Effects of Incubational Oxygen Concentration on Fatty Acid Metabolism and Heme Synthesis in Broiler Breeder Embryos

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Two experiments were conducted to evaluate the effects of incubational oxygen concentration on fatty acid metabolism and heme synthesis in broiler breeder embryos. We measured the hepatic activities of δ-aminolevulinic acid dehydratase (ALAD) and 3-hydroxyacyl-Co-A dehydrogenase (3-HADH) as these enzymes are the limiting steps in heme synthesis and fatty acid metabolism, respectively. We found no differences in ALAD activity between days 17 and 20 of incubation; we hypothesize that this is because heme synthesis is relatively constant at the later periods of embryonic development. 3-HADH activity was higher in chicks hatched from eggs incubated under low oxygen conditions during early incubation, while specific ALAD activity was reduced in this group as compared to controls.

Key words: broiler embryo, fatty acid metabolism, hemopoiesis, oxygen concentration

Introduction

In the broiler industry, improvements in chicken strains and husbandry technology have resulted in shorter production periods, which in turn have increased the importance of the early period of broiler production. It has recently been reported that the effects of early embryonic and neonatal nutrition have significant effects on subsequent growth and other broiler characteristics (Murakami et al., 1992, 1995; Noy et al., 2001; Noy and Sklan, 2001; Suzuki et al., 2008).

The main energy source of chicken embryos is thought to be fat as the respiratory exchange ratio of the developing chicken embryo is about 0.7 (Sato et al., 2006). Efficient energy production from fat would thus improve hatchability and subsequent performance. Chicken embryos respire through the chorioallantoic membrane until the later periods of incubation, when respiration begins in the lung, at which point the embryonic energy source also switches from fat to carbohydrate (Romanoff, 1960; John et al., 1988). Thus, fat metabolism in the chicken embryo might begin to decline at this later period of incubation; it is also possible that the change in energy source might be due to the decreased oxygen supply from the immature lung and the declining the chorion.

If the oxygen supply were to remain high enough, the chicken embryo should be able to produce energy from fat even at a later developmental period, when typically use of this energy source would be diminishing. To increase oxygen supply, human athletes typically increase their red blood cell count by training in low oxygen conditions. It has previously been shown that incubation in high CO₂ conditions during either the early or the late period of development accelerated hatching, and led to increased numbers hatching as well as increased subsequent growth of the chickens (Smit et al., 2006; Bruggeman et al., 2007; Everaert et al., 2007). While high CO₂ conditions indicate there is a low oxygen concentration, these reports did not specifically investigate the effects on oxygen transport and fatty acid metabolism.

This study was designed to investigate the effects of a low oxygen concentration during the early period of incubation of broiler embryos on hemopoiesis and fatty acid metabolism. δ-Aminolevulinic acid dehydratase (ALAD) was chosen to study hemoglobin synthesis, as it is the limiting step in the reaction catalyzing ALA to porphobilinogen. Similarly, 3-hydroxyacyl-Co-A dehydrogenase (3-HADH) was chosen, as it is a limiting step in the catalysis of β-oxidation of fatty acids.

Materials and Methods

Animals

All eggs were obtained from the same breeder flock of Chunky strain broilers and laid within a 24-h period. Eggs were incubated at 37.8°C and over 60% relative humidity (RH).
In experiment 1, 24 eggs were selected on Day 17 of incubation, and eggs were divided into 4 groups of 6 eggs each. Livers from the embryos were collected on Days 17 (group 1), 18 (group 2), 19 (group 3), and 20 (group 4) of incubation, from which hepatic ALAD activity was assayed. In experiment 2, eggs were divided into two groups (control vs. experimental) with 6 eggs each before incubation. Each hatching egg was housed in an airtight cylinder type chamber made of acrylic fiber. Eggs of the control group were incubated under 20.9% oxygen concentration. The experimental group was incubated from Day 0 until Day 10 of incubation under 20.2% oxygen which was achieved by addition of CO₂ at 8527.2 ppm, and then incubation continued under the same conditions as the control group. At hatching, livers were collected from the neonatal chicks.

