Buletin Peternakan 48 (2): 215-220, August 2024

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X
Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v48i3.91485

Efficacy of Iron-Rich Premix Mineral Supplementation on Egg Yolk's Fe Content and Egg Quality

Chusnul Hanim*, Muhammad Fathin Hanif, and Ali Agus

Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia

Article history
Submitted: 6 December 2023
Accepted: 23 January 2024

* Corresponding author:
E-mail: c.hanim@ugm.ac.id

ABSTRACT

Iron's significance in human health and diseases has been extensively examined in recent reviews, leading to the consensus that iron insufficiency is a worldwide issue requiring immediate attention. Fe-enriched eggs are significant for delivering this essential trace mineral to humans. This study aimed to assess the effects of adding premix minerals on the physical and chemical quality of eggs and the yolk's iron levels. 1,680 Lohman brown laying hens, aged 31 weeks (body weight: 1.70±0.11 kg, egg production average: 81.2 %), were divided into two groups (n = 840) and fed different diets for six weeks. The diets included a basal diet (CON) and a diet enriched with a 2.5 g / kg food premix mineral (PM-Fe). The findings indicated that there was no impact on the physical and chemical quality of the eggs. However, compared to the control diet, the addition of premix significantly enhanced the iron level in the yolk after 42 days ($p<0.05$). To summarize, adding 2.5 g per kg of premix mineral (which contains 12.6 g per kg of iron premix) can result in a 23.4% rise in iron content in the diet and a 15.7% increase in iron content in the egg yolk.

Keywords: Chemical egg quality, Fe, Feed additive, Laying hens, Physical egg quality

Introduction

The estimated prevalence of anemia among women of reproductive age in 2019 was 29.9% (95% uncertainty interval (UI) 27.0%, 32.8%). It corresponds to an approximate population of around 500 million women between the ages of 15 and 49. The condition was shown to affect 29.6% (95% uncertainty interval [UI]: 26.6%, 32.5%) of non-pregnant women of reproductive age and 36.5% (95% UI: 34.0%, 39.1%) of pregnant women (WHO, 2019). The Indonesian Ministry of Health (2018) disclosed that the prevalence of anemia, or insufficient blood levels, among pregnant women in Indonesia remains significantly high at 48.9%. It is also associated with a higher incidence of miscarriage and restricted growth of the fetus, resulting in low birth weight, premature birth, fetal mortality, and anemia in the first year of life due to inadequate iron storage (Zhao *et al.*, 2022).

Eggs have a vital role as a staple meal in the daily diet of humans. In addition to being rich in high-quality protein, eggs also provide essential nutrients such as vitamins, minerals, and other bioactive components (Lesnierowski and Stangierski, 2018; Sanlier and Üstün, 2021). Iron (Fe) is a crucial mineral found in eggs that has a vital role in human health (Korish and Attia, 2020). Iron (Fe) is essential for various metabolic processes, encompassing oxygen respiration, detoxification of pharmaceuticals and foreign chemicals, control of reactive oxygen species (ROS), and the synthesis and breakdown of diverse compounds, including nucleic acids, hormones, neurotransmitters, heme, and myelin (Mezzaroba *et al.*, 2019; Grzeszczak *et al.*, 2020).

Iron deficiency may occur due to a decreased iron supply (Soppi, 2018). Iron absorption issues can occur in the gastrointestinal tract due to several factors, such as inflammatory bowel diseases, Helicobacter pylori infections, cancer, congenital or acquired transferrin deficiency, coeliac disease, and increased need for iron, such as during pregnancy. Iron deficiency is more common in women during pregnancy and breastfeeding, children and adolescents during periods of rapid growth, those following a vegetarian diet, and the elderly population (Grzeszczak *et al.*, 2020).

In modern times, food serves the purpose of not only fulfilling hunger and providing essential nutrients for humans but also preventing nutrition-related diseases and enhancing the overall physical and mental health of consumers (Siró *et al.*, 2008). Creating an iron-rich egg with the addition of mineral supplements could be a potential solution for combating iron deficiency in humans. The approach entails enhancing the iron

(Fe) concentration in eggs by injecting supplementary minerals into the layer diet.

