

Research Article

Variations in Yolk Mineral Element Contents from Different Chicken Rearing Systems: Eggs Analyzed by Inductively Coupled Plasma Mass Spectrometry

S.B. Zhu, Q.Y. Zhao, B.L. Liu, L. Wang and S.J. Liu

College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, Daqing 163319, P.R. China

Abstract: We analyzed the contents of the major elements calcium (Ca) and phosphorus (P), the trace elements zinc (Zn), copper (Cu), manganese (Mn), selenium (Se), cadmium (Cd) and lead (Pb) of chicken eggs from free-range and conventional rearing systems using inductively coupled plasma mass spectrometry. The Ca, Cu, Zn and Se contents of the conventional eggs were significantly higher than those of free-range eggs and the Mn and Pb contents of the conventional eggs were significantly lower. The P and Cd values were no different between the two rearing systems. Our results give an indication of mineral element levels in eggs from hens kept in different husbandry systems.

Keywords: Conventional, egg-yolk, free range, ICP-MS, trace elements

INTRODUCTION

Hens eggs are increasingly considered an important source of nutrients (Surai and Sparks, 2001) and conventional and free-range rearing systems are two main rearing systems. With increasing living standards, more consumers now favor eggs originating from free-range systems as they believe that such eggs taste better and have a higher nutritional value (Rodic *et al.*, 2006). However, this cognition is not on account of any measurements of the specific qualities of free-range eggs over conventional eggs (Hidalgo *et al.*, 2008). It is necessary to inform producers and consumers about how free-range system influence the nutritional composition and quality of eggs, but there is limited number of studies in this regard.

Free-range eggs are commonly anticipated to have better nutritional values than conventional eggs because the chickens have free access to the outdoors and to food consumption. Considering the increasing concern regarding free-range eggs, some studies have recently shown the effects of different rearing systems on egg quality (Kucukyilmaz *et al.*, 2012; Cerolini *et al.*, 2005; Minelli *et al.*, 2007; Hidalgo *et al.*, 2008; Samman *et al.*, 2009; Rizzi *et al.*, 2006). However, data on the levels of macro- and microminerals in eggs from free-range housing systems are very scarce (Van Overmeire *et al.*, 2009; Matt *et al.*, 2009; Giannenas *et al.*, 2009).

Yolk is the richest portion of the egg in terms of trace elements (Austic and Nesheim, 1990). Therefore, the aim of this study was to assess differences in mineral concentrations in the yolk of chicken eggs

originating from different housing systems: conventional and free range.

MATERIALS AND METHODS

Animals, housing and diets: The eggs originated from two different husbandry systems, conventional and free range. In total, 198 30-week-old Jing-Hong Brown laying hens were used. The trial was performed in Daqing in the Heilongjiang Province of China, which is in the North Temperate Zone (latitude 45°46', longitude 124°19').

In the conventional system, 108 hens, housed in an automatically controlled henhouse, were randomly assigned to three replications of triple deck batteries equipped with linear feeders and nipple drinkers. Each replication consisted of six cages and the hens of six adjacent cages in the same deck were considered as a replication, each layer measuring 50 cm long, 60 cm wide and 56 cm high, providing 500 cm² of floor space per bird. These hens were given a basic commercial ration with added minerals and vitamins.

Ninety free-range hens were reared in a yard with self-sown grass and soil. In this system, birds were divided into three groups. Each group of 30 birds had access to a range area of 30×50 m² during daytime (June-July). The range area was divided by metal fences and contained a food trough and a bell drinker: feed and water were provided *ad libitum*. The compositions of the diets for conventional and free-range system are shown in Table 1.

Corresponding Author: S.J. Liu, College of Animal Science and Veterinary Medicine, Heilongjiang Bayi Agricultural University, Daqing 163319, P.R. China, Tel.: +86-459-6819195; Fax: +86-459-6819190

This work is licensed under a Creative Commons Attribution 4.0 International License (URL: <http://creativecommons.org/licenses/by/4.0/>).

