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Effects of dietary trace element supplementation on performance of laying hens and mineral content of egg yolk

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Abstract

In this experiment, 320 laying hens of Hy-Line W-36 strain were used in a 2 x 2 x 2 factorial design (eight treatments and eight birds in each pen). The treatments were 0 and 400 mg/kg iron salt, 0 and 450 mg/kg iodide, and 0 and 0.1 mg/kg vitamin B_{12}. There were five replicates of each treatment combination and the birds were from 26 to 39 weeks old. The interaction of iron salt and iodide, the use of 450 mg/kg iodide and 0 levels of iron salt increased the iodide content of yolks. In the treatments with 400 mg/kg of iron salt combined with iodide and with 400 mg/kg of iron salt with 0 levels of iodine salt this caused a sharp decrease in the iodide content of the yolk. In the interaction of treatments containing iron salt and vitamin B_{12}, the lowest amount of cobalt and the highest level of Iodide were observed at the 0 levels of both supplements, whereas the combined use of iron salt and vitamin B_{12} increased the level of cobalt and decreased the yolk iodine content. In the three-way effects between these salts, yolk iron and cobalt content increased, and the highest amount of Iodine was observed in the third treatment (without vitamin B_{12} and iron). Overall the use of iron salt, iodine, and vitamin B_{12} could improve the performance of hens and the composition of egg yolk.

Keywords: cobalt, ferrous sulfate, iodine, vitamin B_{12}, yolk
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Introduction

Numerous studies have shown that trace elements affect the performance of livestock significantly, including that of birds. Nevertheless, the nutrition of birds, in particular laying hens, can vary, depending on factors such as type of the bird, genetic variation, location, time of year, stage of growth and production. Elements such as iron, iodide and vitamin B_{12} are vital to the overall health of birds. Minerals and vitamins that are good antioxidants can improve the internal and external quality of the eggs considerably (El-Hack *et al.*, 2018).

Iron (Fe) is important to the performance of laying hens and the optimal dose has been demonstrated to be 75 to 80 ppm per kg of poultry feed (Liao *et al.*, 2017). The biochemical processes involved include electron transport, peroxidase and catalase activities, oxygen transport and storage (haemoglobin and myoglobin), porphyrin metabolism, collagen synthesis, lymphocyte and granulocyte function, and neurotransmitter anabolism and catabolism (Abd El-Hack *et al.*, 2020; Hamidi *et al.*, 2021). According to the NRC (1994), 80 ppm of iron per kg of feed per day was recommended for the optimal performance of Hy-Line W-36.

Iodine is an essential microelement for humans and animals, and is required for the synthesis of triiodothyronine, thyroxine, and tyrosine (iodinated amino acid). Iodine deficiency reduces the production of thyroid hormones, causing morphological and functional changes of the thyroid gland and thus reducing the secretion of thyroxin (Pearce, 2017; Gaurav *et al.*, 2021).

Naber and Squires (1993) showed that vitamin B_{12} supplementation could increase the B_{12} content of the egg. Increasing the dietary concentration of vitamin B_{12} from 0.5 to 16 Mg/kg for 28 to 70 week old commercial white Leghorn chickens improved vitamin B_{12} level of the yolk by eleven-fold.

The mineral composition of egg can be enriched through manipulating the diet (Abd El-Hack *et al.*, 2020; Macit *et al.*, 2021; Rattanawut *et al.*, 2021). According to recent studies, there is a dearth of information about the main effects of iron, iodine and vitamin B_{12} on functional characteristics of laying hens. Therefore, this study was designed i) to investigate the possibility of enhancing the yolk quality with the

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administration of potassium iodide, vitamin B_{12}, and ferrous sulfate, ii) to improve the iodine, cobalt, and iron contents of eggs through dietary augmentation, and iii) to determine the effects of these nutrients on egg quality and poultry production. The experiment was implemented by providing supplemental iron salts, iodine and vitamin B_{12} to Hy-Line layers.

Materials and Methods

The protocol (no. 1361-IAU. 11.17.2019) was approved by the Experimental Animal Ethics Committee of Maragheh Islamic Azad University. The experiment was conducted at Aghlary Farm Centre, Khoy, West Azerbaijan Province, between 18 November 2019 and 17 January 2020.