Materials and Methods

Study location and ethical clearance

The experiment was conducted at PT. Sentra Gemilang Mulia, Layer Farm in Sedayu, Bantul Regency, Special Region of Yogyakarta, Indonesia and obtained prior consent from the Ethical Commission of the Faculty of Veterinary Medicine at Universitas Gadjah Mada, Indonesia, under reference number 98/EC-FKH/Eks/2023.

Birds, treatments, and sample collection

A total of 1680 Lohman brown hens, aged 31 weeks (body weight of 1.70±0.11 kg, hen day egg production average of 81.2 %), were randomly divided into two groups, with each group consisting of 6 replicates of 140 hens. The first group was provided with the standard diet and served as the control. The second group received a control meal that was fortified with a premix mineral at a concentration of 2.5 g/kg diet (containing inorganic Fe 12.6 g/kg premix) for 31-37 weeks (6-week treatment). PT Agromix Lestari Yogyakarta, Indonesia, supplied the premix mineral (Twin Booster Unggas®). The birds were kept in laying cages with a 16-hour light and 8-h dark cycle, following summer conditions. The same management methods were applied, including ventilation, moisture control, and temperature regulation. A single egg was retrieved from each replicate on days 21 and 42 for the purpose of assessing chemical egg quality. There were a total of 6 eggs per treatment group. An assessment of the physical quality of eggs was conducted every three days, using two eggs per replication and a total of 12 eggs per treatment group.

Physical egg quality

The egg shape index was calculated as the ratio of vertical and horizontal diameter of the egg using Egg Form Coefficient Measurement Instrument (FHK, Fujihira Industry Co., Ltd., Tokyo, Japan). Eggshell strength was measured using a pressure gauge (FHK, Fujihira Industry Co., Ltd., Tokyo, Japan). The height of the albumen was measured as the distance between the metal plate and electrode placed on top of the thickest part of the egg. Using a micrometer (FHK, Fujihira Industry Co., Ltd., Tokyo, Japan), the Haugh units were assessed by the following formula: 100 log (H + 7.57)1.7 · W0.37), where H = albumen height (mm) and W= egg weight (g). The weights of the shell, albumen, and egg yolk were measured using a High-Precision Digital Lab Weight with a capacity of 500 g/0.01 g (YongXuan, China). Yolk color was determined using the Roche yolk color fan scores (RYCF; F. Hoffman-La Roche, Basel, Switzerland), and colors were scored according to 15 sample colors ranging from 1 (the lightest) to 15 (the darkest).

Chemical egg quality

An examination was undertaken to estimate the moisture, protein, fat, and ash content of the egg yolk using proximate analysis, following the guidelines set by AOAC (2005). In order to assess the cholesterol levels in egg yolk, 5 g of yolk samples were combined with 10 milliliters of a 0.25 Normal potassium hydroxide (KOH) solution and immersed in a bath at a temperature of 80°C for 3 hours. Following the chilling process, 20 mL of ethanol was introduced and subsequently subjected to extraction with a 20 mL mixture of diethyl ether and petroleum benzene (1:1) for 24 hours. The uppermost layer solution was subjected to concentration in a water bath at a temperature of 40ºC. The resulting oil was dissolved by means of a 5 mL solution of Toluene and agitated vigorously for 15 s. A volume of 0.25 mL of the supernatant was extracted and mixed with a 1 mL solution of toluene. Subsequently, a volume of 1 µL of the solution was introduced into the GC vial and juxtaposed with the reference solution.

The cholesterol analysis was performed using gas chromatography-mass spectrometry (GC-MS) on an Agilent 7890B autosampler Series Gas Chromatograph (Agilent Technologies, Palo Alto, CA, USA) fitted with a BP-5 capillary column (30 m×320 µm×0.25 µm). Austin, Texas, United States). The temperature program's experimental parameters were as follows: The program started at an initial temperature of 250°C for 15 min, and then it was gradually increased to 290°C at a rate of 20°C per minute. The experiment employed ultrahigh-purity helium as the carrier gas, which was supplied at a flow rate of 30 mL/min. The temperatures of the injector, interface, and ion source were measured and found to be 280°C, 280°C, and 290°C, respectively.