Table 1: Ingredients and composition of the diet of conventionally reared hens

| Ingredients | Composition % | |
|-------------------------------|---------------|------------|
| | Conventional | Free-range |
| Maize, grains | 63.5 | 62.0 |
| Soybean meal | 18.8 | 20.0 |
| Wheat bran | 1.5 | 6.5 |
| Rice bran | | 5.0 |
| Limestone | 8.0 | 2.0 |
| Dicalcium phosphate | 1.0 | 0.5 |
| Sodium chloride | 4.0 | 4.0 |
| Methionine | 1.2 | |
| Vitamin and Mineral premix | 2.0 | |
| Total | 100 | 100 |
| Metabolizable energy(Mcal/kg) | 2.75 | 2.72 |
| Crude protein (%) | 16.50 | 16.2 |
| Trace element analysis | | |
| Ca (%) | 3.82 | 3.5 |
| P (%) (Total) | 0.54 | 0.55 |
| Se (ug/kg) | 0.40±0.02 | 0.09±0.01 |
| Zn (mg/kg) | 89.7±3.2 | 63.5±2.2 |
| Cu (mg/kg) | 21.17±1.2 | 10.9±0.9 |
| Mn (mg/kg) | 108.3±3.2 | 26.41±3.2 |
| Cd (ug/kg) | 0.12±0.02 | 0.2±0.02 |
| Pb (ug/kg) | 0.57±0.02 | 0.52±0.02 |

Table 2: Instrumental parameters for the ICP-MS

| | Parameters |
|--------------------------|------------|
| RF power | 1300 Watts |
| Sample depth | 6 mm |
| Torch-H | -0.3 mm |
| Torch-V | -0.3 mm |
| Carrier gas | 0.65 L/min |
| Nebuliser pump | 0.1 rps |
| S/C temp | 2°C |
| Oxide (CeO/Ce) | ≤1.0% |
| Doubly charged (Ce2+/Ce) | ≤1.0% |
| Nebuliser type | Babington |

Egg sample collection commenced 6 weeks after feeding on the dietary treatments and lasted for 2 weeks. Twenty eggs per housing system were analyzed; each egg sample was analyzed in triplicate. The eggs were selected randomly on the same day from each system. All eggs collected during that period were stored at 4°C, pending further handling.

Mineral analysis: The trace elements phosphorus (P), calcium (Ca), manganese (Mn), copper (Cu), zinc (Zn), selenium (Se), cadmium (Cd) and lead (Pb) were determined using inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500s; Agilent Technologies, Waldbronn, Germany). The instrumental parameters of the equipment used are summarized in Table 2. Elements were determined in the acid digest of the samples as follows. Samples (0.5 g) of wet yolk were soaked in 5 mL concentrated HNO₃ (65%). The samples were heated in a microwave digestion unit fitted with a control panel using a defined schedule of temperature. Losses of volatile element compounds did not occur as the tubes were sealed during heating. The samples were then diluted to a volume of 20 mL with ultrapure water (Milli-Q Water Purification Systems, USA). Standard solutions of single elements were obtained from High Purity Standards (Charleston, SC,

Table 3: Trace element concentrations in yolks of eggs from the two different husbandry systems (fresh samples)

| Element | Conventional | Free-range |
|-------------|-------------------------|-------------------------|
| Ca (mg/kg) | 1058±122 ^a | 946±129 ^b |
| P (mg/kg) | 4152±133 | 4058±121 |
| Se (µg/kg) | 311.4±15.6 ^a | 111.9±9.9 ^b |
| Zn (µg /kg) | 26360±185 ^a | 24906±468 ^b |
| Cu (µg /kg) | 1117±123 ^a | 946±73 ^b |
| Mn (µg /kg) | 861±74 ^a | 1379±135 ^b |
| Cd (µg /kg) | 1.547±0.210 | 1.365±0.209 |
| Pb (µg /kg) | 19.96±3.24 ^a | 45.50±6.42 ^b |

Values are means±S.E.M; ^{a,b}: Numbers with different superscripts in the same row are significantly different (p<0.05)

USA) and used to derive calibration curves. Several standard reference materials were used to validate the analytical procedure; these included the standard element solutions and egg powder, NIST-RM 8415 (LGC Standards; Promochem, Wesel, Germany). All chemicals used were of analytical grade. Spike recoveries typically varied between 90% and 106%. The limits of detection were as follows: Ca 0.55 mg/kg, P 0.01 mg/kg, Zn 0.05 mg/kg, Cu 0.02 mg/kg, Mn 0.002 µg/kg, Se 0.008 µg/kg, Pb 0.55 µg/kg and Cd 0.004 µg/kg, Quantification limits for Ca, P, Zn, Cu, Mn, Se, Pb and Cd were 0.68, 0.01, 0.05, 0.02, 0.007, 0.024, 2.0 and 0.013, respectively.

