Content and uptake of minerals in the yolk of broiler embryos during incubation and effect of nutrient enrichment

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ABSTRACT Although embryo and chicken growth and development rely on mineral nutrition, information on mineral levels in the egg compartments during incubation is limited. Accordingly, we examined P, Ca, Fe, Zn, Cu, and Mn levels in the yolk of breeder eggs during incubation and the effect of embryonic mineral (with specific nutrients) enrichment on yolk mineral levels and consumption. First, fertile eggs were examined on day of setting (DOS), embryonic day (E) 11, E13, E15, E17, E19, E20, and day of hatch (DOH) for the mineral content in the yolk (and albumen on DOS) by inductively coupled plasma atomic emission spectroscopy. Results showed that on DOS, the yolk is the major origin for Mn, P, Fe, Ca, Cu, and Zn. Interestingly, P, Fe, Zn, Cu, and Mn were mostly consumed from the yolk until E17, after which their consumption was very low. Consumption of P was constant until E17 and then decreased until E20. Consumption of Fe, Zn, Cu, and Mn was medium to mild until E11, increased between E11 and E17, and minimal between E17 and DOH. Enrichment treatment, where fertile eggs were divided into 2 groups [nonenriched (control) and enriched (with minerals, vitamins, and carbohydrates on E17 using the in ovo feeding method)] showed that the enriched group had higher Fe, Zn, Cu, and Mn levels than the nonenriched group and exhibited higher consumption of Fe, Zn, and Mn between E20 and DOH. Analysis of the shell mineral composition along incubation showed that the shell released low amounts of P, Fe, and Mn in comparison with the yolk mineral content. Therefore, we concluded that the shell is a minor source of these minerals. Studying the mineral resources and consumption of embryos can lead to a better understanding of the mineral limitations of embryos during incubation. Additionally, because minerals are important for the development of the embryo, the higher mineral levels and consumption observed in the enriched group may affect the development of critical organs, such as the skeletal system.

Key words: mineral, yolk, shell, embryo, broiler

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INTRODUCTION

The importance of minerals to the growth and development of embryos and chickens is supported by many publications stating that a mineral deficiency can cause skeletal, immune, and cardiovascular system disorders; poor shell quality; reduced hatchability; and increased mortality (Wilson, 1997; Kidd, 2003; Angel, 2007; Dibner et al., 2007). Levels of Ca and P are associated with most bone abnormalities (Underwood and Suttle, 2001; Dibner et al., 2007). Moreover, Ca is crucial for eggshell quality (Landauer, 1967; Romanoff and Romanoff, 1972) and for intracellular signal transduction (Chang et al., 1996), whereas P is a critical component of lipids, proteins, and nucleic acids (Richards and Packard, 1996). Zinc plays a role in the development of the immune system of the broiler embryo (Kidd et al., 1992; Kidd, 2003) and plays regulatory roles in bone development, such as its association with the changes in gene transcription that accompany endochondral ossification (Oviedo-Rondon and Ferket, 2005; Dibner et al., 2007). Copper is involved in the synthesis of hemoglobin, erythrocuprein, and other plasma proteins, some of which participate in Fe transport, as reflected by the fact that Cu deficiency can result in signs of anemia (Leeson, 2009). Copper also plays a role in many enzyme systems, such as cytochrome oxidase, which is key for oxidative phosphorylation (Leeson, 2009). The importance of Cu to the developing bone is evidenced by its role in the crosslinking of collagen and elastin, which gives the bone its tensile strength and elasticity (Dibner et al., 2007). Iron has an essential role in cellular oxidative energy metabolism, mainly because it

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is a component of the oxygen transport proteins myoglobin and hemoglobin and of specific redox enzymes (Chang et al., 1996). Manganese is essential for formation of the bone cartilage model (Gilbert, 1997); Mn deficiency can result in embryonic abnormalities and reduced hatchability (Caskey and Norris, 1940; Landauer, 1967). In addition, Mn-deficient embryos exhibit differences in the microscopic structure of the bones (Caskey et al., 1944).

Because of the importance of all of these minerals, their levels in layer and broiler diets are critical for quality and performance (Wilson, 1997). Although the mineral nutrition of the hen has a pronounced effect on progeny performance, studies have shown that increasing the concentration (feeding in excess) of most minerals in the diet of hens has little or no effect on mineral concentrations in the egg (Naber, 1979; Angel, 2007).

The hen deposits minerals in the egg via 2 routes: the ovary to the yolk and the oviduct to the albumen, shell, and shell membrane (Richards and Packard, 1996). Because these routes provide the only mineral source for the embryo during incubation, proper development of the broiler embryo depends on the correct deposition of minerals by the hen into the egg (Wilson, 1997).