Fatty acid content of egg yolk

A 5 g yolk sample was placed into a test tube, and then 10 mL of 37% HCl was added. The mixture was heated at 80ºC for 3 h. After cooling, the mixture solution was subjected to extraction using a combination of 25 mL diethyl ether and petroleum ether in a 1:1 ratio. The mixture was vortexed, and the uppermost layer (oil) was evaporated employing a water bath under an N2 gas environment. A volume of 0.5 mL of oil was introduced into a small test tube that was tightly sealed. Subsequently, 1.5 mL of methanolic sodium solution was added. The mixed solution was heated at 60°C for 10 minutes with shaking. After cooling, 2 mL of Boron trifluoride methanoate was added and heated at 60°C for 10 min and cooled again. The mixture was extracted with 1 mL of Heptana and 1 mL of saturated NaCl. The top layer formed was put into a GC vial as much as 1 µL.

The measurement of fatty acid content was conducted using a gas chromatograph (Agilent 7890B autosampler Series, Agilent Technologies, Palo Alto, CA, USA) equipped with an HP-88 capillary column (100 m×0.3 µm×0.2 µm, Agilent

Table 1. Ingredients and nutrient composition of experimental layer diets

Feed ingredient	CON	PM-Fe
Yellow corn	49.36	49.24
Multifeed® concentrate	36.43	36.33
Rice bran	12.85	12.82
Feed additive	1.37	1.35
Premix[1]	-	0.25
Total	100	100
Nutrient composition		
Gross energy (Kcal/kg)	3525	3528
Crude protein (%)	18.0	18.3
Crude fiber (%)	4.5	4.0
Extract ether (%)	6.1	5.8
Calcium (%)	3.4	3.1
Phosphor (%)	0.38	0.41
Fe (ppm)	128	158

CON: animals fed basal diet, PM-Fe: animals fed basal diet mixed with a premix mineral at a concentration of 2.5 g/kg diet; SEM: standard error of the mean, *p-value*: indicates that the values are significantly different.

[1] Mineral premix provided: Ca 200 g /kg; Na 88,1 g/kg; P 24.6 g/kg; Fe 12.6 g/kg; Mg 2.0 g/kg, Zn 1.8 g/kg; Mn 1.4 g/kg; K 725 mg/kg; Cu 721 mg/kg; S 144 mg/kg; Co 58.3 mg/kg; Se 182 µg/kg.

Table 2. Nutrient composition of multifeed® concentrate used in layer diet

Nutrient	Composition
Moisture (% max)	12
Ash (% max)	35
Crude protein (% min)	36
Extract Ether (%min)	3
Crude fiber (% max)	8
Calcium (% min)	9
Phosphor (% min)	0.5
Methionine (% min)	0.8
Lysine (% min)	1.7
Threonine (% min)	1.1
Tryptophan (% min)	0.34

Concentrate ingredients: rice bran, corn gluten meal, soybean meal, meat bone meal distillers dried grains with solubles, palm oil, essential amino acid.

Technologies, Palo Alto, CA, USA). The technique employed was gas chromatography-mass spectrometry (GC-MS). The experimental settings for the temperature program were as follows: The program was initiated with a starting temperature of 100°C for 5 minutes, after which it was increased to 240°C at a rate of 4°C per minute. The carrier gas utilized in this experiment was ultrahigh purity helium, which was maintained at a flow rate of 30 mL/min. The temperatures of the injector, interface, and ion source were recorded at 280°C, 260°C, and 240°C, respectively.

Iron (Fe) content in egg yolk

0.5 g of yolk sample was placed into a vessel, and 10 mL of 65% HNO3 was added. Program in a microwave digester (CEM Corporation: MARS 6iWave with EasyPrep Vessel, USA) with the Food method indicated on the instrument. The deconstructed solution was allowed to cool to room temperature, and then the solution was diluted to 25 mL in a volumetric flask. Blank preparation (10 mL of 65% HNO_3 solution without sample) was also deconstructed by the same method as the sample. The Fe test method in this study is based on SNI 8910: 2021 (SNI, 2021). Blank solution and deconstructed samples were aspirated with an Atomic Absorption Spectrophotometer or F-AAS (Thermo scientific iCE 3000 Series, Thermo Fisher Scientific Inc., USA) at a metal wavelength of 248.3 nm, with air combustion gas - C_2H_2.