Statistical analysis: Data are expressed as the mean±standard deviation. The mineral contents of the conventional and organic eggs were compared and analyzed using the Student's *t* test procedure of SPSS (IBM Corp., Armonk, NY, USA). The effects of husbandry system on egg and yolk weights were analyzed similarly. Significant differences were determined at p<0.05.

RESULTS

There were significant effects of the husbandry system on the weights of eggs and yolks (p<0.05). Egg weights were 58.5±2.8 g and 54.3±3.2 g and yolk weights were 16.5±1.7 g and 16.1±1.6 g for the conventional and free-range systems, respectively.

Some of the mineral contents in the free-range and conventional eggs showed significant differences, as shown in Table 3. The Ca, Cu, Zn and Se contents of the conventional eggs were significantly higher than free-range eggs. However, the Mn and Pb values were significantly higher in free-range eggs. The P and Cd values did not differ between the eggs from the two rearing systems.

DISCUSSION

Hens in the free-range system were exposed to more variable environmental conditions with more external stimulation compared with the stable conditions in the conventional system. Zn plays a very important role in poultry nutrition, particularly as a component of a number of metalloenzymes, which play

important roles in the hen's immune response. The differences in Zn concentrations of eggs did not arise mainly from the ingested feed ingredients or additional supplementation of minerals. This was probably because the hens in the free-range system used Zn to support their immune system against external stressors, hence depositing less of this element in the egg.

We anticipated that the free-range eggs would contain more Ca and P than conventional ones because the hens had free access to soil and tiny stones. However, the conventionally produced eggs had more Ca content than those originating from the free-range system and the P concentration did not differ between the eggs from the two rearing systems. This was probably because higher amounts of Ca and P had been absorbed to the long limb bones in free-range hens that were engaged in a wide range of physical activities, such as flying, wing flapping, walking and perching (Waheed *et al.*, 1985; Nisianakis *et al.*, 2009; Abdulkhaliq *et al.*, 2012).

The conventionally produced eggs contained more Se than did the free-range eggs. This might be because Daqing is a selenium-deficient area and the grain, grass, soil and tiny stones that the free-range hens ingested would also be short of this element (Xu *et al.*, 1986). However, there was plenty of additional supplementation of minerals in the diets of the conventionally reared hens and the Se concentration of eggs can be modified through the diet (Sparks, 2006). Consequently, the free-range eggs contained less Se than did the conventionally produced eggs. Our results are in agreement with Giannenas's report (Giannenas *et al.*, 2009).

Here, the free-range eggs contained more Mn and Pb than did conventionally produced eggs. However, the concentrations of heavy metals found in the eggs of our study (Table 3) were far below the toxic limits reported in the literature (Waegeneers *et al.*, 2009). With the rapid development of industries and the increased burning of coal and gasoline, increased amounts of Mn and Pb are discharged into the air and fall on the ground. Free-range hens that were reared outdoors were able to access such polluted diets. Therefore, free-range eggs can contain more Mn and Pb than conventional eggs. Others have also found increased concentrations of heavy metal compounds in eggs from hens kept in free-range systems (Van Overmeire *et al.*, 2009).

Better appreciation of the range of concentrations of egg minerals that are naturally achieved by domestic chickens kept under free-range systems at non-polluted sites would provide guidance in formulating dietary supplements for free-living birds and reference values for the effects of pollution.

ACKNOWLEDGMENT

This research was supported by the Program for Innovation Research Team in University of Heilongjiang Province of China (No. 2010td05).

