed with calcium iodide (Yalçinet al., 2004).Christensen et al. (1991) reported that in a study with two commercial strains of turkey the amount of iodine increased from 0.7 to 4.2 mg/kg, in which the hatchability in mother flocks statistically increased. Also Damaziak et al. (2018) stated that the use of a laying hen diet supplemented with iodine up to 10ppmcompletely inhibit the Salmonella growth until day 10 of incubation and impart high level of resistance to the vitelline membrane against the growth of Salmonella in case of eggs stored at 30◦C.Shah and Dwivedi (2022) reported that, *Actinobacteria* such as *Streptomyces* and *Propionibacteria* are among the efficient producers of B vitamins and can serve as probiotics to increase the productivity in the poultry industry through improving growth performance and immune response of poultry. Research has shown that the utilization coefficient of inorganic trace elements was about 30% (Behroozlak et al., 2019). With these aspects the effects of different levels of iron, iodine and vitamin B_{12} supplementation on egg quality and antioxidant capacity of eggs at different times of storage in laying hens evaluated in this study.

MATERIALS AND METHODS

Birds and Housing

Three hundred twenty white Leghorn hens of w-36 strains from the age of 28 to 41 weeks for approximately 13 weeks in the form of a completely randomized design with a factorial arrangement of 2×2×2 with food rations including 3 factors. Which are 2 levels of iodine (zero and 450 mg/kg) and 2 levels of iron (zero and 400 mg/kg) and 2 levels of vitamin B12 (zero and 0.1 mg/kg). Each experimental group consisted of 5 replications, where 2 pens together were considered as one experimental unit, which included a total of 40 experimental units. The chickens were kept with 17 hours of artificial lighting and optimal temperature and proper ventilation. Food rations were balanced according to the guidelines of the target strain and using the UFFDA ration software. The experimental hens was freely access to food and water. The study was carried out in an industrial poultry farm located in Khoy city called Aqlari in the months of November and December of 2018 and January of 2019 which are among the coldest months of the year. This farm has 2 halls that each hall has 4 rows of 3-story cages with cage dimensions of 40×35×30. Experimental hens were selected from the third row in the middle of the hall and from the cages in the middle row. After 10 days of habituation, data collection started.

Experimental Diets and Treatments

The diet used for experimental hens is shown in Table 1. The target ration is balanced according to the 2016 version of the white leghorn strain manual using

Table 1. Feed ingredients and nutrient composition of the basal diet.

Ingredients	Content (%)	Nutrients	
Corn	58.1	Crude protein, %	15.88
Soybean Meal	22.18	Calcium, %	4.42
Oyster Shell	10	Available phosphorus, %	0.52
Wheat Bran	6	Sodium, %	0.31
Soybean Oil	2	Methionine, %	0.58
Dical. Phos	1.6	Lysine, %	0.94
Mineral Premix[1]	0.03	Methionine + cysteine, %	1.36
Vitamin Premix[2]	0.03	Argenine %	1.06
L-lysine HCL	0.025	Linoleic acid, %	1.91
Common Salt	0.025	Threonine, %	0.68
DL-Methionine	0.013	Tryptophan, %	0.31
Total	100	Metabolizable energy, kcal/kg	2700

[1]Mineral supplement per kg of diet: 2.4 mg of copper, 0.34 mg of iodine, 30 mg of iron, 29.76 mg of manganese, 0.08 mg of selenium, 25.87 mg of zinc. [2]Vitamin supplement per kg of diet: 3520Iu Vitamin A, 0.59 mg vitamin B1, 1.6 mg vitamin B2, 13.86 mg niacin, 13.3 mg pantothenic acid, 1 mg B6, 0.06 mg biotin, 80 mg choline, 0.004 mg vitamin B12, 0.19 mg B9, 1000IU vitamin D3, 8.8 IU vitamin E, 0.88 mg k3, all calculations are evaluated based on the requirements of (1994) NRC.

the UFFDA ration software.

Furthermore, the experimental treatments were contained different dietary supplementations. Hens in control group received a basal diet with no supplementation whereas hens in treat 2 received basal diet supplemented with 400 mg Fe/kg as $FeSO_4$, treat 3 included basal diet + 450 mg I/kg as KI, treat 4 contained basal diet + 0.1 mg B_{12}vit/kg, treat 5 were basal diet + 400 mg Fe/kg as $FeSO_4$ + 450 mg I/kg as KI, treat 6 included basal diet + 400 mg Fe/kg as $FeSO_4$ + 0.1 mg B_{12}Vit/kg, treat 7 contained basal diet + 450 mg I/kg as KI + 0.1 mg B_{12}Vit/kg and treat 8 were basal diet + 400 mg Fe/kg as $FeSO_4$ + 450 mg I/kg as KI + 0.1 mg B_{12}Vit/kg, respectively.

