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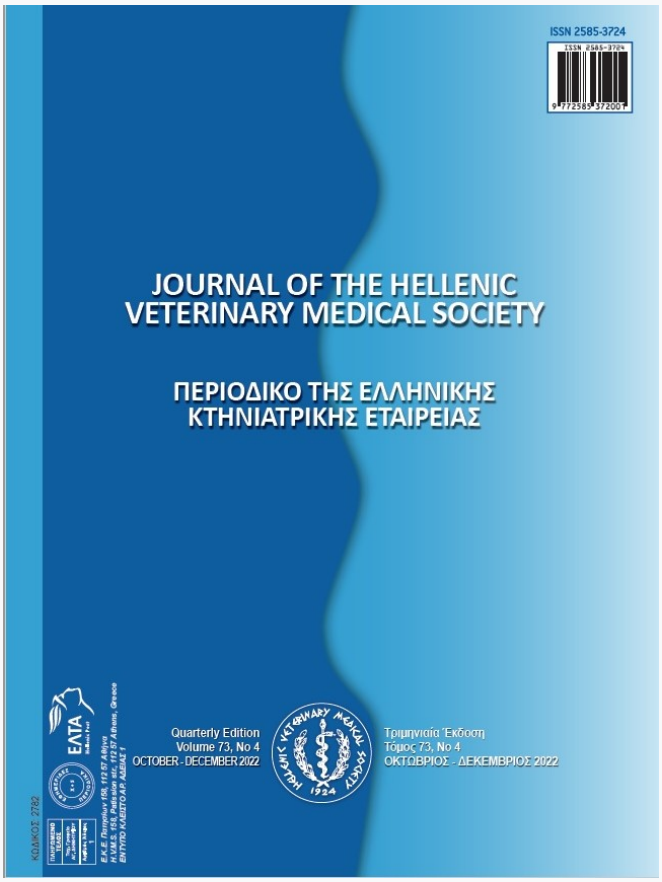

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Influence of Organic Iron Enriched Diets on Performance, Egg Quality, Blood Haematological and Biochemical Constituents in Quails

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ABSTRACT: The current research was carried out to investigate the effect of diets enriched with different levels of organic iron on performance, egg quality, blood haematological parameters, and serum biochemical constituents in laying Japanese quails. In the study, 120 female quails at 20 weeks of age were allocated to 5 treatment groups with 6 replicates of 4 quails (249±11g) in each. Experimental diets were formed by adding 0, 50, 100, 150, and 200 mg/kg of iron (iron-glycine) to the diet containing 96 mg/kg of iron. Performance parameters of quails were not affected by the supplementation of organic iron to diet (P>0.05). The supplementation of organic iron affected as quadratic only the eggshell breaking strength among the egg external quality parameters, and it was maximum at 150 mg/kg organic iron addition (P<0.01). Egg yolk a* value, which is one of the egg internal quality parameters, was linearly affected by organic iron addition, but other parameters were not affected (P<0.01). The administration of organic iron to the diet linearly increased the serum iron level(P<0.01), the serum phosphorus level was affected quadratically(P<0.05) and reached a maximum at 150 mg/kg. Among the blood haematology parameters, white blood cell and monocyte counts linearly increased with the addition of organic iron to the diet (P<0.01), but other parameters were not affected. According to the results obtained from current research, it can be said that enrichment of laying quail diets with organic iron up to 150 mg/kg level improved eggshell breaking strength without affecting performance, however, it negatively affected blood parameters by increasing white blood cell and monocyte without changing blood red cell parameters.

Keywords: Egg quality, haematology, organic iron, performance, quail, serum.

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INTRODUCTION

Iron is an essential element of vital importance in all animals. The reason for that is of iron is found in the haemoglobin structure in the organism, it carries out many functions such as oxygen and electron transport (Leeson and Summers, 2001), enzymatic activities (Chen et al., 2008), acts as prooxidant (Rajpathaket al., 2009), energy production (Mackenzie et al., 2008), and collagen synthesis (McDowell, 2003).

Since vegetable raw materials are insufficient in terms of iron due to factors such as soil, climate and phytate content of the plant (Yu et al., 2000; Gupta et al., 2008), iron deficiency occurs in poultry fed with these materials (Gupta et al., 2008).In these mentioned feed sources, both the iron level and its bio-availability are low, as well as high calcium, phytic acid, and iron levels in the diet affects its availability (Vieira, 2008; Gibson et al., 2010; Xie et al., 2019a,b).

