***EFFICACY OF IRON-RICH PREMIX MINERAL SUPPLEMENTATION ON EGG YOLK'S FE CONTENT AND EGG QUALITY***

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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, 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 grams per kilogram of premix mineral (which contains 12.6 grams per kilogram 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**

The experiment was conducted at PT. Sentra Gemilang Mulia in Indonesia and obtained prior consent from the Ethical Commission of the Faculty of Veterinary Medicine at Universitas Gadjah Mada, Indonesia, under reference number

**Birds, Treatments, and Sample Collection** A total of 1680 Lohman brown hens, aged 31 weeks, 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-hour 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** Various egg quality parameters were measured, such as egg weight, egg length, egg breadth, eggshell thickness, eggshell straightness, eggshell weight, albumen index, albumen weight, yolk index, yolk weight, and haugh unit.

**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 grams 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 degrees Celsius 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 seconds. 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 minutes, 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** A 5-gram 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 hours. 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 minutes and cooled again. The mixture was extracted with 1 mL of Heptan 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 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% HNO3 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 - C2H2.

**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. Figure 1 shows that the dietary PM-Fe had no significant impact on egg weight during 42 days. 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 quality and fatty acid content**

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 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 Brunet et al. (1999), rats who were fed a meal containing 3% carbonyl Fe for 12 weeks exhibited a notable increase in cholesterol and triglyceride levels compared to the control group of animals. In addition, they analyzed essential enzymes responsible for regulating cholesterol levels and observed a decline in the functioning of cholesterol 7a-hydroxylase and 3-hydroxy-3-methylglutaryl-Co A reductase, along with an elevation in acyl-Co A–cholesterol acyltransferase activity.

**Fe content in yolk**

Figure 2 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 (FeSO4) did not lead to increased iron content in the egg yolk compared to iron supplementation.

It is anticipated that the presence of heme iron will result in higher iron absorption than inorganic forms (Henry and Miller, 1995). Heme is produced through the proteolysis of myoglobin and hemoglobin. The soluble form of iron (Fe) that heme contains allows the gastrointestinal tract to absorb it. The solubility of heme Fe safeguards it from various factors that might impede the absorption of inorganic Fe. Protein improves the ability of animals to absorb heme iron, as demonstrated by Hallberg et al. in 1979. The metalloporphyrin, known as heme Fe, is transported into the absorptive cell of the small intestine without undergoing any changes. Subsequently, heme oxygenase cleaves the heme molecule, resulting in the release of inorganic Fe (Uzel and Conrad, 1998). The majority of iron present in the yolk is bound to phosvitin (Greengard et al., 1964).

**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.

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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 |
| Premix1 | - | 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 |

1Mineral 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 multi feed 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

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 straightness (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 straightness (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 |

Table 4. Effect premix mineral supplementation rich in Fe on chemical 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 |

Table 5. Effect premix mineral supplementation rich in Fe on fatty acids 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 |

Table 6. Effect premix mineral supplementation on Fe content of egg yolk

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variables | Con | PM-Fe | SEM | *p-value* |
| **Day 0** |  |  |  |  |
| Fe (ppm) | 54.6 | 59.6 | 2.1 | 0.37 |
| **Day 21** |  |  |  |  |
| Fe (ppm) | 57.4 | 64.7 | 1.7 | 0.05 |
| **Day 42** |  |  |  |  |
| Fe (ppm) | 56.8 | 65.7 | 1.8 | 0.03 |

Figure 1. Effect premix mineral supplementation rich in Fe on eggshell straightness

Figure 2. Effect premix mineral supplementation rich in Fe on eggshell thickness

Figure 3. Effect premix mineral supplementation rich in Fe on eggshell weight

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Figure 4. Effect premix mineral supplementation rich in Fe on egg yolk Fe level