Results and Discussion

Physical egg quality

The dietary interventions had no impact on the physical characteristics of the egg, as indicated in Table 3. In contrast to the findings of Sarlak *et al.* (2021), the study period revealed that the supplementation of high Fe premix resulted in a reduction in thickness and a decreased percentage of egg shells. Nevertheless, the addition of PM-Fe has a tendency to elevate the weight of albumen after 21 days, with a statistically significant p-value of 0.05. Our findings corroborate the findings of Xie *et al.* (2019), indicating that the introduction of different iron sources resulted in a significant rise in both Haugh unit and egg weight compared to a control group. While not thoroughly examined, the authors ascribed the heightened effects, correlated with the rise in succinate dehydrogenase activity, to an augmentation in protein synthesis within the egg.

Chemical egg yolk quality

On day 21, the dietary treatment with PM-Fe decreased the moisture content and raised the cholesterol level in the yolk ($p<0.05$, Table 4). The dietary interventions had no effect on the fatty acid composition of the yolk, as shown in Table 5. Supplementing with PM-Fe did not results in any meaningful difference in the chemical quality on day 42. The investigation demonstrated a positive association between the intake of iron supplements and the level of yolk cholesterol. Consistent with

Table 3. Effect premix mineral supplementation rich in Fe on physical egg quality

Variables	CON	PM-Fe	SEM	*p-value*
Day 21				
Egg weight (g)	61.7	61.8	0.40	0.85
Egg shape index	76.5	76.3	0.40	0.81
Eggshell thickness (mm)	0.35	0.35	0.01	0.94
Eggshell strength (mPa)	0.41	0.44	0.01	0.23
Eggshell weight (g)	6.7	6.7	0.09	0.95
Albumen index	0.14	0.14	0.00	0.32
Albumen weight (g)	38.2	40.1	0.47	0.05
Yolk index	0.43	0.44	0.01	0.47
Yolk weight (g)	14.6	13.8	0.22	0.09
Yolk color	10.5	10.7	0.13	0.54
Haugh unit	97.5	99.0	1.1	0.53
Day 42				
Egg weight (g)	61.4	62.0	0.32	0.35
Egg shape index	77.3	77.0	0.39	0.71
Eggshell thickness (mm)	0.34	0.35	0.01	0.34
Eggshell strength (mPa)	0.40	0.41	0.01	0.71
Eggshell weight (g)	7.0	7.1	0.11	0.57
Albumen index	0.14	0.14	0.01	0.80
Albumen weight (g)	41.6	42.5	0.58	0.49
Yolk index	0.44	0.43	0.00	0.10
Yolk weight (g)	14.9	14.78	0.17	0.74
Yolk color	10.8	11.2	0.08	0.05
Haugh unit	97.2	96.8	0.07	0.16

CON: animals fed basal diet, PM-Fe: animals fed basal diet mixed with a premix mineral at a concentration of 2.5 g/kg diet; SEM: standard error of the mean, *p-value*: indicates that the values are significantly different.

Table 4. Effect premix mineral supplementation rich in Fe on chemical egg yolk quality

Variables	CON	PM-Fe	SEM	*p-value*
Day 21				
Moisture (%)	47.5	46.7	0.17	0.02
Protein (%)	30.8	31.0	0.26	0.73
Fat (%)	57.3	57.5	0.28	0.78
Cholesterol (%)	0.69	0.80	0.03	0.03
Ash (%)	0.06	0.06	0.00	0.90
Day 42				
Moisture (%)	47.4	47.4	0.19	0.90
Protein (%)	30.0	29.9	0.21	0.76
Fat (%)	57.8	57.1	0.29	0.16
Cholesterol (%)	0.56	0.59	0.04	0.73
Ash (%)	0.04	0.04	0.00	0.59

CON: animals fed basal diet, PM-Fe: animals fed basal diet mixed with a premix mineral at a concentration of 2.5 g/kg diet; SEM: standard error of the mean, *p-value*: indicates that the values are significantly different.