The freshly laid egg is composed of about 60% albumen, 30% yolk, and 10% shell, depending on the layer's age and genetic background. The yolk contains about 50% water, 33% fat, and 17% protein (Romanoff, 1967); the albumen contains about 88% water and supplies the protein for embryonic development (Romanoff, 1967); and the shell contains mostly minerals (Richards and Packard, 1996).

The major mineral source for the embryo during incubation is the yolk, which contains most of the P, Zn, Cu, Mn, and Fe in fresh eggs (Richards and Packard, 1996; Richards, 1997). The albumen is a major source of Na and K and contains low levels of Fe, Cu, Mn, and Zn (Richards and Packard, 1996; Richards, 1997). During incubation, the albumen content mixes with the yolk sac content and the amniotic fluid (Romanoff, 1967). Although the shell contains minerals and may be an important mineral reserve for the embryo, most of the shell content is probably unavailable to the embryo, and to date, the release of only Ca and Mg from the shell has been documented (Packard and Packard, 1991; Richards, 1991). Knowing the mineral contribution of the shell to the other egg compartments would enable a better understanding of the embryo's mineral metabolism.

Data on mineral levels in the egg compartments during incubation are very limited. Furthermore, today's "high-metabolism, fast-growing" broiler embryos and chickens (Tona et al., 2004) may reach levels of mineral deficiency that can lead to metabolic disorders (Angel, 2007). The purpose of the current study was to examine the levels of several minerals (P, Ca, Fe, Zn, Cu, and Mn) in the fertile breeder egg yolk on day of setting [**DOS**; embryonic day (**E**) 0], during the incubation period (E11, E13, E15, E17, E19, and E20), and on day of hatch (**DOH**; E21) and to examine the effect of embryonic mineral, vitamin, amino acids, and carbohydrate enrichment on yolk mineral levels and consumption.

Table 1. Breeder flock diets

Item	Experiment 1	Experiment 2
Protein (%)	14.5	15.0
Lysine (%)	0.68	0.70
Sulfur amino acids (%)	0.60	0.62
Ca (%)	3.20	2.80
Total P (%)	0.56	0.62
Mn (mg/kg)	140	140
Additive Zn (mg/kg)	110	110
Additive Fe (mg/kg)	40	40
Additive Cu (mg/kg)	10	10
Total fat (%)	3.00	3.50
Total fiber (%)	3.50	3.50
Ash (%)	10.0	10.0
Salt (%)	0.35	0.35
Moisture (%)	11.0	11.0
Linoleic acid (%)	1.20	1.30
Metabolic energy (kcal/kg)	2,700	2,700
Age of hen (wk)	50	38

MATERIALS AND METHODS

Experiment 1

General Procedure. The Ethics Committee of the Faculty of Agriculture of Hebrew University approved experiments 1 and 2. In experiment 1, fertile eggs (n = 150; average weight = 71.56 ± 4.38 g) from Cobb 500 hens (50 wk of age), fed according to Cobb (2008; Table 1), were obtained from Brown Hatchery (Hod Hasharon, Israel) and incubated in a Petersime incubator (Zulte, Belgium) in the Animal Science building at the Robert H. Smith Faculty of Agriculture, Food and Environment (Rehovot, Israel) according to routine procedures (37.8°C and 56% RH).

On DOS, E11, E13, E15, E17, E19, E20, and DOH, 8 eggs were randomly selected for mineral analyses. The yolk sac of each egg was collected and the yolk content was separated from the yolk sac membrane, weighed, and sampled. The albumen was weighed and sampled only on DOS.

Mineral Analysis. Samples (100–150 mg) from each yolk's contents or albumen were weighed and digested with a mixture of 2 mL of 30% H_2O_2 and 4 mL of 70% HNO_3 inside a 50-mL plastic tube for 6 h in a 95°C bath. The digested samples were analyzed for their mineral content using an inductively coupled plasma atomic emission spectroscopy (**ICP-AES**) instrument (Spectro Arcos, Kleve, Germany).