Albumen quality

Height meter was used to determine the height of albumen. In order to determine the haugh (HU) unit, 5 eggs from each replicate were randomly selected and after numbering, they were taken to the laboratory and first weighed with a digital scale with an accuracy of ±0.001 grams and then broken on a glass surface. The height of albumen from a certain point was calculated for all eggs using a height measuring device and the HU unit using the following formula (haugh, 1937):

$$HU = 100 \times Log\,(H + 7.57 - 1.7\,W^{0.37})$$

In this equation, H is the height of the albumen (mm) and W is the weight of the egg (grams).

Albumen Index

It was calculated as the average between the maximum and minimum diameter of albumin.

Shell Quality

To determine the quality of the egg shell, two factors of the shell contain shell thickness and shell strength were measured. To determine the shell thickness, 5 eggs from each replicate were selected, numbered and broken. Then, the inside contents were completely washed with water and dried well with a paper towel, and then the thickness of the shell was calculated using a micrometer. A strength tester was used to determine the shell strength (Mertens et al., 2006).

Yolk color

Color fan method is a common method to measure yolk color and is accepted as a standard in most countries of the world. Color fan consists of 15 color stencils that are fan-shaped. Each stencil has a color that is measured visually. The range of colors is from pale yellow (number 1) to reddish orange (number 15). By comparing the color of egg yolks with a color template, scores were given according to the observed colors.

Yolk index and egg shape index

To calculate the yolk index, the height of the yolk was measured first using a height meter, and then its diameter was measured with a caliper (in centimeters). The result of dividing the height of the yolk by the diameter of the yolk represents the yolk index. For the egg shape index, like the yolk, the length and width of the egg were measured with a caliper.

Egg shape index= 100 ×W/L

W is the width and L is the length of the egg

After 13 weeks, 4 eggs were randomly taken from each replicate to determine the concentration of iron, iodine and cobalt inside the eggs and were taken to the laboratory. From the 3 treatments and the pure treatments, 4 eggs were transferred to the laboratory in the first days, 28 days and 56 days with an interval of 28 days to measure the antioxidant capacity and the effect of storage on the eggs. The storage conditions of the eggs were kept in a refrigerator at 4 degrees Celsius and 60% humidity for the effect of storage. The laboratory chosen to measure the internal content of eggs, including the amount of iodine, iron, cobalt and antioxidants, is the University Jihad Laboratory of West Azarbaijan Province, located in Urmia city, which is equipped with equipment including a furnace, an atomic absorption device for measuring substances. Mineralogy and spectrophotometry were used to measure the amount of antioxidants using a laboratory kit.

Egg quality

The height of yolk and Albumen was measured using a stand-based device (made in Japan, model. Alimet OSK3470) with an accuracy of 0.01 mm. The haugh unit was calculated using the formula previously presented by haugh (1937). The shell strength (kg/cm2) was obtained using an egg shell strength measuring device (made in Japan, model. OSK-13473). The thickness of the shell was evaluated using a micrometer with an accuracy of 0.01 mm (F.K.H model, made in Japan) in the back part of the egg from three different points (Nobakht et al., 2022).

Minerals Preparation

The experimental diet with the addition of ferro sulfate, potassium iodide and synthetic vitamin B_{12} to the basic diet in 2 levels of iron (zero and 400 mg/kg) and 2 levels of iodine (zero and 450 mg/kg) and 2 levels of vitamin B12 (zero and 0.1 mg/kg) were prepared. In the NRC table, the amount of iron, iodine, and vitamin B_{12} needed by laying poultry is 55 mg per kg, iodine 1.7 mg per kg, and vitamin B_{12} 0.2 mg per kg of diet. Ferro sulfate with the chemical formula $FeSo_4.7H_2O$ in the form of green crystals and soluble in water with the grade of industrial, food and agricultural products was purchased from Iran's Pars Oxid Company, which contains 70% iron. Iodide potassium with the chemical formula KI and the appearance of white solid crystals and molecular weight of 166 g/mol and solubility in water and for use in the food industry, which was purchased from Barnard Company with a purity of 99%. Vitamin B_{12} with the name of cyanocobalamin with 99% purity and food grade was purchased from the representative of Germany's Lohmann Company in Iran with the trade name Microvit and with the chemical formula of $C_{63}H_{88}CoN_{14}O_{14}P$.