**ALAD Activity Analysis**

Liver (0.25 g) was collected and immediately frozen in liquid nitrogen. Frozen samples were homogenized with a homogenizer (Phycotoron, Microtech Nichion, Chiba), and centrifuged at 2°C and 20,000 g for 60 minute. The supernatant was collected; the enzymatic activity retained in this fraction was used to measure porphobilinogen production over 60 min. Porphobilinogen production was quantified using a spectrophotometer (Shimadzu UV mini-1240).

**3-HADH Activity Analysis**

Liver (0.1 g) was collected and immediately frozen in liquid nitrogen. Frozen samples were homogenized with a homogenizer (Phycotoron, Microtech Nichion, Chiba), and centrifuged at 2°C and 2,000 G for 10 min. The supernatant was then collected to measure the kinetics of NADH reduction via spectrophotometer.

**Protein Content Analysis**

Protein content of the liver supernatant was determined using the Bradford method (Protein Assay, Bio-Rad, Hercules, CA, USA) for both enzyme assays.

**Statistics**

Results obtained were analyzed by one-way ANOVA using the General Linear Models procedure of SAS® software (SAS Institute, 2012). When differences among the means were significant, the means were separated using Tukey’s multiple range test. In experiment 2, means were compared with the Student’s t-test. Statements of significance are based on \( P < 0.05 \), unless otherwise stated.

**Results**

**Experiment 1**

Weights of both the embryos and the embryonic livers increased with age, with the most rapid increase seen between days 19 and 20 of incubation \( (P < 0.05) \). No significant difference in either the total or the specific hepatic ALAD activity was found during any of the time points investigated \( (P > 0.05) \; \text{Table 1}. \)

**Experiment 2**

The average O₂ concentration within the chambers of the control group was 20.9%, with a range of 20.7–21.0%. The experimental group had an average O₂ concentration of 20.2%, ranging from 20.0–20.3%. There were no significant differences in the weights of either the embryos or their livers between the groups. However, protein content in the liver was lower in the low oxygen concentration group than in control group \( (P < 0.05) \). Specific ALAD activity was higher in the control group than in the low oxygen concentration group \( (P < 0.05) \), but total ALAD activity was not \( (\text{Table 2}) \). Hematocrit values trended higher in the low oxygen concentration group, but did not obtain significance. Specific 3-HADH activity was higher in the low oxygen concentration group than in the control \( (P < 0.05) \).

**Discussion**

This study investigated whether incubation of broiler breeder embryos, under low oxygen conditions, during the early period of incubation improved fatty acid metabolism at the time of hatching.
We concluded that there was no significant difference in either specific or total hepatic ALAD activity from embryonic days 17 to 20. A previous report has shown that the specific ALAD activities of F1 chickens (White Leghorn × Rhode Island Red) were constant from 5 to 20 days of embryonic development (Tsushima and Yamada, 1988), and the levels of activity found were similar to those of our present study. Thus, it is possible that the maximum activity level had already been achieved.

We next compared both ALAD and 3-HADH activities in embryos incubated under low oxygen concentrations as compared to embryos incubated under normal conditions. We found no significant differences in liver weight between the two groups, but noted that there was a trend towards heavier weight in the low O2 group. We hypothesize that this might be due to the high fat content of the liver, and its ability to be used as an energy source. We also noted that the protein contents in the livers of the control group were higher than those of the low O2 group.

We found that the chicks that hatched from eggs incubated in lower oxygen concentrations had higher 3-HADH activity than the controls, but lower specific ALAD activity and no significant difference in total ALAD activity. While normal-to-low levels of heme synthesis, as indicated by the ALAD levels, in a reduced-oxygen environment seem to indicate reduced levels of oxygen transport, it should be noted that low oxygenation was only maintained until day 10 of incubation. When blood oxygen levels decrease, the kidneys secrete erythropoietin (Vlaskia et al., 2008) to promote production of new red blood cells. Thus, at the early stage of incubation in low oxygen, the chicken embryos might increase hematopoiesis to compensate for the effects of the reduced oxygenation. Alternatively, broiler strains that consume high levels of O2 are known to have higher fat consumption than strains that consume lower levels of O2 (Chwalibog et al., 2007). Thus, the hemopoiesis in later periods of incubation, when the embryos initially incubated under low oxygen concentrations are supplied with more O2, this might result in reduced ALAD activity.

These results suggested that the hemopoiesis of chicken embryos during hatch was reduced by low oxygen incubation during former period, while β oxidation of fatty acids were elevated.

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