Experiment 2

General Procedure. Fertile eggs (n = 300; average weight = 67.32 ± 3.31 g) from Cobb 500 hens (38 wk of

Table 2. Mineral amounts in the enrichment solution

Mineral	Chemical form	Concentration (mg/mL)	Enrichment (mg/egg)
Fe	Organic[1]	1.6	0.96
Zn	Organic[1]	1	0.6
Mn	Organic[1]	0.6	0.36
Ca	Organic Ca–β-hydroxy-β-methylbutyrate	0.6	0.36
Cu	Organic[1]	0.03	0.018
P	KH_2PO_4	1.4	0.845

[1]Bioplex (Alltech, Lexington, KY).

age), fed according to Cobb (2008; Table 1), were obtained from Brown Hatchery and divided into 2 groups of similar weight distribution: the enriched group and the nonenriched group (control). The eggs were then incubated as in experiment 1. On E17, the amniotic fluids of the enriched group were enriched by in ovo feeding methodology (Uni and Ferket, 2003, 2004) with 0.6 mL of a solution containing 800, 182, and 0.8 IU of vitamins A, D, and E (Phibro, Ridgefield Park, NJ), respectively; 4% maltodextrin (Sigma-Aldrich, St. Louis, MO); and various minerals in different chemical forms (Table 2).

The solution was prepared on the day of enrichment (E17) using a sterile 0.15% NaCl solution as a diluent and a mineral concentration selected based on preliminary results and osmolarity restrictions. Mineral concentrations in the enrichment solution were verified by ICP-AES analysis.

On DOS and E17 (before the enrichment), 8 eggs (independent of their group) were randomly selected, whereas on E18, E19, E20, and DOH, 8 eggs were randomly selected from each group. The yolk content of each selected egg was separated from the yolk sac membrane, weighed, and sampled. In addition, on DOS and DOH, the shell of each of the randomly selected eggs was collected, separated from the shell membranes, cleaned, weighed, and sampled.

Mineral Analysis. Shell samples were ashed in a Bifa furnace (Ramat Gan, Israel) at 600°C for 10 h; a sample of the ashes from each shell was weighed and taken for mineral analysis. Yolk and ashed shell samples (100–150 mg) were digested as in experiment 1 and analyzed for mineral content using an ICP-AES instrument.

Statistical Analysis

In experiment 1, data were subjected to 1-way ANOVA. In experiment 2, data for E18 to DOH were subjected to 2-way ANOVA with a model that included embryonic day (E18, E19, E20, and DOH) and group (enriched and control) and their interaction as main effects. Values are presented as means ± SE. Differences among means were tested by contrasts using *t*-test. All statistical analyses were carried out using JMP 7 software (SAS Institute Inc., Cary, NC). Differences were considered statistically significant at $P < 0.05$.

RESULTS

Mineral Content in the Yolk and Albumen on DOS

All results were processed and calculated as mean total weight in the yolk, albumen, or shell by multiplying the concentration of every examined mineral in the sample by the total weight of the tissue. Table 3 presents the mineral amounts in the yolk and albumen on DOS. Figure 1 presents the mineral distribution of the examined minerals between the yolk and the albumen on DOS: 96.53, 94.46, 87.77, 87.12, 76.41, and 62.63% of the Mn, P, Fe, Ca, Cu, and Zn, respectively, originated from the yolk, whereas only 3.47, 5.53, 12.24, 12.88, 23.59, and 37.37%, respectively, originated from the albumen.

Mineral Contents in the Yolk During Incubation

Figure 2 presents the yolk content and uptake (represented by the slope) of the examined minerals from the yolk on DOS, E11, E13, E15, E17, E19, E20, and DOH (E21). The level of P (Figure 2a) on DOS was 111.7 mg, followed by significant decreases to 74.90 mg on E11 and 21.53 mg on E17. On E19, E20, and DOH, no significant difference in P level was seen. On DOS, the yolk contained 28.89 mg of Ca (Figure 2b) followed by a significant decrease to 21.89 mg on E11. Between E11 and DOH, no significant change in Ca level occurred. The Fe level (Figure 2c) on DOS was 2.78 mg, followed by a significant decrease to 1.39 mg on E11. Between E13 and DOH, no significant decrease in Fe level was observed. The Zn level (Figure 2d) on DOS was 0.99 mg, followed by a significant decrease to 0.51 mg on

Table 3. Mineral content (±SD) in the yolk and albumen on day of setting

Mineral	Yolk	Albumen
P (mg)	111.7 (±16.84)	6.55 (±5.19)
Ca (mg)	28.89 (±6.19)	4.27 (±3.78)
Fe (mg)	2.78 (±1.89)	0.39 (±0.47)
Zn (mg)	0.99 (±0.41)	0.59 (±0.45)
Cu (μg)	31.8 (±13.12)	9.82 (±7.03)
Mn (μg)	21.62 (±7.23)	0.78 (±0.8)