Statistical analysis

At the end of the experiment, statistical analysis of data was done using SAS software version 3.9 and GLM procedure. The experiment was carried out in the form of a completely randomized design with a factorial arrangement of 2×2×2 with 2 levels of iodine, 2 levels of iron and 2 levels of vitamin B_{12}. The results related to antioxidants and storage conditions were completely randomized with a factorial arrangement including the first factor (control, iron, iodine, vitamin B_{12} and the combination of all 3) and the second factor (zero, 4 weeks and 8 weeks storage time). Before analyzing the data, the normality of the data was evaluated using the Kolmogorov-Smirnov test. In this model:

$$Y_{ijkl}=\mu+A_i + B_j +C_k + AB_{ij} + AC_{ik} + BC_{jk} + ABC_{ijk} + \varepsilon_{ijkl}$$

Y_{ijkl}= measured value for each piece; μ = community average; A_i= effect of iron;B_j= effect of iodine; C_k= effect of vitamin B_{12};AB_{ij}= interaction effect of iron and iodine; AC_{ik} = interaction effect of iron and vitamin B_{12}; BC_{jk}= interaction between iodine and vitamin B_{12}; ABC_{ijk}= interaction effect of iron, iodine and vitamin B_{12}; ε_{ijkl}= experimental error;

Data were analyzed using GLM procedure of SAS software version 9.3, and the means were compared with Tukey test at the level of 0.05.

RESULTS AND DISCUSSION

The effects of using different levels of iron, iodine, and vitamin B_{12} on egg parameters are presented in Tables 2, 3, and 4 during the first, second, and third 20 days of the experiment, respectively.

As it shown in table 2, the treatment containing 0.1 mg vitamin B_{12} with 450 mg of iodine per kg of the diet of laying hens had significant effects on the shell thickness ($p<0.05$), but the other treatments had no effect on egg indices in the first 20 days of the experiment. Also, it can be seen in the table 2 that the index of shell thickness in which with addition of iodine to the diet contained vitamin B_{12}, the thickness of the shell had increased, which is 7% more than treatment without iodine, and it can be indicated that vitamin B_{12} with iodine could increase the thickness of the egg shell. Also, the addition of vitamin B_{12} increases the shape index, but this increase is not significant ($p>0.05$).

Table 2. Effects of treatments on egg indices in the first 20 days of the experiment.

Treatments	large diameter (cm)	Small diameter (cm)	Shape Index	Yolk diameter (cm)	shell strength (gr/cm^2)	Egg weight gr	Albumen height (mm)	Yolk height (mm)	shell thickness (mm)	Yolk color	HU
Fe											
0	5.303	4.081	0.766	4.314	2.468	60.441	7.015	16.882	0.3498	3.7	82.495
400	5.396	4.109	0.761	5.572	1.892	61.672	6.876	15.664	0.3523	4	81.362
SEM	0.0243	0.012	0.0034	0.5531	0.5732	0.5146	0.2481	0.5447	0.0041	0.1497	1.5316
P-value	0.0643	0.1176	0.3417	0.1179	0.4829	0.1005	0.6944	0.1238	0.6722	0.166	0.6043
Iodine											