Accordingly, this element is supplemented in the diet with premix in order to ensure that the iron needs of the poultry are exactly covered. Commonly, iron sulphate, which is a cost-efficient and low bioavailability source (form), is generally used in premixes (Ma et al., 2014; Xie et al., 2019a).For that purpose, addition of amino acid chelates (such as iron glycine) to diet instead of inorganic forms or enrichment diets in terms of iron become importance for the sustainability of the performance. Xieet al., (2019a) stated that the lower level (20 mg/kg) of organic source (iron glycine) can be used instead of iron sulphate for the permanence of egg quality in laying hens. Results reported by Sarlak et al. (2021) were similar. In another study (Paik et al.,2009), it was stated that there was an improvement in egg weight and Haugh unit in laying hens fed with diets enriched with organic iron source (iron proteinate). Compared to other essential trace elements, the number of studies on iron in poultry is less. Therefore, the aim of this research was to examine the effects of practical diets enriched with organic iron on performance, internal and external qualities of egg, blood haematology and serum biochemical constituents in layer quails.

MATERIALS AND METHODS

Ethical Approval

Criteria specified by the National Institute of Health Guide for the Care and Use of Laboratory Animals were followed during the study period.

Animals and Feed Materials

In the present experiment, 120 female Japanese quails at the age of 20 weeks were fed *ad-libitum* for 70 days with 5 treatment diets that included 0, 50, 100, 150 or 200 mg/kg organic iron (iron-glycine) to the basal diet based on corn-soybean meal (Table 1).The study was conducted in 5 experimental groups consisting of 6 replicates, each containing 4 female quails. A 16-hour lighting program was applied to quails.

Method

Determination of performance parameters

At the beginning of the experiment, the quails were placed in the cages by group weighing and at the end of the experiment, the groups were weighed again, to determine changes in body weight. Experimental feeds were given to the treatment subgroups by weighing and feed intake (FI) were assessed as g/day/quail. Eggs collected at the same time of each day were recorded and egg production (EP) was calculated as %. Egg weight (EW), on the other hand, was determined as g by weighing all eggs collected in the last three days of the experiment.From these data, also, egg mass (EM)

Table 1. Basal diet and its calculated nutrient contents

Ingredients	g/kg	Nutrient contents	g/kg
Corn	544.0	Metabolizable energy, kcal ME/kg	2900
Soybean meal (46%)	343.7	Crude protein	200
Soybean oil	36.7	Calcium	25.0
Limestone	56.0	Available phosphorus	3.5
Dicalcium phosphate	11.5	Lysine	10.9
Salt	3.5	Methionine	4.5
Premix[1]	2.5	Cystine	3.7
DL methionine	2.1	Methionine+cystine	8.2
Total	1000.0	Iron	0.96

[1]Premix is supplied that per kg of diet; Manganese: 80 mg, Iron: 60 mg, Copper: 5 mg, Iodine: 1 mg, Selenium: 0.15 mg, Vitamin A: 8.800 IU, Vitamin D_3: 2.200 IU, Vitamin E: 11 mg, Nicotine acid: 44 mg, Cal-D-Pan: 8.8 mg, Riboflavin: 4.4 mg, Thiamine: 2.5 mg, Vitamin B_{12}: 6.6 mg, Folic acid: 1 mg, Biotin: 0.11 mg, Choline: 220 mg.

was calculated as g/day/quail with the formula *(EP x EW) / 100* and feed conversion ratio (FCR) was determined as g feed/g egg *with FI / EM* formula.

Determination of egg quality parameters

During the experiment, broken, cracked and damaged eggs were recorded and calculated as % of the number of eggs. Egg internal and external quality parameters were determined from all eggs collected in the last three days of the experiment. Eggshell breaking strength was assessed by applying supported-systematic pressure to the blunt of the eggs (Egg Force Reader, Orka Food Technology, Israel).Immediately after the determination of the eggshell breaking strength, the eggs were broken on a clean, glass surface, and after the residues in the eggshell were cleaned, the shells were dried at room temperature for three days and weighed, and relative weights were calculated as a ratio (%) of the egg weight. Eggshell thickness was calculated by averaging the measurements obtained from three sections (equator, blunt, and pointed parts) of the eggshell using a micrometre (Mitutoyo, 0.01 mm, Japan).Eggs, which external quality characteristics were determined, were broken on a surface and their albumen and yolk heights were measured with a height gauge and their length and width were measured with the calliper. The parameters calculated from these data and the formulas used are follows: albumen index with the formula *albumen height/ ((albumen width + albumen length) / 2) × 100*, yolk index with the *(yolk height / yolk diameter) × 100,* and finally Haugh unit with the *100 x log (albumen height + 7.57-1.7 x EW$^{0.37}$).*In addition,L*, a* and b* a values of yolk were measured with a colorimeter (MinoltaChromaMeter CR 400 (MinoltaCo., Osaka, Japan) (Romero et al., 2002).