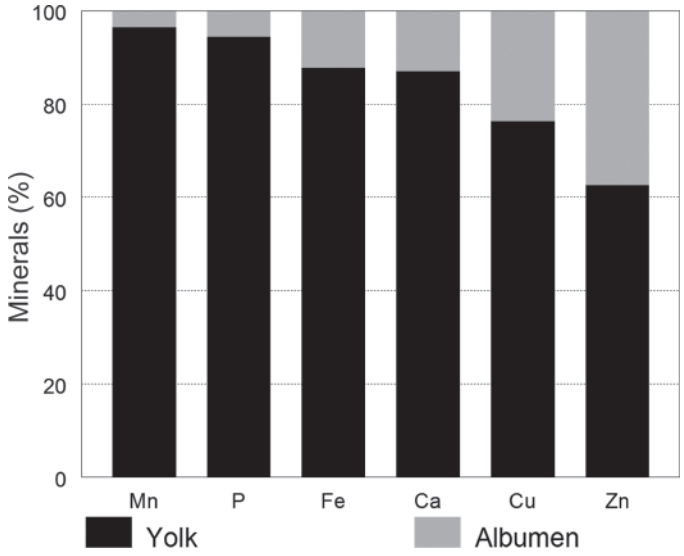

Figure 1. The distribution of the minerals between the yolk and albumen on day of setting.

E13. Between E17 and DOH, no significant decrease in Zn level occurred. The Cu level (Figure 2e) on DOS was 31.8 μg, followed by significant decreases to 22.65 μg on E11 and 12.57 μg on E13. Between E15 and DOH, no significant decrease in Cu level was seen. The Mn level (Figure 2f) on DOS was 21.62 μg, followed by a significant decrease to 12.36 μg on E11. Between E15 and DOH, no significant decrease in Mn level was seen.

Mineral Relative Consumption

The total relative consumption of each mineral during incubation (Figure 3) was calculated by dividing the amount of consumed mineral by its initial amount on DOS using the formula