0	5.337	4.106	0.766	4.342	1.731	61.33	6.903	16.945	0.347	3.94	81.492
450	5.335	4.08	0.761	5.544	2.63	60.78	6.987	15.602	0.355	3.76	82.365
SEM	0.0243	0.0122	0.0034	0.553	0.5732	0.5146	0.248	0.5447	0.004	0.1497	1.5316
P-alue	0.9655	0.2206	0.3417	0.1341	0.2757	0.4578	0.8117	0.0909	0.1415	0.4014	0.6897
B_{12}											
0	5.355	4.094	0.762	4.316	2.602	60.291	6.938	16.78	0.352	3.875	81.661
0.1	5.32	4.096	0.766	5.57	1.759	60.822	6.952	15767	0.35	3.825	82.196
SEM	0.0243	0.0122	0.0034	0.553	0.5732	0.5146	0.2481	0.5447	0.0041	0.1497	1.5316
P-value	0.3695	0.8995	0.3838	0.1187	0.3067	0.5239	0.9677	0.1977	0.7755	0.8148	0.8066
Fe + Iodine											
0 + 0	5.294	4.082	0.768	4.331	1.757	60.586	6.974	16.771	0.351	3.69	82.084
400 + 0	5.312	4.081	0.764	4.298	3.18	60.296	7.055	16.993	0.349	3.71	82.906
0 + 450	5.379	4.13	0.764	4.353	1.706	62.074	6.832	17.119	0.342	4.19	80.9
450 + 400	3.358	4.088	0.759	6.79	2.08	61.27	6.92	14.21	0.362	3.81	81.823
SEM	0.0344	0.0173	0.0049	0.7821	0.8106	0.7278	0.3509	0.7703	0.0057	0.2117	2.167
P-value	0.5785	0.2606	0.8227	0.1241	0.5222	0.7263	0.9926	0.0505	0.0807	0.3518	0.9815
Fe + B_{12}											
0 + 0	5.294	4.091	0.769	4.311	3.139	60.634	6.966	16.683	0.352	3.54	82.146
0.1 + 0	5.312	4.072	0.762	4.318	1.797	60.348	7.063	17.081	0.348	3.86	82.845
400 + 0	5.409	4.098	0.754	4.321	2.064	61.948	6.91	16.876	0.352	4.21	81.177
400 + 0.1	5.328	4.121	0.769	6.822	1.721	61.396	6.842	14.452	0.353	3.79	81.547
SEM	0.0344	0.0172	0.0049	0.7821	0.8106	0.7278	0.3509	0.7703	0.0057	0.2117	2.1661
P-value	0.1565	0.2377	0.1207	0.1208	0.542	0.9099	0.8152	0.0764	0.6473	0.09	0.9398
Iodine + B_{12}											
0 + 0	5.381	4.114	0.762	4.345	1.737	62.308	7.044	16.846	0.355^{a}	4.04	81.939
0 + 0.1	5.295	4.098	0.77	4.34	1.725	60.352	6.763	17.043	0.338^{b}	3.84	81.045
450 + 0	5.322	4.075	0.761	4.288	3.466	60.274	6.833	16.714	0.349^{ab}	3.71	81.383
0.1 + 450	5.349	4.095	0.762	6.801	1.794	61.292	7.142	14.49	0.362^{a}	3.81	83.347
SEM	0.0344	0.0173	0.0049	0.7821	0.8106	0.7278	0.3509	0.7703	0.0057	0.2116	2.166
P-value	0.1019	0.3109	0.4061	0.1174	0.3136	0.2181	0.4059	0.1259	0.0122	0.4837	0.5142
Fe + Iodine +B_{12}											
0 + 0 + 0	5.334	4.109	0.768	4.325	1.642	61.864	7.217	16.621	0.364	3.66	83.27
0 + 0 + 0.1	5.255	4.055	0.767	4.337	1.871	59.308	6.732	16.921	0.337	3.72	80.898
0 + 450 + 0	5.254	4.072	0.771	4.297	4.636	59.404	6.716	16.746	0.34	3.42	81.021
0.1 + 450 + 0	5.37	4.089	0.758	4.299	1.724	61.188	7.394	17.241	0.358	4	84.792
0 + 0 + 400	5.429	4.118	0.756	4.364	1.832	62.752	6.871	17.071	0.346	4.42	80.608
0.1 +0 + 400	5.329	4.142	0.773	4.342	1.579	61.396	6.793	17.166	0.339	3.96	81.192
0 + 450 + 400	5.39	4.077	0.752	4.279	2.296	61.144	6.949	16.681	0.357	4	81.745
0.1 + 450 + 400	5.327	4.1	0.766	4.172	1.864	61.396	6.89	16.802	0.366	3.62	81.901
SEM	0.0486	0.0244	0.0069	1.1061	1.1463	1.0293	0.4962	1.0894	0.0081	0.2993	3.063
P-value	0.2592	0.3056	0.6399	0.1161	0.3679	0.3551	0.4205	0.0992	0.2281	0.6068	0.4537