the findings of Whittaker and Chanderbhan (2001), the elevation of dietary iron result in an increase in plasma lipid hydroperoxide and LDL-cholesterol levels while having no impact on HDL-cholesterol or triglyceride levels. In a study conducted by Marquez Ibarra *et al.* (2016), a high-iron diet increased cholesterol significantly, with no significant changes to triglyceride concentrations. the persistent overabundance of iron in the diet, lipid peroxidation, hampered plasma lipid transport, and hence elevated cholesterol. Reactive oxygen species can harm proteins, lipids, and cell membranes as a result of oxidative stress brought on by an excess of stored iron. The ensuing lesions may harm organs such as the pancreas and liver as well as tissue (Kundu *et al.*, 2013; Rajpathak *et al.*, 2009). Graham *et al.* (2010) also suggested that hepatic iron overload could increase hepatic cholesterol biosynthesis

Fatty acid content of egg yolk

The effects of different feed sources on egg yolk fatty acids composition is shown in Table 5. The omega fatty acids profile of the egg yolk wasn't affected by Fe supplementation. These results are in line with Buckiuniene *et al.* (2016), neither organic nor inorganic iron supplementation affected the fatty acid profile of egg yolk, but supplementation of 144 mg/kg organic Fe had a positive effect on lipid stability. This should also be visible in the indicators of lipid peroxidation.

Fe content in yolk

Figure 1 illustrates the impact of dietary treatment on the iron (Fe) level in the yolk on day 21 ($p=0.05$) and day 42 ($p<0.05$). The addition of dietary supplements resulted in an increase in the iron concentration in the yolk. Paik *et al.* (2009) found that the addition of Fe-SP 100 resulted in a 16.6% rise in the iron content of the yolk, whereas Fe-Met showed a 13.1% increase, and Fe-SP 200 showed an 8.3% increase. The concentration of iron (Fe) in the egg yolk showed a rise after a period of two weeks, with a notable and statistically significant increase observed only after five weeks of treatment. The effectiveness of iron supplementation depends on the particular type of iron used, as explained by Park *et al.* (2004). In their study, adding iron (Fe) at parts per million (ppm) concentrations in different organic forms (Fe-Met or Availa-Fe) or an inorganic form ($FeSO_4$) did not lead to increased iron content in the egg yolk compared to iron supplementation.

Table 5. Effect premix mineral supplementation rich in Fe on fatty acids of egg yolk

Variables	CON	PM-Fe	SEM	*p-value*
Day 21				
Omega 3 (% relative)	0.87	0.98	0.03	0.13
Omega 6 (% relative)	3.8	3.8	0.06	0.74
Omega 9 (% relative)	11.4	17.7	3.04	0.34
Day 42				
Omega 3 (% relative)	1.2	1.2	0.02	0.80
Omega 6 (% relative)	4.4	4.2	0.06	0.23
Omega 9 (% relative)	11.8	11.6	0.16	0.43

CON: animals fed basal diet, PM-Fe: animals fed basal diet mixed with a premix mineral at a concentration of 2.5 g/kg diet; SEM: standard error of the mean, *p-value*: indicates that the values are significantly different.

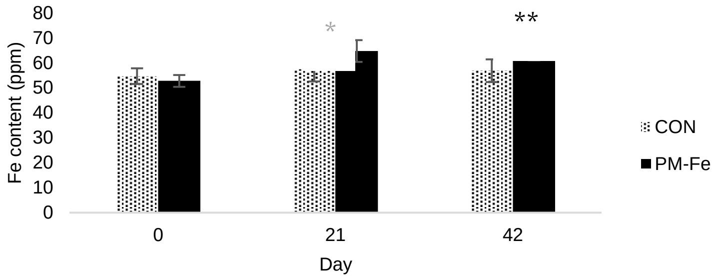

Figure 1. Effect premix mineral supplementation rich in Fe on egg yolk Fe level. CON: animals fed basal diet, PM-Fe: animals fed basal diet mixed with a premix mineral at a concentration of 2.5 g/kg diet; SEM: standard error of the mean, $p<0.05$: indicates that the values are significantly different**,** * : *p-value* = 0.05, ** : *p-value* <0.05.