REFERENCES

- Abdulkhaliq, A., K.M. Swaileh, R.M. Hussein and M. Matani, 2012. Levels of metals (Cd, Pb, Cu and Fe) in cow's milk, dairy products and hen's eggs from the West Bank, Palestine. *Int. Food Res. J.*, 19(3): 1089-1094.
- Austic, R.E. and M.C. Nesheim, 1990. *Poultry Production*. 13th Edn., Lea and Febiger, Philadelphia.
- Cerolini, S., L. Zaniboni and R. La Cognata, 2005. Lipid characteristics in eggs produced in different housing systems. *Ital. J. Anim. Sci.*, 4: 520.
- Giannenas, I., P. Nisianakis, A. Gavrill, G. Kontopidis and I. Kyriazakis, 2009. Trace mineral content of conventional, organic and courtyard eggs analysed by inductively coupled plasma mass spectrometry (ICP-MS). *Food Chem.*, 114: 706-711.
- Hidalgo, A., M. Rossi, F. Clerici and S. Ratti, 2008. A market study on the quality characteristics of eggs from different housing systems. *Food Chem.*, 106: 1031-1038.
- Kucukyilmaz, K., M. Bozkurt, C. Yamaner, M. Cinar and R. Konak, 2012. Effect of an organic and conventional rearing system on the mineral content of hen eggs. *Food Chem.*, 132: 989-992.
- Matt, D., E. Veromann and A. Luik, 2009. Effect of housing systems on biochemical composition of chicken eggs. *Agron. Res.*, 7(2): 662-667.
- Minelli, G., F. Sirri, E. Folegatti, A. Meluzzi and A. Franchini, 2007. Egg quality traits of laying hens reared in organic and conventional systems. *Ital. J. Anim. Sci.*, 6: 728-730.
- Nisianakis, P., I. Giannenas, A. Gavriil, G. Kontopidis and I. Kyriazakis, 2009. Variation in trace element contents among chicken, turkey, duck, goose and pigeon eggs analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). *Biol. Trace Elem. Res.*, 128: 62-71.
- Rizzi, L., G. Simioli, G. Martelli, R. Paganelli and L. Sardi, 2006. Effects of organic farming on egg quality and welfare of laying hens. *Proceeding of European Poultry Conference*. Verona, Italy.
- Rodic, V., L. Peric, N. Vukelic and N. Milosevic, 2006. Consumers attitude towards chicken meat produced in extensive systems. *Proceeding of European Poultry Conference*. Verona, Italy.
- Samman, S., F.P. Kung, L.M. Carter, M.J. Foster, Z.I. Ahmad, J.L. Phuyal *et al.*, 2009. Fatty acid composition of certified organic, conventional and omega-3 eggs. *Food Chem.*, 116(4): 911-914.
- Sparks, N.H.C., 2006. The hen's egg- Is its role in human nutrition changing? *World Poultry Sci. J.*, 62: 308-315.
- Surai, P.F. and N.H.C. Sparks, 2001. Designer eggs: From improvement of egg composition to functional food. *Trends Food Sci. Tech.*, 12: 7-16.

- Van Overmeire, I., N. Waegeneers, I. Sioen, M. Bilau, S. De Henauw, L. Goeyens, L. Pussemier and G. Eppe, 2009. PCDD/Fs and dioxin-like PCBs in home-produced eggs from Belgium: Levels, contamination sources and health risks. *Sci. Total Environ.*, 407(15): 4419-4429.
- Waegeneers, N., M. Hoenig, L. Goeyens and L.D. Temmerman, 2009. Trace elements in home-produced eggs in Belgium: Levels and spatiotemporal distribution. *Sci. Total Environ.*, 407: 4397-4402.
- Waheed, S., I. Fatima, A. Mannan, M.S. Chaudhary and I.H. Quershi, 1985. Trace element concentration in egg-yolk and egg-white of farm and domestic chicken eggs. *Int. J. Environ. An. Ch.*, 21: 333-344.
- Xu, C., Y. Fu, Z. Xu, D. Xia, X. Sun and Pengyi, 1986. Selenium content and distribution in soil and feed in Heilongjiang Province. *J. Northeast Agric. Coll.*, 12(17): 399-405.