Changes in broiler chick tissue concentrations of lipid-soluble antioxidants immediately post-hatch

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A B S T R A C T
The antioxidant protection of the chicken (Gallus gallus) embryo during incubation and early postnatal development plays an important role in chick viability. To assess the antioxidant capacity of the newly hatched chick, we determined the concentrations of vitamin A, vitamin E, carotenoids and coenzyme Q10 in the major tissues of chicks which had been held in an incubator for up to 36 h post-hatch. Concentrations of total carotenoids and free retinol and retinol esters in the tissues did not differ significantly over the 36 h period post-hatch (p>0.05). In contrast concentrations of vitamin E (α-tocopherol, γ-tocopherol, and α-tocotrienol and γ-tocotrienol) in various tissues (liver, heart, brain and leg muscle) decreased significantly in chicks that had been held in the incubator for 36 h when compared to younger chicks that were held for up to 18 h. Comparatively high concentrations of coenzyme Q10 were detected in the yolk sac membrane, liver and heart, and the concentrations being dependent on age of chicks, the highest value being recorded 18 h post-hatch. In most of the tissues studied, coenzyme Q10 concentrations decreased substantially between 18 and 36 h post-hatch. This study demonstrated that there are tissue-specific changes in the concentrations of the major antioxidants (vitamin E and coenzyme Q10) during the 36 h post-hatch.

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1. Introduction

Traditional breeding programmes were designed to produce a broiler with the potential to maximise growth, yield and feed efficiency. These traits can however compromise health status since there is a trade-off between growth- and health-related traits (Siegel et al., 2001). Chick viability is an important factor in determining profitability and, from fertilisation to placement at the broiler farm, factors such as egg quality, egg storage conditions, incubation conditions and post-hatch environment will all affect chick quality (Decypere et al., 2001).

The 21 day incubation period and the 10 day post-hatch period of the chick comprises about 50% of a 2 kg broiler’s lifespan in current intensive production systems. Therefore, anything that hinders or promotes growth and development during this neonatal period will have a marked effect on overall performance and health of poultry (Ferket, 2006). So the success of embryonic development depends on the interactions between egg composition and the conditions of egg incubation. During chick embryo development there is an antioxidant/prooxidant balance in the tissues which supports normal embryonic development and post-hatch chick viability. It has been suggested that an accumulation of the natural antioxidants like vitamins A, E and carotenoids as well as an increase in GSH-Px activity in the embryonic liver may have an adaptive significance, evolving to protect unsaturated lipids against peroxidation during the stress imposed by hatching (Surai, 2002). It seems likely, that in postnatal development there is a different strategy in relation to antioxidant defence compared to the embryonic chicken (Surai, 2000). For example, during mammalian prenatal development the antioxidant system is considered to be immature (Allen and Venkatraj, 1992; Fantel, 1996) with maturation occurring in the postnatal period. Probably the same is true in avian embryo development i.e. an embryo relies on natural antioxidants accumulated in the egg yolk to protect tissues during hatching against lipid peroxidation.

Antioxidant protection at hatching time is considered to be an important determinant of chick viability during first post-hatch days (Surai, 1999a, 2000). Depending on the so-called spread of hatch (i.e. the time from the first to last chick to hatch) it would not be uncommon for a significant percentage of any hatch to spend up to 36 h in the hatchery having emerged from the egg. Given the relatively high temperature and humidity in the hatchery, it is easy to make the argument that the chick may be under chronic oxidative stress during this holding time. However antioxidant defences during this important time have not been studied yet.

The objective of our work, therefore, was to investigate the content of commonly recognised antioxidants (vitamin A, E, total carotenoids...
and coenzyme Q10 in the tissues of chicks held in the incubator for times ranging from 0 to 36 h.

2. Material and methods

2.1. Chicks (Gallus gallus)

In the incubator 100 Hubbard Flex eggs (10 eggs on each of 10 trays) were marked and incubated according to standard hatchery practices (37.5 °C). The humidity of incubator was about 58–60% during 1–18 days. After day 18, the humid was raised to 65%. From each tray 1 chick was taken for laboratory analyses at 0, 18 and 36 h after hatching (in total 30 chicks).