It is anticipated that the presence of heme iron will result in higher iron absorption than inorganic forms (Henry and Miller, 1995). Iron absorbed from intestinal-mucosal cells is transported to the portal blood of liver, and thus Fe content in plasma from the hepatic portal vein can accurately reflect the Fe transport and absorption from the intestinal lumen (Bai *et al.*, 2021). On the brush border of enterocytes, various iron import proteins are present, and specific pathways of absorption have been described for the two ionic forms of iron (Fe^{2+} and Fe^{3+}; both being non-heme iron molecules) and also for iron associated with heme (heme iron) (Fuqua *et al.*, 2012). Iron is stored in large amounts in the body, primarily in reticuloendothelial cells of the liver, spleen, and bone marrow. Dietary demands of Fe can be modulated to increase or to decrease its rate of absorption through different known pathways according to the body's status of Fe (Grotto, 2008). More than 90% of the iron in eggs is estimated to be bound to phosvitin in the yolk (Choi *et al.*, 2004).

Conclusion

The addition of 30 ppm of iron (Fe) could enhance the iron concentration in the yolk by 15.7%. The addition of Fe immediately increased the content of yolk cholesterol. However, additional research is required to evaluate the distribution of iron in the chicken's body.

Conflict of interest

The authors have no conflict of interest to declare. All authors have seen and agree with the contents of the manuscript.

Funding statement

This research was supported by PT Agromix Lestari Yogyakarta, Indonesia.

Acknowledgment

The authors express their gratitude to the director of PT Agromix Lestari Yogyakarta, Indonesia for supplying the premix mineral utilized in this study. We want to extend our appreciation for the generous support provided by PT. Sentra Gemilang Mulia, Indonesia, who allowed us to utilize their open-housed barn and laying hens for our research.

Author’s contribution

The authors confirm contribution to the paper as follows: study conception and design: C. H. and A. A.; draft manuscript preparation: C. H., M. F. H. and A. A.; data collection: M. F. H.; analysis and interpretation of results: A. A. and M. F. H.; and All authors reviewed the results and approved the final version of the manuscript.

Ethics approval

The experiment was obtained prior consent from the Ethical Commission of the Faculty of Veterinary Medicine at Universitas Gadjah Mada, Indonesia, under reference number 98/EC-FKH/Eks/2023.

References

AOAC. 2005. Official Methods of Analysis of the Association of Official Analytical Chemists

20th ed. Assoc. off. Anal. Chem. Washington, D.C

Bai, S., S. Cao, X. Ma, X. Li, X. Liao, L. Zhang, M. Zhang, R. Zhang, S. Hou, X. Luo and L. Lu. 2021. Organic iron absorption and expression of related transporters in the small intestine of broilers. Poult. Sci. 100: 101-182.

Buckiuniene, V., M. A. Grashorn, R. Gruzauskas, V. Kliseviciute, A. Raceviciute-Stupeliene, G. Svirmickas, S. Bliznikas, A. Miezeliene and G. Alencikiene. 2016. Effect of organic and inorganic iron in the diet on yolk iron content, fatty acids profile, malondialdehyde concentration, and sensory quality of chicken eggs. European Poultry Science/Archiv für Geflügelkunde. 80.

Choi, I., C. Jung, H. Seog, and H. Choi. 2004. Purification of phosvitin from egg yolk and determination of its physicochemical properties. Food Sci. Biotechnol. 13: 434–437.

Fuqua, B.K., C. D. Vulpe, and G. Anderson. 2012. Intestinal iron absorption. J. Trace Elem. Med. Biol. 26: 115–119

Graham, R. M., A. C. Chua, K. W. Carter, R. D. Delima, D. Johnstone, and C. E. Herbison. 2010 Hepatic iron loading in mice increases cholesterol biosynthesis. Hepatology. 52: 462–471.

Grotto, H. Z. W. 2008. Metabolismo do ferro: Uma revisão sobre os principais mecanismos envolvidos em sua homeostase. Revista Brasileira de Hematologia e Hemoterapia. 30: 390 – 397.