Table 3. Effects of treatments on egg indices in the second 20 days of the experiment.

Treatments	large diameter (cm)	Small diameter (cm)	Shape Index	Yolk diameter (cm)	shell strength (gr/cm^2)	Egg weight gr	Albumen height (mm)	Yolk height (mm)	shell thickness (mm)	Yolk color	HU
Fe											
0	5.673	4.384	0.77	4.527	2.584	61.529	7.832	17.515	0.583	3.35	87.447
400	5.690	4.369	0.769	4.509	2.245	62.150	8.163	17.693	0.310	3.6175	89.121
SEM	0.02216	0.01671	0.00345	0.02550	0.16569	0.8851	0.19700	0.14531	0.19571	0.0793	1.1099
P-value	0.5749	0.6811	0.8709	0.6097	0.1583	0.4034	0.2435	0.395	0.3326	0.0233	0.2941
Iodine											
0	5.686	4.385	0.767	4.510	2.541	61.627	7.679	17.497	0.306	3.447	86.504
450	5.676	4.394	0.771	4.526	2.288	62.052	8.315	17.711	0.5876	3.52	90.064
SEM	0.02216	0.01671	0.00345	0.02550	0.16569	0.5188	0.19700	0.14531	0.19571	0.0793	1.1099
P-alue	0.7494	0.7121	0.4914	0.6683	0.2893	0.5667	0.0291	0.3051	0.3171	0.523	0.0302
B_{12}											
0	5.696	4.394	0.768	4.5011	2.364	61.975	8.043	17.617	0.306	3.475	88.504
0.1	5.667	4.385	0.770	4.5355	2.465	61.704	7.952	17.591	0.5874	3.492	88.064
SEM	0.02216	0.01671	0.00345	0.02550	0.16569	0.5188	0.19700	0.14531	0.19571	0.0793	1.1099
P-value	0.3602	0.7058	0.7454	0.3474	0.6709	0.7146	0.7461	0.8978	0.3178	0.8771	0.7814
Fe + Iodine											
0 + 0	5.673	4.389	0.771	4.512	2.712	61.22	7.605	17.441	0.301	3.3	87.511
450 + 0	5.672	4.379	0.769	4.542	2.456	61.838	8.059	17.590	0.864	3.4	87.383
400 + 0	5.7	4.380	0.764	4.508	2.37	62.035	7.753	17.553	0.311	3.595	89.496
450 + 400	5.680	4.408	0.773	4.509	2.121	62.266	8.572	17.832	0.310	3.64	88.746
SEM	0.03134	0.02363	0.00488	0.03607	0.23432	0.73369	0.27860	0.20550	0.27678	0.1122	1.5696
P-value	0.7735	0.4321	0.2772	0.6924	0.9882	0.7935	0.5164	0.7546	0.3157	0.808	0.4487
Fe + B_{12}											
0 + 0	5.684	4.394	0.770	4.494	2.57	61.706	7.848	17.5742	0.302	3.15^{b}	87.511
400 + 0	5.661	4.375	0.769	4.561	2.598	61.352	7.815	17.4576	0.863	3.55^{b}	87.383
0 + 0.1	5.708	4.394	0.767	4.508	2.159	62.244	7.237	17.6614	0.310	3.8^{a}	89.496
400 + 0.1	5.672	4.394	0.771	4.509	2.332	62.057	8.088	17.7248	0.311	3.435^{ab}	88.746
SEM	0.03134	0.02363	0.0488	0.03607	0.23432	0.73369	0.27860	0.20550	0.27678	0.1122	1.5696
P-value	0.8371	0.6934	0.5705	0.37	0.759	0.9099	0.8353	0.6644	0.3191	0.0018	0.8443
Iodine + B_{12}											
0 + 0	5.701	4.366	0.763	4.527	2.466	61.602	7.725	17.5094	0.304	3.61	86.891
0 + 0.1	5.672	4.404	0.772	4.493	2.616	61.653	7.633	17.4854	0.307	3.285	86.118
450 + 0	5.691	4.421	0.774	4.474	2.263	62.348	8.360	17.7262	0.308	3.34	90.117
0.1 + 450	5.662	4.366	0.768	4.577	2.314	61.756	8.271	17.697	0.867	3.7	90.011
SEM	0.03134	0.02363	0.00488	0.03607	0.23432	0.73369	0.27860	0.20550	0.27678	0.1122	1.5696
P-value	0.9975	0.0583	0.1029	0.0648	0.834	0.664	0.9955	0.99	0.3229	0.0046	0.8333
Fe + Iodine +B12											
0 + 0 + 0	5.693	4.384	0.768	4.539	2.66	61.424	7.659	17.507	0.298	3.22^{b}	86.642
0 + 0 + 0.1	5.653	4.395	0.773	4.486	2.764	61.016	7.550	17.374	0.304	3.38^{b}	85.89
0 + 450 + 0	5.675	4.403	0.772	4.448	2.48	61.988	8.037	17.640	0.307	3.08^{c}	88.380
0.1 + 450 + 0	5.67	4.356	0.765	4.636	2.432	61.688	8.080	17.540	0.422	3.72^{a}	88.869
0 + 0 + 400	5.709	4.348	0.757	4.516	2.272	61.78	7.791	17.511	0.311	4^{a}	87.139
0.1 +0 + 400	5.690	4.412	0.772	4.500	2.468	62.29	7.715	17.596	0.310	3.19^{b}	86.340
0 + 450 + 400	5.707	4.44	0.776	4.500	2.046	62.708	8.684	17.811	0.309	3.6^{a}	91.853
0.1 + 450 + 400	5.654	4.376	0.770	4.519	2.196	61.824	8.461	17.853	0.311	3.68^{a}	91.152
SEM	0.04433	0.03344	0.00690	0.05101	0.33138	1.03759	0.39400	0.29063	0.39142	0.1587	2.219
P-value	0.584	0.472	0.7149	0.1639	0.9107	0.6125	0.7906	0.9277	0.3256	0.368	0.857

As it cleared in Table 3, the treatment containing 400 mg Fe/kg of diet of laying hens and the treatment containing all three supplemented substances (iron, iodine and vitamin B12) improved egg yolk color index significantly ($p<0.05$), but the other treatments had no effect on the egg indices at the second 20 days of the experiment ($p>0.05$).

Also, as it shown in table 4, the treatment containing 400 mg Fe/kg with 0.1 mg vit B_{12}/kg of diet of laying hens and the treatment containing all three supplemented substances (iron, iodine and vitamin B_{12}) improved egg yolk color index significantly ($p<0.05$), but the other treatments had no effect on the egg indices at the third 20 days of the experiment ($p>0.05$).

Table 4. Effects of treatments on egg indices in the third 20 days of the experiment.