Blood haematological analysis

At the end of the experiment, blood were collected from the wing veins of 6 quails randomly selected from each treatment group (one quail from each subgroup) into heparinized tubes for blood haematological analysis. Blood haematological analyses were performed in an auto-analyser in a commercial laboratory according to Campbell (1988).

Serum biochemical analysis

At the end of the experiment (10thweek), 3 ml of blood was taken from one random quail (30 in total) of similar body weight from each subgroup to determine serum parameters. Blood were centrifuged at 4000 rpm for 10 minutes and serum were extracted. The serums were stored at -20^{o}C until analysis, and the concentrations of glucose, triglyceride, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, albumin, globulin, urea, creatinine, calcium, phosphorus and iron in the serum were determined (Choi et al., 2013) in an auto-analyser device using commercial kits in a commercial laboratory (DDS® SpectrophotometricKits, Diesis DiagnosticSystemsCo., Istanbul, Turkey).

Statistical analysis

Data were analysed in the SPSS 18.0 software package (SPSS Inc., Chicago, IL, USA) with model of one-way ANOVA, using the group mean as an experimental unit. A probability value of $P<0.05$ was considered statistically significant. Orthogonal polynomial contrasts were used to evaluate the significance of linear and quadratic models to determine the response of the dependent variable to an increasing organic iron level.

RESULTS AND DISCUSSION

Performance Parameters

Effects of organic iron supplemented to commercial quail diets at different levels (0, 50, 100, 150 or 200 mg/kg) on performance were demonstrated in Table 2. Administration of organic iron to laying quail diets did not significantly affect body weight change,

Table 2. Influence of dietary supplementation of organic iron on the performance parameters in layer quails

	Organic iron (mg/kg)						*P*-value of contrast	
Parameters	0	50	100	150	200	SEM*	Linear	Quadratic
Body weight change, g	-3.17	-11.92	-2.00	1.17	-5.52	2.056	0.188	0.051
Egg production, %	90.43	92.00	89.13	89.91	92.02	0.739	0.841	0.464
Egg weight, g	12.30	12.27	12.02	12.38	11.46	0.135	0.097	0.289
Egg mass, g/d/quail	11.12	12.29	10.71	11.12	10.54	0.151	0.226	0.697
Feed intake, g/d/quail	29.45	30.67	28.13	29.34	29.02	0.268	0.205	0.742
Feed conversion ratio, g feed/g egg	2.65	2.74	2.63	2.64	2.77	0.038	0.627	0.543

*Standard error means

EP, EW, EM, FI and FCR (P>0.05). These results are agreement with the reports of Buckiuniene et al. (2016), Bai et al. (2018), Gou et al. (2020) who stated that the addition of iron to the diet did not affect performance in laying birds, but not Paik et al. (2009), Xie et al. (2019a), Sarlak et al. (2021) who clarified that it affect positively performance. Iron is a mineral involved in the protein and energy metabolism, and transport of oxygen in animals (Leeson and Summers, 2001; Mackenzie et al., 2008). With these properties, it is a mineral that can affect the productivity of poultry. However, in the current study, the reason why enriched the diet with organic iron did not affect performance could be because the iron level in the basal diet was sufficient for the performance of quails.

Egg quality parameters

Effects of organic iron supplementation to commercial quail diets on the egg external quality were given in Table 3, and the effects on egg internal quality were shown in Table 4.

Damaged egg rate, relative eggshell weight, and eggshell thickness were not affected by the treatments(P>0.05). However, addition of iron-glycine to laying quail diets quadratically affected the eggshell breaking strength (P<0.01). Eggshell breaking strength improved with the supplementation of organic iron to the diet and reached maximum at 150 mg/kg, but it was minimum at the level of 200 mg/kg addition. In a related study, Gou et al. (2020) explained that addition of iron to the diets linearly and quadratically affected eggshell breaking strength, and it was maximum at 58 mg/kg and it minimum at 100 mg/kg level in broiler breeders. Sarlak et al. (2021), on the other hand, stated that the addition of inorganic iron(iron-sulphate) at the level of 30 and 60 mg/kg and organic iron (iron-glycine) at the level of 30, 60, and 120 mg/kg increased the eggshell breaking strength in laying hens. However, Paik et al. (2009), Seo et al. (2010), Taschetto et al. (2017), Xie et al. (2019b) noted that the supplementation of organic iron at the level of 0-200 mg/kg to the diet of laying hens did not affect the eggshell breaking strength.