Three hundred and twenty Hy-Line W-36 hens were used in a 13-week study. They were 26 to 39 weeks old and weighed from 1.48 to 1.52 kg. Treatments were arranged in a 2 x 2 x 2 factorial design. There were five replicates with eight birds in each replicate. Two adjacent cages (cage dimension 40 × 20 × 25 cm) containing four hens were considered an experimental unit. The hens received 16 hours/day of artificial lighting, with ventilation maintaining the building at a natural ambient temperature. Feed and water were provided ad libitum throughout the experiment. After an adaptation period of 10 days, the number of eggs produced, egg weight, and the amount of feed consumed were recorded daily. Feed conversion ratio (FCR) was calculated from average daily feed intake and the average egg mass produced per day (MP). The percentage of days on which a hen produced an egg (PP) was also calculated. After 13 weeks, 20 eggs were randomly selected from each treatment and were analysed for their cobalt and iron contents (Research Department of Chromatography, Iranian Academic Centre for Education, Culture and Research (ACECR), Urmia, West Azerbaijan, Iran).

A basal corn-soybean meal basal diet was formulated according to the National Research Council (1994) for laying poultry (Table 1). Experimental diets were obtained by supplementing the basal diet with synthetic 0 and 400 mg/kg of iron (Fe), 0 and 450 mg/kg of iodine (I), and 0 and 0.1 mg/kg of vitamin B_{12}.

Table 1 Ingredients and chemical composition of basal diet (%, as fed basis)

Ration	%	Nutrient	
Corn	58.10	Crude protein, %	15.88
Soybean meal	22.18	Calcium, %	4.42
Oyster shell	10.00	Available phosphorus, %	0.52
Wheat bran	6.00	Sodium, %	0.31
Soybean oil	2.00	Methionine, %	0.58
Dicalcium phosphate	1.60	Lysine, %	0.94
Mineral premix[1]	0.03	Methionine + cysteine, %	1.36
Vitamin premix[2]	0.03	Arginine, %	1.06
L-Lysine hydrochloride	0.025	Linoleic acid , %	1.91
Salt	0.025	Threonine, %	0.68
DL-Methionine	0.013	Tryptophan, %	0.31
Total	100	Metabolizable energy, kcal/kg	2700

[1] Copper: 2.4 mg, iodine: 0.34 mg, iron: 30 mg, manganese: 29.76 mg, selenium: 0.08 mg, zinc: 25.87 mg per kg of diet
[2] Vitamin A: 3520 IU, vitamin B_1: 0.59 mg, vitamin B_2: 1.6 mg, niacin: 13.86 mg, panothenic acid: 13.3 mg vitamin B_6: 1 mg, biotin: 0.06 mg, choline: 80 mg, vitamin B_{12}: 0.004 mg, vitamin B_9: 0.19 mg, vitamin D_3: 1000 IU, vitamin E: 8.8 IU, vitamin K_3: 0.88 mg per kg of diet

To analyse the mineral content of the yolk, 2.5 g of the yolk sample was weighed and placed inside the crucible. The crucible was then placed a kiln and heated to a temperature of 550 °C. After the whole ash was prepared, the crucibles were allowed to cool, and then a solution of nitric acid was used to dissolve the ash at 75 °C. The content of each crucible was transferred to a 25 cc balloon using a funnel, and the vessel was rinsed several times with distilled water, which was also added to the balloon. Measurements of cobalt and iron were done in the Research Department of Chromatography, Iranian Academic Centre for Education, Culture and Research (ACECR) at Urmia, Iran. Standards containing 1000 ppm iron and cobalt were prepared. The atomic absorption rate of the samples was recorded using atomic absorption apparatus

(Analyst 800, PerkinElmer, Inc., Waltham, Massachusetts, USA). The amounts of cobalt and iron in each sample were calculated with a calibration curve and expressed per 100 g of the sample.

After 13 weeks, 20 eggs were randomly selected from each treatment and the iodine content of the egg yolk was measured by kinetic spectrophotometry (Haap *et al.*, 2017; Todorov *et al.*, 2018; Tufarelli *et al.*, 2021). Iodine was also measured in the Research Department of Chromatography, ACECR.

The data were analysed using the PROC GLM procedure of SAS 9.4 software (SAS Institute Inc., Cary, North Carolina, USA). When the variance was unequal across treatments, the data were log-transformed before they were analysed (Milani *et al.*, 2013). Means were compared with Tukey's multiple range test at $\alpha = 0.05$.