All chicks were dissected and tissue mass were assessed \( n = 10 \). After that, tissues (liver, residual yolk (YSR), yolk sac membrane (YSM), lung, brain, heart, leg and breast muscle) from 5 chicks per group were taken for biochemical analysis \( n = 5 \). Carotenoids, vitamins A, E and coenzyme Q10 were assessed in tissues.

The study was approved by the SAC Animal Ethics Committee.

2.2. Analytical work

Vitamins in tissues were determined by high-performance liquid chromatography (HPLC) as previously described \( \text{Surai et al., 2001} \). An aliquot of tissue was vortexed in 0.7 mL 5% NaCl, then 1 mL ethanol was added and homogenized (1 min), during homogenization 2 mL hexane was added. Samples were then centrifuged and the hexane phase, containing the carotenoids, was collected. Extraction with hexane was repeated and the combined hexane phase was dried under \( \text{N}_2 \) gas, and then re-dissolved in dichloromethane/methanol (1:1 v/v). Carotenoids, vitamin A (retinol and retinol esters), E and coenzyme Q10 were quantified by HPLC.

Vitamin E (\( \alpha-, \gamma-, \delta-, \) tocopherols and \( \alpha-, \gamma- \)-tocotrienols) were determined as previously described \( \text{Surai and Speake, 1998a, b} \) using an HPLC system (Shimadzu Liquid Chromatography, LC-10AD, Japan Spectroscopic Co Ltd. with JASCO Intelligent Spectrofluorometer 821-FP) fitted with a Spherisorb, type S30DS2, 3 μ C18 reverse phase HPLC column, 15 cm × 4.6 mm (Phase Separations Limited, UK). Chromatography was performed using a mobile phase of methanol/water (97:3, v/v) at a flow rate of 1.05 mL/min. Fluorescence detection of tocopherols and tocotrienols involved excitation and emission wavelengths of 295 and 330 nm respectively. Standard solutions of tocopherols and tocotrienols in methanol were used for instrument calibration and tocoul was used as an internal standard.

Coenzyme Q10 was analysed in the same extract by injecting 50 μL into the same HPLC system, but using a Vidas 201TP54 column (5 μm, 25 cm × 4.6 mm) and mobile phase ethanol/methanol/2-propanol (70:15:15 by volume) and flow rate 1.5 mL/min with a diode array detection at 275 nm (Matilla and Kumpulainen, 2001). Coenzyme Q10 (Sigma) standard was used for calibration.

Total carotenoids were analysed by injecting 10 μL into the same HPLC system, using Waters NH2 column (5 μm, 25 cm × 4.6 mm) and mobile phase methanol/water (97:3 by volume) and with a flow rate of 1.5 mL/min with a diode array detection at 444 nm \( \text{Surai et al., 1998a, b} \).

Retinol and retinol esters were determined by injecting 10 μL into the HPLC system, but using Waters superspherisorb ODS 2, 5 μ C18 reverse phase HPLC column, 25 cm × 4.6 mm (Phase Separations Limited, UK) with a diode array detection at 325 nm \( \text{Karadas et al., 2005} \).

2.3. Statistical analysis

Statistical analysis was carried out by the General Linear Models (GLM) procedure of SAS (statistical Software for Windows 6.12, 1996; SAS Inc., Cary, NC, USA). Significant differences between treatment means were examined by Duncan’s multiple range tests. Significance was assigned as a level of \( p < 0.05 \) unless otherwise stated.

3. Results

The body mass (36.21 ± 0.94; 36.85 ± 2.52; 35.32 ± 0.73 g) and liver mass (0.92 ± 0.04; 1.02 ± 0.05; 1.00 ± 0.05) g of chicks (0, 18 and 36 h respectively) were not affected by the age of birds (different holding times in the incubator). However, the masses of the yolk sac membrane (YSM) (0.33 ± 0.05; 0.18 ± 0.02; 0.14 ± 0.012) g and residual yolk (YSR) (4.07 ± 0.30; 3.76 ± 0.20; 2.85 ± 0.16) g were reduced significantly \( (p < 0.05) \) for chicks held in the incubator for 18 h or more.