Among the egg internal quality parameters, albumen index, yolk index, Haugh unit, and L* and b* values of yolk did not considerably affected by the administration of organic iron to the diet(P>0.05). Yolk a* value was linearly reduced by treatments(P<0.01) and it was minimum at 200 mg/kg iron-glycine addition to the diet. Since iron is included in the structure of haemoglobin and myoglobin, which colouring red to meat, it is known that added iron to the diet increases a* value (redness) of meat (Lin et al., 2020). In addition, it has been reported that the supplementation of iron to the diet increased (Sarlak et al., 2021) or did not affect (Paik et al., 2009; Seo et al., 2010; Gou et al., 2020; Xie et al., 2019b) the colour of egg yolk(Roche scale).However, in the literature, there aren't data examining the effect of dietary iron level (additional) on a* value, which is a measure of greenery and redness of egg yolk. Therefore, it is obvious that the linear decrease in the egg yolk a* value(increasing greenery) by the risen iron level in the diet should be investigated in more detail.

Table 3. Influence of dietary supplementation of organic iron on the egg external quality in layer quails

Parameters	Organic iron (mg/kg)					SEM*	*P*-value of contrast	
	0	50	100	150	200		Linear	Quadratic
Damaged egg rate, %	0.28	0.35	0.82	0.14	0.00	0.155	0.503	0.242
Eggshell breaking strength, kg	1.39	1.49	1.55	1.61	1.36	0.034	0.796	*0.006*
Relative eggshell weight, %	8.07	8.19	7.99	8.16	8.33	0.073	0.359	0.466
Eggshell thickness, µm	217	218	214	221	221	1.9	0.466	0.534

*Standard error means

Table 4. Influence of dietary supplementation of organic iron on the egg internal quality in layer quails

Parameters	Organic iron (mg/kg)					SEM*	*P*-value of contrast	
	0	50	100	150	200		Linear	Quadratic
Albumen index	3.38	3.16	3.63	3.02	2.97	0.093	0.128	0.333
Yolk index	50.87	53.24	53.31	53.10	52.80	0.503	0.311	0.201
Haugh unit	95.84	94.94	97.91	93.26	93.11	0.642	0.100	0.229
L* value of yolk	51.96	53.51	53.93	53.31	53.98	0.290	0.061	0.237
a* value of yolk	0.20	-0.28	-0.53	-0.20	-0.65	0.092	*0.008*	0.347
b* value of yolk	24.76	27.23	24.29	25.40	25.46	0.401	0.878	0.810

*Standard error means

Blood haematology

Effects of organic iron supplemented to commercial quail diets at different levels (0, 50, 100, 150 or 200 mg/kg) on blood haematological parameters were shown in Table 5.Among the blood haematological parameters, white blood cell and monocyte counts were linearly affected by the administration of organic iron to the diet($P<0.01$), this effect was not observed in other parameters($P>0.05$).White blood cell concentration showed a linear increase with the supplementation of iron-glycine to the diet and it was maximum at the level of 150 mg/kg. Supplementation of organic iron did linearly affect the monocyte level of laying quails, and while it was minimum with added organic iron at 50 mg/kg, it reached maximum at 200 mg/kg. Seo et al. (2010) indicated that the administration of 100 mg/kg iron proteinate to laying hen diets did not affect serum white blood cell and monocyte levels. There is evidence that iron inhibits intracellular monocyte accumulation (Li and Frei, 2006; Scott et al., 2021). Therefore, enrichment of the diet with iron could diminish the number of intracellular monocytes in quails and caused an increase in monocytes in the blood. In parallel, it is seen that the increase in monocyte, which is one of the white blood cells, also increases the counts of white blood cell.

Serum biochemical constituents

Supplementation of organic iron to the laying quail diets did not statistically affect serum biochemical constituents except for phosphorus and iron levels. Serum phosphorus level was quadratically affected by the treatments ($P<0.05$), and it was maximum in the group fed with diet added at lowest level (50 mg/kg) of iron-glycine, while it was minimum in the group added at highest level (200 mg/kg) of iron-glycine. This result was not in harmony with the reports demonstrated by Xieet al. (2019a).

Serum iron level of laying quails linearly increased the addition of organic source of iron to the diet and it reached a maximum at 200 mg/kg level. Similarly, Xie et al. (2019a) notified that the serum iron level augmented with the administration of organic source of iron (iron-glycine) at the level of 60 and 80 mg/kg to the laying hen diets. In addition, Bai et al. (2018) stated that high iron supplementation provided an increase in serum iron level. Similar results were reported by Guoet al. (2020) and Sarlak et al. (2021).