Grzeszczak, K., S. Kwiatkowski, and D. Kosik-Bogacka. 2020. The role of fe, zn, and cu in pregnancy. Biomolecules. 10: 1–33.

Henry, P. R. and E. R. Miller. 1995. Iron bioavailability. In: Bioavailability of Nutrients for Animals. Elsevier. p. 169–199.

Indonesian Ministry of Health. 2018. Laporan Nasional Riskesdas 2018, Jakarta.

Korish, M. A. and Y. A. Attia. 2020. Evaluation of heavy metal content in feed, litter, meat, meat products, liver, and table eggs of chickens. Animals. 10.

Kundu D, A. Roy, T. Mandal, U. Bandyopadhyay, E. Ghosh, and D. Ray. 2013. Relation of iron stores to oxidative stress in type 2 diabetes. Niger. J. Clin. Pract. 16: 100–103.

Lesnierowski, G. and J. Stangierski. 2018. What’s new in chicken egg research and technology for human health promotion? - A review. Trends Food Sci. Technol. 71: 46–51.

Márquez-Ibarra, A., M. Huerta, S. Villalpando-Hernández, M. Ríos-Silva, M. I. Díaz-Reval, H. Cruzblanca, E. Mancilla, and X. Trujillo. 2016. The effects of dietary iron and capsaicin on hemoglobin, blood glucose, insulin tolerance, cholesterol, and triglycerides, in healthy and diabetic Wistar rats. PloS one. 11, e0152625.

Mezzaroba, L., D. F. Alfieri, A. N. Colado Simão, and E. M. Vissoci Reiche. 2019. The role of zinc, copper, manganese and iron in neurodegenerative diseases. Neurotoxicology. 74: 230-241.

Paik, I., H. Lee, and S. Park. 2009. Effects of organic iron supplementation on the performance and iron content in the egg yolk of laying hens. J. Poult. Sci. 46: 198–202.

Park, S. W., H. Namkung, H. J. Ahn, and I. K. Paik. 2004. Production of iron enriched eggs of Laying Hens. Asian-Australas J. Anim. Sci. 17: 1725–1728.

Rajpathak, S. N., J. P. Crandall, J. Wylie-Rosett, G. C. Kabat, T. E. Rohan, and F. B. Hu. 2009. The role of iron in type 2 diabetes in humans. Biochem Biophys Acta. 1790: 671–681.

Sanlier, N. and D. Üstün. 2021. Egg consumption and health effects: A narrative review. J. Food. Sci. 86: 4250–4261.

Sarlak, S., A. S. Tabeidian, M. Toghyani, A. D. F. Shahraki, M. Goli, and M. Habibian. 2021. Effects of replacing inorganic with organic iron on performance, egg quality, serum and egg yolk lipids, antioxidant status, and iron accumulation in eggs of laying hens. Biol. Trace Elem. Res. 199: 1986–1999.

Siró, I., E. Kápolna, B. Kápolna, and A. Lugasi. 2008. Functional food. Product development, marketing and consumer acceptance—A review. Appetite. 51: 456–467.

SNI. 2021. How to test for metal content in solid waste, sediment and soil test samples by acid deconstruction method using an Atomic Absorption Spectrometer (AAS) or an Inductively Coupled Plasma Optical Emission Spectrometric (ICP-OES). Badan Standardisasi Nasional, Jakarta.

Soppi, E. T. 2018. Iron deficiency without anemia - a clinical challenge. Clin. Case Rep. 6: 1082–1086.

Whittaker, P. and R. F. Chanderbhan. 2001. Effect of increasing iron supplementation on blood lipids in rats. Br. J. Nutr. 86: 587–592.

WHO. 2019. Anemia in women and children. Geneva.

Xie, C., H. A. M. Elwan, S. S. Elnesr, X. Y. Dong, and X. T. Zou. 2019. Effect of iron glycine chelate supplementation on egg quality and egg iron enrichment in laying hens. Poult. Sci. 98: 7101–7109.

Zhao, D., C. Zhang, J. Ma, J. Li, Z. Li, and C. Huo. 2022. Risk factors for iron deficiency and iron deficiency anemia in pregnant women from plateau region and their impact on pregnancy outcome. Am. J. Transl. Res. 14: 4146-4153.