$$C = \frac{A_{consumed}}{A_{DOS}} \times 100, \quad [1]$$

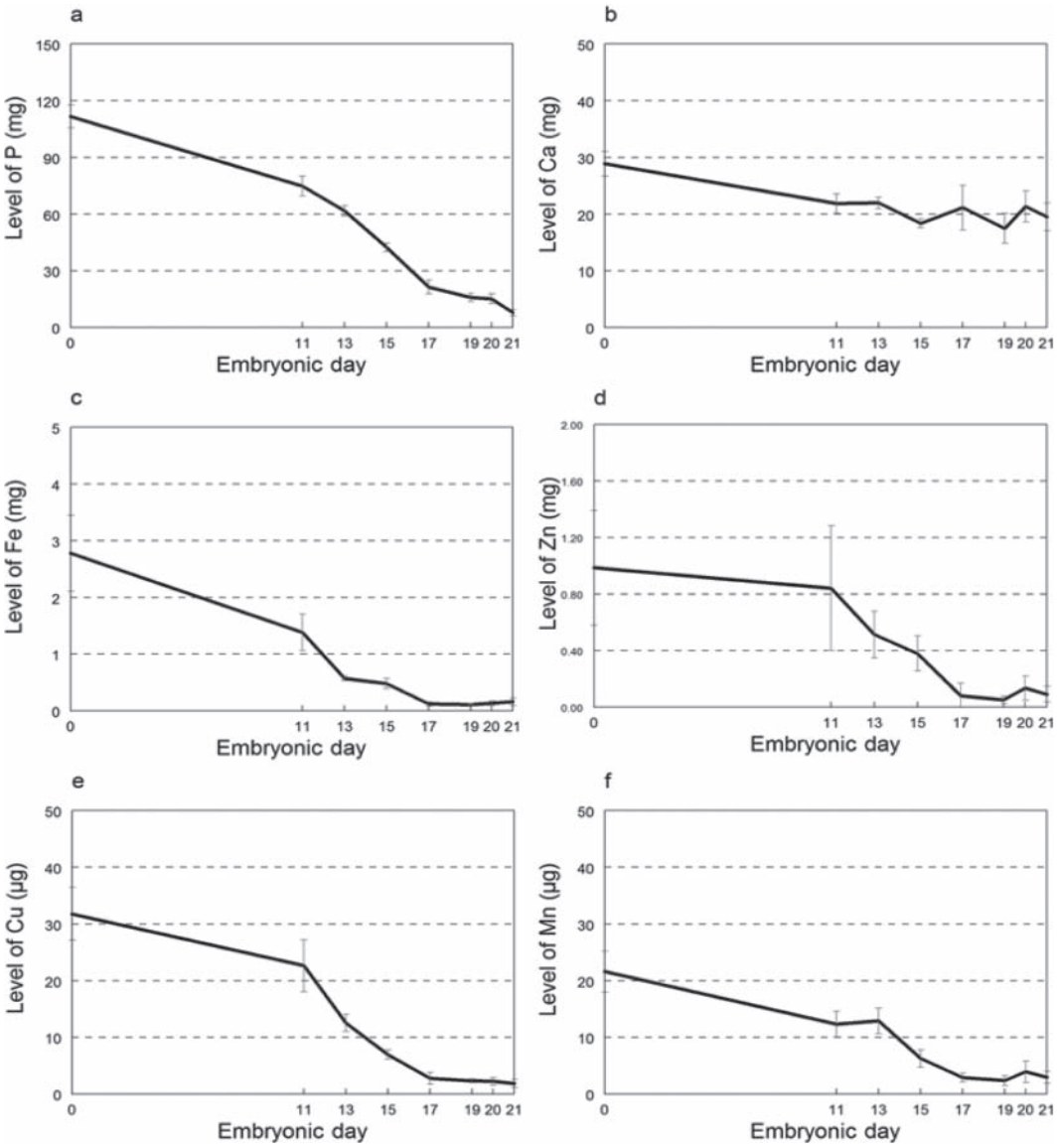

Figure 2. Yolk content (±SE) and uptake of P (a), Ca (b), Fe (c), Zn (d), Cu (e), and Mn (f) from the yolk during incubation.

Table 4. Mean amount (±SE) of specific minerals in the shell on day of setting (DOS) and day of hatch (DOH)

Mineral	Amount on DOS[1]	Amount on DOH[2]	Amount released	*P*-value
Ca (mg)	2,746 (±150.0)	1,903 (±101.8)	843	0.0005
P (mg)	10.09 (±0.80)	7.34 (±0.40)	2.75	0.0115
Fe (μg)	57.92 (±24.19)	0.52 (±0.37)	57.2	0.032
Zn (μg)	15.81 (±3.26)	10.44 (±1.85)	NS	0.19
Mn (μg)	0.94 (±0.23)	0.26 (±0.07)	0.68	0.0203
Cu (mg)	0	0		

[1]Average shell weight on DOS: 8.74 ± 0.93 g.

[2]Average shell weight on DOH: 5.67 ± 0.41 g.

where C is the percentage of relative consumption during incubation, A_{DOS} is the combined amount in the yolk and albumen on DOS, and $A_{consumed}$ is the total amount consumed from the yolk and albumen during incubation, which was calculated using the formula

$$A_{consumed} = A_{DOS} - A_{DOH}, \quad [2]$$

where A_{DOH} is the amount of each mineral in the yolk on DOH.

The calculation of total relative consumption of each mineral during incubation (Figure 3) showed that 95.49, 94.93, 94.18, 93.31, 86.68, and 41.09% of the Cu, Fe, Zn, P, Mn, and Ca, respectively, was consumed by DOH, whereas only 4.51, 5.07, 5.81, 6.69, 13.32, and 58.9%, respectively, was stored in the residual yolk at hatch.

Shell Mineral Content

Table 4 presents the amounts of Ca, P, Fe, Mn, Zn, and Cu in the eggshell on DOS and DOH. On DOS, Ca was found in the highest amount in the shell, which also contained some P and small amounts of Fe, Zn, and Mn. No Cu was found in the shell. By DOH, the examined minerals (except for Zn and Cu) showed significantly reduced levels in the shell.

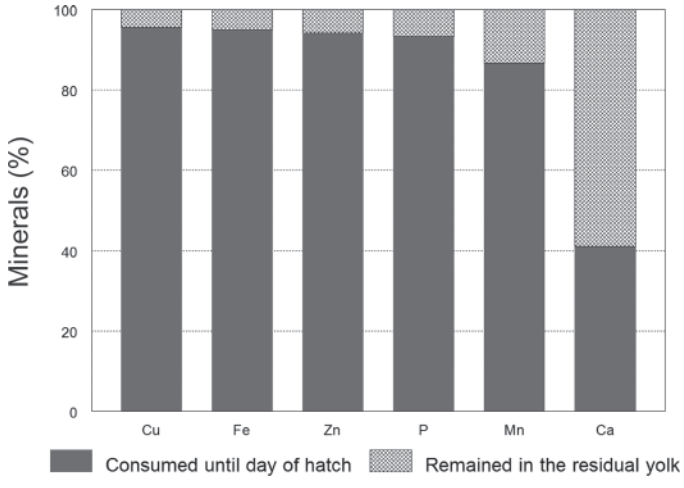

Figure 3. Relative consumption of Cu, Fe, Zn, P, Mn, and Ca during incubation.