It was expected that the serum iron level would increase with the increasing iron level in the diet. Surprisingly, however, despite an antagonistic relationship between iron and phosphorus (Ashmead and Zunino, 1993), serum phosphorus levels increased with the addition of organic iron to the diet at 50, 100, and 150 mg/kg. This increase in serum phosphorus level can be due to insufficient iron level for the antagonist between the two elements. As a matter of fact, when Table 6 is examined, it is seen that the serum phosphorus level tends to decrease with the addition of 200 mg/kg iron in the diet. It can also be predicted that this decrease in serum phosphorus level will be exacerbated by the addition of higher levels of iron to the diet.

CONCLUSIONS

In this study, the performance parameters of lay-

Table 5. Influence of dietary supplementation of organic iron on the blood haematological parameters in layer quails

	Organic iron (mg/kg)						*P*-value of contrast	
Parameters	0	50	100	150	200	SEM*	Linear	Quadradic
White Blood Cell, $10^3/\mu L$	14.26	17.42	18.10	21.43	19.25	0.671	*0.001*	0.093
Neutrophil, $10^3/\mu L$	0.632	0.753	0.656	0.989	0.803	0.0535	0.125	0.673
Lymphocyte, $10^3/\mu L$	13.73	15.78	13.46	17.14	15.65	1.608	0.355	0.870
Monocyte, $10^3/\mu L$	0.058	0.043	0.122	0.100	0.147	0.0121	*0.004*	0.799
Eosinophil, $10^3/\mu L$	0.195	0.178	0.294	0.236	0.215	0.0287	0.648	0.472
Basophil, $10^3/\mu L$	1.300	0.528	1.048	0.488	0.835	0.2280	0.569	0.566
Erythrocyte, $10^6/\mu L$	2.97	3.07	2.94	2.67	3.10	0.063	0.777	0.321
Haemoglobin, g/dL	16.52	17.73	16.96	17.68	17.09	0.472	0.473	0.914
Haematocrit, %	43.05	45.10	44.14	42.25	47.18	0.599	0.173	0.301
Mean corpuscular volume, μm^3	146	147	150	150	147	1.2	0.525	0.223
Mean corpuscular haemoglobin, pg	55.90	58.12	57.70	55.76	57.20	0.871	0.970	0.692
Red cell distribution width, %	10.90	10.63	11.58	11.22	10.98	0.163	0.522	0.372
Platelet, $10^3/\mu L$	29.94	43.55	38.22	41.56	28.52	3.470	0.848	0.144
Haemoglobin/ Lymphocyte	1.22	1.16	1.50	1.07	1.18	0.147	0.754	0.517

*Standard error means

Table 6. Influence of dietary supplementation of organic iron on the serum parameters in layer quails

	Organic iron (mg/kg)						*P*-value of contrast	
Parameters	0	50	100	150	200	SEM*	Linear	Quadratic
Glucose, mg/dL	294	287	255	280	295	6.2	0.933	0.057
Triglyceride, mg/dL	1197	1290	1318	1164	1231	33.1	0.813	0.414
Cholesterol, mg/dL	183	159	172	145	164	4.9	0.118	0.247
AST, U/L	259	251	238	240	281	11.6	0.714	0.218
ALT, U/L	1.50	1.33	1.67	2.50	2.00	0.251	0.245	0.939
Total protein, g/dL	4.10	3.77	4.30	4.18	4.20	0.098	0.385	0.952
Albumin, g/dL	1.45	1.40	1.58	1.50	1.47	0.035	0.602	0.442
Globulin, g/dL	2.65	2.37	2.72	2.68	2.73	0.070	0.340	0.634
Urea, mg/dL	11.33	10.17	11.33	9.67	12.50	0.472	0.585	0.199
Creatinine, mg/dL	0.308	0.275	0.297	0.680	0.292	0.0805	0.522	0.612
Calcium, mg/dL	20.20	17.78	22.02	20.23	19.12	0.590	0.945	0.150
Phosphorus, mg/dL	7.60	8.70	8.30	8.53	7.07	0.256	0.487	*0.039*
Iron, µg/dL	389	495	509	541	610	18.7	*<0.001*	0.632

*Standard error means, AST: aspartate aminotransferase, ALT: alanine aminotransferase.

ing quails were not affected by the addition of organic iron to the diet. Eggshell resistance and white blood cell counts increased with the addition of organic iron to the diet at the level of 150 mg/kg. In addition, serum iron concentration linearly increased with rising dietary organic iron supplementation. These results showed that laying quails can be fed with diets enriched with organic iron at the level of 150 mg/kg.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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