Results and Discussion

Mean concentrations of iron, cobalt and iodide in the egg yolk are shown in Table 2. The three-factor interaction was significant for iron and iodide, The main effects of vitamin B_{12} and iodide were significant for the cobalt content of the egg yolks, which was increased when supplemental B_{12} or iodide was provided. The significance of these interaction effects required that the interacting treatments should be considered simultaneously in interpreting the results. The highest levels of iron in the yolk were found when i)iodide was supplemented without iron and B_{12}, ii) iron was supplemented without iodide and B_{12}, and iii) the diet was augmented with iron, iodide and B_{12}. Conversely, the lowest levels of iron in the yolk were found when i) no supplemental nutrients were provided, and ii) supplemental iron was provided with iodide or B_{12}. The Iodine content of the egg yolk was highest when the diet was supplemented with iodide and without additional iodide or B_{12} and lowest when the diet was unsupplemented. The remaining treatments produced responses that were intermediate between these extremes.

Table 2 Means for effects of dietary supplementation with potassium iodide, vitamin B_{12} and iron sulphate on the iodine, cobalt, and iron content of egg yolk from the treated hens

Treatments			Response variables		
Iron	Iodide	Vitamin B_{12}	Iron	Cobalt	Iodine
0	0	0	5.73 ± 1.16^{d}	0.07 ± 0.03^{b}	0.32 ± 0.02^{g}
0	0	0.1	10.76 ± 1.16^{cd}	0.20 ± 0.03^{ab}	0.45 ± 0.02^{ef}
0	450 mg/kg	0	17.31 ± 1.16^{abc}	0.08 ± 0.03^{b}	1.18 ± 0.02^{a}
0	450 mg/kg	0.1	11.81 ± 1.16^{bcd}	0.18 ± 0.03^{ab}	0.88 ± 0.02^{b}
40 mg/kg	0	0	19.14 ± 1.16^{a}	0.07 ± 0.03^{b}	0.41 ± 0.02^{f}
40 mg/kg	0	0.1	7.26 ± 1.16^{d}	0.14 ± 0.03^{ab}	0.50 ± 0.02^{de}
40 mg/kg	450 mg/kg	0	6.38 ± 1.16^{d}	0.12 ± 0.03^{ab}	0.57 ± 0.02^{d}
40 mg/kg	450 mg/kg	0.1	19.10 ± 1.16^{ab}	0.24 ± 0.03^{a}	0.70 ± 0.02^{c}

^{a,b,c,d,e,f} within a column, values with a common superscript did not differ with probability $P < 0.05$

The effects of the various supplements on the performance of laying hens are shown in Table 3. Mean egg weight and feed intake were not affected by the dietary supplements ($P > 0.05$). The PP, MP, and FCR were all affected by the three-factor interaction among the treatments ($P < 0.05$) with the observed differences in MP and FCR probably being attributable to the treatment effects on PP. The PP was highest from hens that were supplemented with iron, iodide and vitamin B_{12} and lowest for hens that were provided only supplemental iodide. The remaining treatments produced intermediate responses that were not significantly different from either extreme value. As with PP, hens that received all three dietary supplements had the highest MP and hens that received supplemental iodide produced the lowest MP. All of the remaining treatments produced values similar to the extreme values, with the exception that the supplementation with iron alone resulted in higher MP than supplementation with iodide alone.

Table 3 Means for effects of dietary supplementation with potassium iodide, vitamin B_{12} and iron sulphate on the performance of laying hens

Treatments			Response variables				
Iron	Iodide	Vitamin B_{12}	Egg weight, g	PP, %	Feed intake, g	MP, g	FCR
0	0	0	62.19 ± 0.51	79.8 ± 1.5^{ab}	110.0 ± 1.1	49.53 ± 0.92^{ab}	1.79 ± 0.04^{ab}
0	0	0.1	61.85 ± 0.51	79.8 ± 1.5^{ab}	112.4 ± 1.1	49.22 ± 0.92^{ab}	1.83 ± 0.04^{ab}
0	450 mg/kg	0	61.87 ± 0.51	74.8 ± 1.5^{b}	110.0 ± 1.1	46.15 ± 0.92^{b}	1.91 ± 0.04^{a}
0	450 mg/kg	0.1	61.52 ± 0.51	81.0 ± 1.5^{ab}	110.0 ± 1.1	49.90 ± 0.92^{ab}	1.77 ± 0.04^{ab}
40 mg/kg	0	0	62.91 ± 0.51	81.2 ± 1.5^{ab}	110.4 ± 1.1	50.85 ± 0.92^{a}	1.74 ± 0.04^{b}
40 mg/kg	0	0.1	61.63 ± 0.51	79.6 ± 1.5^{ab}	112.6 ± 1.1	49.12 ± 0.92^{ab}	1.83 ± 0.04^{ab}
40 mg/kg	450 mg/kg	0	62.38 ± 0.51	80.8 ± 1.5^{ab}	112.4 ± 1.1	50.17 ± 0.92^{ab}	1.80 ± 0.04^{ab}
40 mg/kg	450 mg/kg	0.1	62.05 ± 0.51	84.6 ± 1.5^{a}	111.6 ± 1.1	52.49 ± 0.92^{a}	1.70 ± 0.04^{b}