Treatments	large diameter (cm)	Small diameter (cm)	Shape Index	Yolk diameter (cm)	shell strength (gr/cm²)	Egg weight gr	Albumen height (mm)	Yolk height (mm)	shell thickness (mm)	Yolk color	HU
Fe											
0	5.718	4.410	0.767	4.911	2.217	62.473	7.308	17.678	0.284	3.61	84.363
400	5.693	4.407	0.772	4.548	1.921	61.941	7.329	17.583	0.290	3.65	84.518
SEM	0.0192	0.0118	0.0032	0.2556	0.1228	0.4582	0.1130	0.1138	0.0037	0.08003	0.74213
P-value	0.3624	0.8273	0.2929	0.3230	0.0981	0.4178	0.8948	0.5581	0.217	0.7261	0.8835
Iodine											
0	5.703	4.413	0.772	4.572	2.087	62.368	7.345	17.686	0.2857	3.515	84.576
450	5.709	4.404	0.768	4.887	0.052	62.046	7.292	17.575	0.2889	3.745	84.306
SEM	0.0192	0.0118	0.0032	0.2556	0.1228	0.4582	0.1130	0.1138	0.0037	0.08003	0.74213
P-alue	0.8351	0.5762	0.4766	0.3905	0.8439	0.6227	0.4737	0.4952	0.5460	0.0505	0.7985
B_{12}											
0	5.711	4.410	0.7703	4.895	2.126	61.331	7.448	17.761	0.291	3.65	85.124
0.1	5.700	4.407	0.7704	4.564	2.013	62.083	7.188	17.500	0.283	3.61	83.757
SEM	0.0192	0.0118	0.0032	0.2556	0.1228	0.4582	0.1130	0.1138	0.0037	0.08003	0.74213
P-value	0.6748	0.8365	0.9827	0.3677	0.5206	0.7045	0.1129	0.1150	0.1467	0.7261	0.2022
Fe + Iodine											
0 + 0	5.699	4.413	0.771	4.544	2.261	62.448	7.277	17.615	0.284	3.46	84.160
400 + 0	5.7384	4.408	0.746	5.278	2.174	62.498	7.338	17.741	0.283	3.76	84.566
0 + 450	5.7076	4.414	0.773	4.600	1.912	62.288	7.412	17.757	0.287	3.57	84.992
450 + 400	5.6769	4.400	0.772	4.496	1.931	61.594	7.246	17.409	0.294	3.73	84.045
SEM	0.0271	0.0168	0.0045	0.3614	1.1738	0.6480	0.1598	0.1610	0.0052	0.01131	1.04953
P-value	0.2237	0.7999	0.4766	0.2553	0.7620	0.5700	0.4812	0.1504	0.4512	0.5407	0.5237
Fe + B_{12}											
0 + 0	5.715	4.4124	0.770	5.206	2.361	62.462	7.522	17.128	0.304	3.44[b]	85.659
400 + 0	5.722	4.409	0.765	4.616	2.074	62.484	7.093	17.269	0.290	3.78[a]	83.067
0 + 0.1	5.708	4.409	0.770	4.584	1.891	62.200	7.375	17.268	0.308	3.86[a]	84.589
400 + 0.1	5.678	4.405	0.775	4.512	1.952	61.682	7.283	16.088	0.276	3.91[a]	84.448
SEM	0.0271	0.01668	0.0045	0.3614	0.1738	0.6480	0.1598	0.2879	0.1131	0.1131	1.04953
P-value	0.4916	0.9859	0.3550	0.4787	0.3257	0.6797	0.2979	0.6452	0.4739	0.0020	0.2516
Iodine + B_{12}											
0 + 0	5.738	4.424	0.770	4.436	2.211	63.012	7.483	17.177	0.306	3.91	85.264
0 + 0.1	5.668	4.403	0.773	4.416	1.9626	61.724	7.206	17.264	0.284	3.82	83.888
450 + 0	5.685	4.397	0.769	4.381	2.041	61.650	7.414	17.220	0.264	3.76	84.984
0.1 + 450	5.732	4.411	0.767	5.689	2.064	62.442	7.170	16.093	0.286	3.66	83.627
SEM	0.0271	0.0168	0.0045	0.4091	0.1738	0.6481	0.1598	0.0052	0.1131	0.0495	1.04953
P-value	0.0383	0.2952	0.6169	0.0911	0.3450	0.4400	0.1184	0.3653	1	0.3384	0.9929
Fe + Iodine +B12											
0 + 0 + 0	5.722	4.431	0.774	4.484	2.580	63.060	7.429	17.932	0.294	3.18[b]	84.986
0 + 0 + 0.1	5.676	4.3956	0.767	4.605	1.943	61.836	7.125	17.299	0.274	3.74[a]	83.334
0 + 450 + 0	5.708	4.3936	0.765	5.928	2.142	61.864	7.616	17.847	0.285	3.70[a]	86.333
0.1 + 450 + 0	5.768	4.422	0.764	4.628	2.206	63.132	7.060	17.636	0.282	3.82[a]	82.800
0 + 0 + 400	5.754	4.417	0.766	4.644	1.842	62.964	7.537	17.850	0.284	4[a]	85.542
0.1 +0 + 400	5.660	4.410	0.778	4.556	1.982	61.612	7.287	17.665	0.289	3.14[b]	84.441
0 + 450 + 400	5.662	4.400	0.774	4.523	1.940	61.436	7.212	17.416	0.3004	3.72[a]	83.636
0.1 + 450 + 400	5.696	4.401	0.7712	4.469	1.922	61.52	7.279	17.401	0.288	3.74[a]	84.454
SEM	0.0384	0.0237	0.0064	0.5112	0.2457	0.9165	0.2260	0.2277	0.0074	0.16007	1.48426
P-value	0.8465	0.3951	0.2560	0.3214	0.2261	0.7526	0.3799	0.6978	0.1190	0.0064	0.3720