Yolk Mineral Content After the Enrichment

Figure 4 presents the yolk content and uptake of the examined minerals from the yolk of the enriched (dashed gray line) and nonenriched (solid black line) groups on DOS, E17, E18, E19, E20, and DOH (E21).

The control group (nonenriched) exhibited a pattern similar to that seen in Figure 2: a significant reduction in the yolk content of all examined minerals between DOS and 17E, whereas from E19, significant differences were seen only in Cu and Zn levels (between E19 and E20) and in Ca level (between E20 and DOH). None of the mineral levels showed any significant interaction between sampling day and treatment.

Following enrichment on E17, the enriched and nonenriched groups showed differences in mineral levels: the enriched group had higher levels of Fe, Zn, Cu, and Mn than the nonenriched group, although no significant difference between groups was found for P and Ca.

The enriched group showed Fe levels of 0.35, 0.36 and 0.23 mg on E19, E20, and DOH, respectively, whereas the nonenriched group showed significantly lower levels of 0.16, 0.11, and 0.08 mg, respectively. The Zn levels in the enriched group were 0.17, 0.18, and 0.15 mg on E19, E20, and DOH, respectively, whereas the nonenriched group had significantly lower Zn levels of 0.08, 0.02, and 0.03 mg, respectively. The Cu levels in the enriched group were 1.94 and 3.05 μg on E20 and DOH, respectively, whereas the nonenriched group showed significantly lower Cu levels of 0.13 and 1.15 μg, respectively. The Mn levels in the enriched group were 98.04, 119.12, 131.77, and 88.04 μg on E18, E19, E20, and DOH, respectively, whereas the nonenriched group showed significantly lower Mn levels of 3.32, 2.31, 1.33, and 1.51 μg, respectively.

DISCUSSION

The results of this study demonstrate that most of the minerals were consumed by E17. In the last days of incubation, the amounts of P, Fe, Zn, Cu, and Mn in the yolk were low and the embryo consumed little, if