The concentrations (μg/g fresh tissue) of the free retinol and retinol esters (R-linoleate, R-oleate, R-palmitate and R-stearate) in liver and yolk sac membrane are shown in Table 1. The only significant changes in the concentrations of these compounds however were the retinol concentrations, which decreased after 18 h, and the total vitamin A concentration in the YSM which peaked after 18 h and decreased again by 36 h post-hatch.

The total carotenoids concentrations (μg/g) are presented in Table 2. Generally there were no significant changes in the concentration of carotenoids in the various tissues, the only changes occurring in the brain and heart where there was a tendency for the concentrations to decrease by 36 h post-hatch.

The vitamin E and coenzyme Q10 concentrations are shown in Table 3. As would be predicted the concentrations of vitamin E were greatest in the liver and the YSM with values in other samples being less than 10% of those recorded in the liver and the YSM. In general, in the liver alpha-tocopherol represents about 85.7% of total vitamin E, while gamma-tocopherol, alpha- and gamma-tocotrienol represent 11.4, 2.4 and 0.5 respectively. Vitamin E concentration in the liver is about 3-fold higher than coenzyme Q10 concentration in this tissue. In the YSM this difference is only 2-fold, while in other tissues (lung, heart, brain, breast and leg muscles) coenzyme Q10 concentrations are several fold higher than that of alpha-tocopherol.

The concentration of coenzyme Q10 did vary among tissues, or for liver and yolk sac membrane for the different holding periods \( (p < 0.05) \). The highest concentration was detected in YSM while the lowest was detected in the YSR. Coenzyme Q10 concentration even if it is minor in most tissues, decreased significantly and reached significant level in liver, brain, heart, lung and YSM \( (p < 0.05) \) at 36 h.

4. Discussion

The antioxidant system of the chicken embryo and newly hatched chick has been studied extensively \( \text{Surai, 2002} \). It was shown that it includes fat-soluble antioxidants (vitamin E and carotenoids) originating from the maternal diet \( \text{Surai et al., 1996} \), as well as water-soluble antioxidants (ascorbic acid, glutathione, and uric acid) and antioxidant enzymes (superoxide dismutase, glutathione peroxidase and catalase; \text{Surai, 1999a} \) which are synthesised during embryonic development \( \text{Surai et al., 1998a; Surai, 1999a} \).

We would argue that because hatching places a stress upon the emerging chick that natural antioxidant concentrations (vitamin E and carotenoids) have evolved to reach the maximum to protect the unsaturated lipid in the tissues and so limit lipid peroxidation \( \text{Surai et al., 1998a; Surai, 1999a} \). Furthermore, any delay in accessing food during the first 10 days of post-hatch development has been shown for...
Values are means±S.E.M. (n=5). Means of each factor in columns are not sharing a common superscript differ significantly (P<0.05).

Table 2
Total carotenoids concentration (μg/g) in the day-old chick tissues held at different period in the incubator.

<table>
<thead>
<tr>
<th>Holding time (h)</th>
<th>Liver</th>
<th>Yolk sac membrane</th>
<th>Yolk sac residual</th>
<th>Heart</th>
<th>Lung</th>
<th>Brain</th>
<th>Breast muscle</th>
<th>Leg muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>16.76±0.34a</td>
<td>22.34±3.10a</td>
<td>33.7±0.28b</td>
<td>1.69±0.32a</td>
<td>4.57±1.31a</td>
<td>1.64±0.31b</td>
<td>1.63±0.42a</td>
<td>1.47±0.30a</td>
</tr>
<tr>
<td>18</td>
<td>15.50±2.04b</td>
<td>22.68±2.18b</td>
<td>5.96±1.12c</td>
<td>3.32±0.13a</td>
<td>4.52±0.58a</td>
<td>1.60±0.19a</td>
<td>2.16±0.41a</td>
<td>2.51±0.44a</td>
</tr>
<tr>
<td>36</td>
<td>16.54±1.04b</td>
<td>27.93±2.19b</td>
<td>4.99±1.68a</td>
<td>1.20±0.32b</td>
<td>5.88±0.44b</td>
<td>0.24±0.04b</td>
<td>2.40±0.03b</td>
<td>1.56±0.16b</td>
</tr>
</tbody>
</table>