In our study, adding minerals and vitamins to diet has increased the egg yolk color index and these findings in agreement with other reports (Zurak et al. 2022). Also, it has been reported that the addition of some minerals such as selenium to the diet of laying hens increases the activity of enzymes related to antioxidants, especially the glutathione peroxidase enzyme, which reduces the potentials of cell damage of egg liquid and egg shell, and protects it against free radicals damages (Wakebe, 1998; Dvorskaet al., 2003).Vitamins as natural antioxidants protect the yolk pigments by protecting them against oxidation and preserving the color of the yolk (Omri et al. 2019;Kim and Shin, 2022). Guoet al. (2021) observed that adding vitamin A and K3 to the diet resulted an improvement in egg shell quality and yolk color, as well as the antioxidant status of the egg shell gland of old laying hens.

Egg quality in storage conditions

The effects of using iron and iodine salts, and vitamin B_{12} on albumen and yolk weight indices and antioxidant content in the process of 8 weeks of egg storage are shown in Table 5. As can be seen in table 5, the effect of two factors of supplementation and storage time on egg albumen and yolk weight and the amount of total antioxidant content characteristics has been investigated.

Regarding the albumen weight, the highest amount of this parameter is observed in control group and this difference is significant ($p<0.05$), while no significant difference was seen in other treatments ($p>0.05$). Also, concerning the yolk weight trait, a significant difference be observed between the treatment containing Fe and the treatment containing a mixture of 3 supplements ($p<0.05$), while the other treatments did not show a significant difference ($p>0.05$). Moreover, the results of this experiment showed that by increasing the levels of iron, iodine and vitamin B_{12} (alone and with combination) the antioxidant content of eggs will be improved significantly ($p<0.05$). About the storage time and the effect of different times of storage on egg quality and egg antioxidant content, the results indicated that increasing the storage time caused a decrease in albumen and yolk weight and this decrease was statistically significant ($p<0.05$) and also the antioxidant content of the egg increased significantly with increasing days of storage in the

Table 5: The effects of Fe, I and vit B_{12} on the albumen and yolk weight and antioxidant content in the process of egg storage.

Treatments	Items		
	Albumen weight	Yolk weight	Antioxidant content
Control	62^{a}	19^{ab}	0.38^{d}
Fe	55^{b}	20^{a}	0.68^{b}
I	53^{b}	19^{ab}	0.67^{b}
Vit B_{12}	55^{b}	20^{a}	0.61^{c}
Fe + I + Vit B_{12}	52^{b}	18^{b}	0.77^{a}
SEM	1.0880	0.389	0.0123
P-Value	< 0.0001	0.0180	< 0.0001
Time			
Storage start time	60^{c}	19^{b}	0.68^{a}
4 weeks storage	57^{b}	20^{a}	0.63^{b}
8 weeks storage	49^{a}	19^{b}	0.56^{b}
SEM	0.8427	0.3014	0.0095
P-Value	< 0.0001	0.0008	< 0.0001
Treatments + Time			
Control + Storage start time	65^{ab}	20^{ab}	0.42^{d}
Control + 4 weeks storage	61^{a}	19^{ab}	0.37^{d}
Control + 8 weeks storage	59^{a}	19^{ab}	0.36^{d}
Fe + Storage start time	58^{a}	21^{ab}	0.74^{ab}
Fe + 4 weeks storage	54^{ab}	20^{ab}	0.68^{b}
Fe + 8 weeks storage	52^{ab}	19^{ab}	0.62^{b}
I + Storage start time	61^{a}	18^{bc}	0.76^{ab}
I + 4 weeks storage	56^{ab}	21^{ab}	0.71^{b}
I + 8 weeks storage	42^{c}	20^{ab}	0.56^{c}
Vit B_{12} + Storage start time	59^{a}	19^{ab}	0.66^{bc}
Vit B_{12} + 4 weeks storage	55^{ab}	22^{a}	0.60^{bc}
Vit B_{12} + 8 weeks storage	52^{ab}	19^{ab}	0.57^{c}
Fe + I + Vit B_{12} + Storage start time	56^{ab}	17c	0.83^{a}
Fe + I + Vit B_{12}+ 4 weeks storage	58^{a}	20^{ab}	0.77^{ab}
Fe + I + Vit B_{12}+ 8 weeks storage	41^{c}	18^{bc}	0.70^{a}
SEM	1.8844	0.6740	0.0213
P-Value	0.0004	0.0080	0.0058