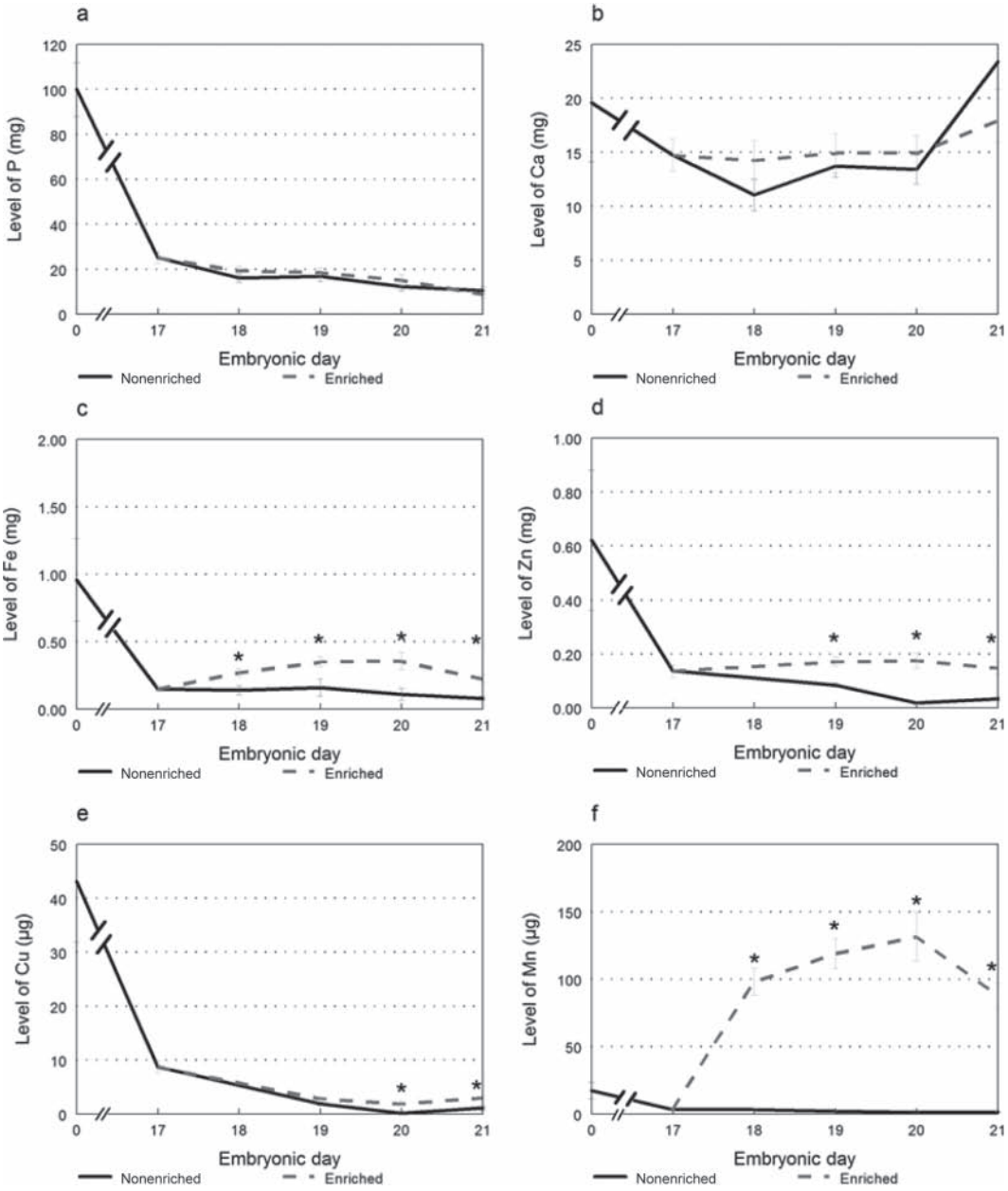

Figure 4. Yolk content (±SE) and uptake of P (a), Ca (b), Fe (c), Zn (d), Cu (e), and Mn (f), from the yolk of the nonenriched (solid line) and enriched (dashed line) groups during incubation. Asterisk indicates significant differences ($P < 0.05$) between treatments within days. Levels of Zn and Cu were not examined on embryonic d 18 because of a technical difficulty.

any, of these minerals. Embryonic enrichment demonstrated that higher mineral levels in the yolk along with addition of vitamins, amino acids, and carbohydrates leads to higher mineral consumption by the embryo, which might affect embryonic development.

The Yolk as a Major Mineral Storage Compartment

The yolk is the major storage compartment for P, Fe, Zn, Cu, and Mn in the egg (Figure 1). Other egg compartments (allantoic fluid, albumen, and amniotic fluid) contain low amounts of these minerals, as noted by Romanoff (1967) who showed that on E17, Fe levels in the allantoic fluid, albumen, and amniotic fluid were 39, 20, and 18.9 μg, respectively, whereas in the yolk it was much higher (680 μg). Another example is P levels, which at E17 were 35-fold higher in the yolk than in the allantoic fluid, albumen, and amniotic fluid combined. Therefore the yolk is the major organ to study and analyze mineral content and uptake during the embryonic period.

Shell Mineral Content and Release

Because the contribution of minerals other than Ca and Mg by the shell has never been fully documented, we also examined mineral release from the shell dur-

ing incubation (Table 3) and found, in agreement with previous publications, that the shell is a major storage compartment for Ca, providing the embryo with Ca during incubation (Packard and Packard, 1991; Richards and Packard, 1996). In addition, we showed, for the first time, that the shell also releases 2.75 mg of P, 57.2 μg of Fe, and 0.68 μg of Mn during incubation. However, the shell's contribution of these minerals is very low relative to the yolk and, therefore, it can be concluded that the shell is a minor source of P, Fe, and Mn and a major source of Ca for the embryo. It is assumed that layer age-related changes occur in the shell mineral composition. However, these changes are probably small relative to the yolk mineral composition. This hypothesis should be further examined. The fluctuations in the Ca graph (Figure 2b) between E11 and DOH can be explained by Ca influx from the shell into the yolk.

Mineral Consumption from the Yolk

Experiment 1 (Figure 2) showed that in the last days of incubation, the yolk contained low levels of P, Fe, Zn, Cu, and Mn. Moreover, consumption (as reflected by the slope) of P, Fe, Zn, Cu, and Mn from the yolk was very low: consumption of P (Figure 2a) was relatively constant between DOS and E17 and then decreased between E17 and E20. Iron (Figure 2c), Zn (Figure 2d), Cu (Figure 2e), and Mn (Figure 2f) showed moderate to mild consumption between DOS and E11, followed by increased consumption between E11 (E13 for Mn) and E17. However, from E17 to DOH, almost no consumption of Fe, Zn, Cu, and Mn was observed.

The mineral consumption trends imply that either mineral limitation or limited mineral demand exist during the last days of incubation. Moreover, because P, Fe, Zn, Cu, and Mn are important for the development of critical systems such as the cardiovascular, immune, and skeletal systems, it is reasonable to assume that their low levels and minimal consumption can impair the developmental potential and performance of chicks.