PP: egg production percentage, MP: mean daily egg mass, FCR: feed conversion ratio
[a,b] within a column, values with a common superscript did not differ with probability $P < 0.05$

At 28 - 36 weeks old, there were no significant differences in feed intake. Saki *et al.* (2019) and Jarosz *et al.* (2021) observed that feed intake decreased significantly with iron supplementation in broiler diets, which is not consistent with the results of the present study. In this study, however, there was no significant effect of iron on feed intake. Supplementing poultry diets with Iodine had no significant effect on eggs and egg mass (Damaziak *et al.*, 2018; Sarlak *et al.*, 2020).

Because of improved production traits, it seems that the microelement requirements recommended by NRC for laying poultry need to be revised. Thus, to meet the fundamental requirements of minerals and vitamins in industrial poultries, it is necessary to conduct some studies. Egg yolk has good potential for enrichment with diets supplemented with various minerals and vitamins. It is possible to increase the vitamin content of eggs significantly through fortifying the laying hen diet with synthetic vitamins, and table eggs can be changed to a rich source of microelements (Bagheri *et al.*, 2019; Kang *et al.*, 2020; Sun *et al.*, 2021).

Cobalt is not considered an essential mineral for hens, even though it may amount to 4% of the composition of the molecule of vitamin B_{12}. Therefore, in this study, cobalt was investigated as an indicator of B_{12}. Table 2 shows that when the diet of laying hens is supplemented with B_{12}, the level of cobalt increases significantly. However, adding vitamin B_{12} to the diet did not affect ($P > 0.05$) cobalt concentration in the liver and yolk (Souza *et al.*, 2020).

Iron is one of the components of materials found in bodies and is involved in many reactions, in metabolism, and in energy transformation (Xie *et al.*, 2019). Sarlak *et al*. (2021) stated that the iron contents of yolk, albumen, and shell rose gradually with increasing concentrations of iron. Damaziak *et al*. (2018) found that the dietary addition of 10 mg iodine/kg caused a beneficial effect on the laying performance of hens in egg production, egg weight, and feed conversion efficiency. Sarlak *et al*. (2020) demonstrated that supplementation of diets with 2 or 4 mg iodine/kg increased the iodine content of eggs without adverse effects on hen performance and egg quality traits. Janist *et al*. (2019) noted that vitamin B_{12} did not affect the production performance and egg quality.

Furthermore, iron and iodide were increased in the egg yolk when the levels of these microelements in diets were elevated. When iron and iodideare added to salt and in the presence of oxygen, the iodine moiety of the double-fortified salt is likely to be unstable owing to evaporation and catalytic oxidation of the negatively charged iodide ion to iodine (Sattarzadeh & Zlotkin, 1999). According to the present result, iron in the diet and the iodine content of the egg were negatively correlated, indicating the antagonistic effect of iron on iodine. According to Table 3, to enrich eggs with iron, special attention should be paid to the level of iodine in the diet or the iodide should be prevented from being evaporated, since reducing the iodine content of eggs lowers their nutritional value.

Conclusions

Generally, the use of iron, iodine, and vitamin B_{12} salt supplements increased the performance of chickens, the egg content, including iron, iodine, and cobalt, and improved some of the economic characteristics of chickens. This study showed that to enrich eggs yolk with iron, special care should be paid to iodine levels in the poultry diets. However, owing to the increasing accumulation of iron and iodine in the eggs, further studies are necessary to evaluate the safety of egg fortification with iron and iodine.

Authors' Contributions

MNA collected the data for this study, YM conducted the statistical analyses, MNA and AN developed the original hypotheses and designed the experiments, All authors have read and approved the finalized manuscript

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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