refrigerator condition (4 degrees of Celsius and 60% humidity) ($p>0.05$).

About the mutual effects of supplements and storage time which there was observed a significant difference in albumen weight in the treatment containing 450 mg I/kg of diet of laying hens, ($p<0.05$) and also in treatment mixture of Fe, I and vitamin B_{12} ($p<0.05$), while other treatments did not show significant difference in albumen weight ($p>0.05$). Besides, the effect of treatment include three supplements (Fe + I + vit B_{12}) showed a significant difference on yolk weight ($p<0.05$) during egg storage time, while other treatments did not show a significant difference ($p>0.05$). Furthermore, the mutual effects of mineral supplements and vitamin B_{12} on the antioxidant content of eggs of laying hens there were significant improvements ($p<0.05$) in treatments containing 450 mg I/kg and a mixture of 3 supplements. Meanwhile, there was no significant difference in the interaction effects between treatments and time in other experimental groups ($p<0.05$).

According to the findings obtained in this experiment, minerals and vitamins did not have much effect on the weight of egg yolk and albumen, and the most important factor could be storage conditions and the loss of moisture and Co_2 through the egg shell and our findings in agreement with other reports (Eke et al., 2013). Therefore, the amount of antioxidants in eggs did not affect the weight of egg yolk and albumen, and it is just the storage conditions that affect the external and internal quality of eggs. Also, according to the decreasing trend of antioxidants in 8 weeks of storage and for treatments without mineral and vitamin supplements, it is a constant trend and covers approximately 12% and numerically it is 0.05 g/kg. But then for the treatments that have two minerals and vitamin B_{12} supplements, this reduction trend was 7% of the initial amount of antioxidants, so therefore, the antioxidants capacity of these eggs increases in storage conditions.

Antioxidants play an important role in maintaining the balance between free radicals produced by the metabolic system or taken from environmental sources and the body's antioxidant system. Damage to the endogenous antioxidant system contributes to the accumulation of free radicals and stimulates lipid peroxidation processes (Singh et al., 2004). Therefore, supplementing the diet with antioxidants is a suitable method to reduce oxidative stress, and in this field, the role of low-requirement elements and vitamins cannot be ignored and it was bold with other researchers (Khan et al., 2014).On the other hand, trace elements and vitamins have an effect on various physiological functions and have a major role in bone and egg shell formation and internal quality of eggs in poultry (Obianwuna et al., 2022). Subsequently deficiency of these elements can have a negative effect on egg quality (Nestor et al., 1985; Lilburn, 1994; Bessei, 2006; Angel, 2007). These factors can reduce production efficiency and ultimately lead to economic losses (Cook, 2000; Cotoet al., 2008). Based on previous studies, the mineral elements and vitamins can increase the antioxidant capacity of eggs and our findings cover that (Ghasemi et al., 2022).

The internal quality of the egg, even at the moment of laying, is affected by some chemical changes in the egg, and the deterioration process begins with the production of CO_2 gas. During the storage period, some physical and chemical changes, which include the increase in the area of the air bag, the decrease in the height of the egg yolk and albumen, which is affected by the loss of water and Co_2 from the shell, cause a decrease in the quality of the yolk and albumen (Arivazhaganet al., 2013). The amount of albumen reducing process during the storage, is highly dependent on the storage conditions (temperature and relative humidity) and shell characteristics (Obianwuna, 2022).

Storage time is usually used to identify eggs that are fresh and suitable for consumer use (according to above, it is not possible to rely only on the number of days after laying) (Rossi et al., 2010) and the supplemental treatments of this experiment could be helpful in stability of egg quality in 4 and 8 weeks of egg storage time.

CONCLUSION

Since our knowledge is still limited in practical usages of mineral and vitamin additives, totally the writers suggest that the proposed levels of Iron, Iodine and Vit B_{12} in this study maybe beneficial to enrichment the eggs of laying hens and therefore it could improve the storage condition of eggs but it needs more studies.

CONFLICT OF INTEREST DECLARATION

The authors declare that they have no known competing financial interests or personal relationship that could have appeared to influence the work reported in this paper.

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