Values are means±S.E.M. (n=5). Means of each factor in columns are not sharing a common superscript differ significantly (P<0.05).
In general, dietary supplementation of coenzyme Q₁₀ does not affect the endogenous synthesis of coenzyme Q₁₀ in tissues. However, oxidative stress (physical exercise, thyroid hormone treatment, cold adaptation, vitamin A deficiency, etc.) is associated with increased coenzyme Q synthesis reflecting cellular adaptation (Ernest and Dallner, 1995). Therefore, coenzyme Q₁₀ synthesis is considered to be an adaptive mechanism in response to stress conditions when other antioxidants are depleted. For example, in vitamin E and Se deficient rats the concentration of coenzyme Q₁₀ was elevated and the coenzyme-Q-dependent reductase system was activated (Navarro et al., 1998).

It is believed that exogenous coenzyme Q protects cells from oxidative stress by conversion into its reduced antioxidant form by cellular reductases. In particular cytosolic NADPH-coenzyme Q reductase is responsible for cellular coenzyme Q redox cycle as an endogenous antioxidant (Kishi et al., 1999). The plasma membrane oxidoreductase and DT-diaphorase are two such systems, likewise, they are overexpressed under oxidative stress conditions (Genova et al., 2003). In addition, the selenoenzyme thioredoxin reductase is an important ubiquinone reductase and can explain how selenium and coenzyme Q, by a combined action, may protect the cell from oxidative damage (Xia et al., 2003). Since coenzyme Q is an essential part of oxidative phosphorylation complex in mitochondria the majority (molar amounts) of endogenous coenzyme Q is found in these organelles. However, exogenous coenzyme Q is usually found in the extra-mitochondrial fractions including lysosomes and Golgi vesicles (Dallner and Sindelar, 2000). From our data it is not clear if the increased concentration of Q₁₀ in the liver at 18 h of chick holding is an adaptive reaction to the stress, but clearly combined with a high vitamin E concentration coenzyme Q₁₀, potentially can take place in antioxidant defences in the chicken liver. On the other hand, decreased coenzyme Q₁₀ concentration at 36 h of chick holding could be a refection of the redistribution of these antioxidants. Alternatively, excessive oxidative stress at this time could cause an imbalance between coenzyme Q₁₀ synthesis, transport and usage.

The presence of high concentrations of coenzyme Q₁₀ in all membranes provides a basis for antioxidant action either by direct reaction with radicals or by regeneration of alpha-tocopherol from its oxidised form. In fact, a protective effect of coenzyme Q₁₀ against lipid peroxidation was shown in fatty acid emulsions, mitochondria, subcellular fractions and whole liver tissues of the newly hatched chick (Br. Poult. Sci. 39, 257–263). From our data it is not clear if the increased concentration of Q₁₀ in the liver at 18 h of chick holding is an adaptive reaction to the stress, but clearly combined with a high vitamin E concentration coenzyme Q₁₀, potentially can take place in antioxidant defences in the chicken liver. On the other hand, decreased coenzyme Q₁₀ concentration at 36 h of chick holding in conjunction with substantial (almost two-fold) decrease in vitamin E concentration in the liver could be a reflection of the redistribution of these antioxidants. Alternatively, excessive oxidative stress at this time could cause an imbalance between coenzyme Q₁₀ synthesis, transport and usage.

In conclusion, this study demonstrated that there are tissue-specific changes in the concentrations of the major antioxidants (vitamin E and coenzyme Q₁₀) during the 36 h post-hatch. Indeed, there is a need for further research in this fascinating area.

Acknowledgments

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References