Minerals in the Residual Yolk on DOH

Most of the minerals in the yolk and albumen on DOS were largely consumed by DOH (Figure 4), leaving low levels of P, Fe, Zn, Cu, and Mn in the residual yolk. Posthatch, the residual yolk is the major energy and nutrient source for the transition period from embryonic phase to hatchling (Noy and Sklan, 1999; Gonzales et al., 2003; Henderson et al., 2008). Moreover, because most hatchlings are fed only at 36 to 48 h posthatch while being subjected to intensive metabolic demands (De Oliveira et al., 2008), the low mineral levels in the residual yolk posthatch may impair the development of critical organs and systems during that period. The results and conclusions from experiment 1 led to the concept of enriching the embryos with minerals by either changing the nutrition of the breeding flocks or providing in ovo enrichment.

Means to Increase the Mineral Content of Eggs

One of the pertinent questions in broiler breeder nutrition is the effect of hen nutrition on the mineral composition of eggs. Previous research has shown that, with the exception of Mn, increasing the concentration of the minerals examined here in the diet of hens has little or no effect on the concentration of those minerals in the egg (Naber, 1979).

A current approach to modifying minerals in the diet of hens involves changing them to organic forms (Flinchum et al., 1989; Richards, 1997; Ao et al., 2006): supplementation of organic Zn, or additive Zn and Mn, to hen diet resulted in improved immunity but not growth (Kidd et al., 1992, 1993). Supplementation of 20 and 40 mg/kg of Zn and Mn as methionine-chelated forms significantly reduced (by half) the incidence of shaky leg and angular defects compared with the inorganic sulfate forms of these minerals (Ferket et al., 1992). Virden et al. (2003) demonstrated that breeders fed supplemental Zn and Mn amino acid complexes have progeny with improved early survival. Bone strength of chicks from hens fed inorganic minerals at 100% NRC (1994) levels was significantly lower than that of chicks from hens fed 8, 17, or 33% of those levels in organic form (Pierce et al., 2009). The benefits of supplementing organic minerals to breeder flocks are probably attributed to the increased mineral amounts in the embryonated egg, as has been demonstrated with organic Se supplementation (Cantor, 1997). Nevertheless, although feeding organic minerals to breeder flocks appears to be a promising approach, it is unclear whether such additions will enhance mineral levels in the egg or how effective this method actually is.

In ovo enrichment is an excellent tool for understanding whether the mineral uptake by the embryo can be increased by embryonic nutritional manipulation. In addition, the in ovo enrichment can be used to understand whether the minimal mineral uptake by the embryo toward hatch is attributable to the observed low levels of the minerals or to the low nutritional requirements of the embryo for those minerals.

In Ovo Enrichment

Examination of the effect of in ovo enrichment of P, Ca, Fe, Zn, Cu, and Mn along with vitamins, amino acids, and carbohydrates showed (Figure 4) that the enrichment resulted in increased Fe, Zn, Cu, and Mn levels in the yolk even though the minerals are supplemented in the amniotic fluid. The gradual increase in yolk Fe, Zn, and Mn levels between E17 and E20 was probably attributable to gradual transfer from the enriched amniotic fluid swallowed by the embryo during

the final days of incubation. As shown by Esteban et al. (1991), the fluids are transferred to the yolk sac from the gastrointestinal tract via the vitelline diverticulum. In addition to higher mineral amounts, consumption of Fe, Zn, and Mn (and possibly P) between E20 and DOH was higher in the enriched group. The increase in Ca levels between E20 and DOH seems to be lower in the enriched group, which could suggest that the supplementation of vitamin D and Ca resulted in an enhanced uptake of Ca from the yolk storage.

The low mineral consumption toward hatch can be manipulated by in ovo feeding of minerals and other specific nutrients. The enrichment solution contained 4% carbohydrates, β-hydroxy-β-methylbutyrate (**HMB**), and vitamins, which were added based on their known effect: vitamin D is known to affect calcium and phosphorus metabolism (DeLuca, 1992), and an in ovo administration of HMB and carbohydrates (such as maltodextrin) was shown to enhance embryonic intestinal development (Tako et al., 2004). The assumed role of vitamin D, HMB, and carbohydrates on mineral metabolism should be further examined.

The presented data on embryo mineral resources and consumption during incubation is important for a better understanding of the nutrition and nutritional limitation of embryos during incubation. The increased mineral consumption in the enriched group could affect embryonic development. An examination of the effect of higher mineral levels and consumption on the developing chick, both pre- and posthatch, will provide a better understanding of the role of minerals and other nutrients in the embryonic development of broilers. For example, in the skeletal system, comparing the mechanical and geometrical properties of bones from the enriched and nonenriched groups could help in understanding the effect of embryonic enrichment (mineral, vitamin, amino acids, and carbohydrates) on bone development.

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