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Thermographic analysis of the radiant heat of chicken and duck eggs in relation to the embryo's oxygen consumption

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ABSTRACT

In eggs, the metabolic activities of the developing embryo produce heat (H) that is dissipated in various forms, including radiation. Given that much of the total heat radiated by an egg (H_{tot}) is heat acquired passively, we asked whether it was possible to detect the fraction produced metabolically (H_{metab}) and the extent of its correlation with the embryo's metabolic rate. In chicken and duck eggs at various incubation ages, under standardized experimental conditions of heat conduction and convection, H_{metab} was measured by thermography as the difference in H_{tot} between fertile and sterile eggs. Then, H_{metab} was correlated to the embryo's oxygen consumption ($\dot{V}_{O_2}$), measured by an open-circuit methodology. Heat loss by water evaporation was found to be less than 3% of the total. During the first half of incubation H_{metab} was too small to be significantly separated from H_{tot}. In the second half of incubation H_{metab} was significant, represented 30–50% of the total energy consumed and correlated linearly with $\dot{V}_{O_2}$ for a good fraction of incubation. We conclude that under standardized conditions of heat conduction and convection, in the second half of incubation thermography offers a simple tool not only to verify the progression of the embryo's incubation but also to estimate its metabolic rate.

1. Introduction

Embryonic development is a continuous transformation of energy in the process of tissue formation and organ growth. In eggs the energy balance is represented by

$$M = A - P \quad (1)$$

where A represents the original energy available in the yolk, P the energy progressively accumulated in embryonic tissues (neglecting the small fraction of excretions and residuals) and M the energy dissipated for the metabolic transformations. M is a low fraction of A because the conversion efficiency ($E_c = P/A$) is typically very high in all classes of developing organisms (Rombough, 2011). The cost M of the conversion processes could be measured by various approaches, as the caloric difference between A and P (Eq. (1)), as the total heat (H) dissipated during development by direct calorimetry, or as the oxygen consumed ($\dot{V}_{O_2}$) during development by indirect calorimetry. Although all equivalent (Kleĭmenov et al., 1984), direct and indirect calorimetries in their various modes of application are rather laborious and may require sophisticated equipment (McLean and Tobin, 1987). Thermography is a rather recent technique to measure the heat dissipated by radiation. A superficial look at a thermographic image of eggs during incubation reveals major differences related to the stage of development (Fig. 1). Therefore, we wondered about the extent of the correlation between the heat of metabolic origin (H_{metab}) radiated by the egg and the embryo's metabolic rate, computed as oxygen consumption.

First, we established standard experimental conditions of heat conduction and convection. Heat loss by water evaporation was computed and found to be minimal. The total heat radiated by the egg (H_{tot}) included the component passively acquired during incubation; therefore, H_{metab} was the difference in H_{tot} between fertile and simultaneously incubated embryo-free eggs. We found that during the first half of incubation H_{metab} was too small to be detected with certainty. In the second half of incubation H_{metab} represented some 30–50% of the total energy consumed and correlated with the embryo's oxygen consumption.

2. Materials and methods

Experiments were conducted on chicken eggs of the White Leghorn variety and on Muscovy duck eggs purchased from a local supplier. After noting the fresh weight, the eggs were placed in incubators (Hova-Bator, Savannah, GA) set at the temperature of

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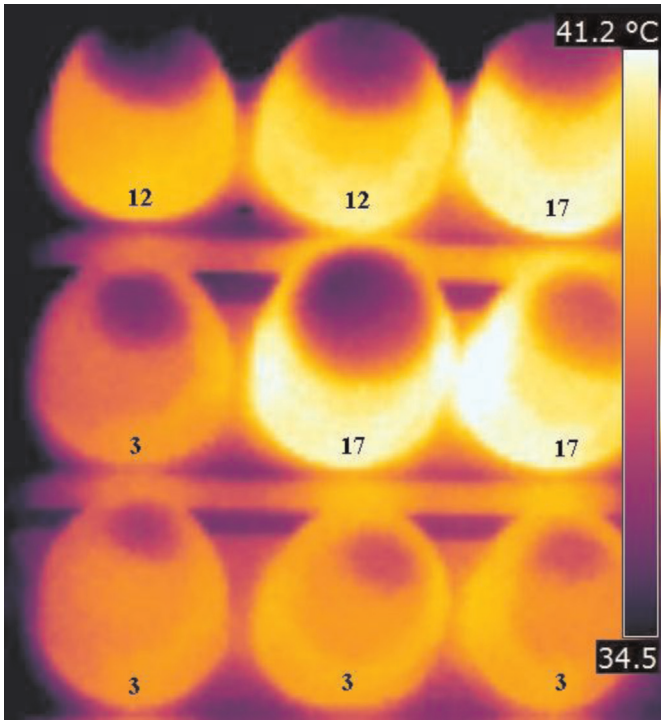

Fig. 1. Thermographic picture of nine chicken eggs in the incubator from the top of the rack. Numbers refer to the incubation age (day, out of 20.5 days). The cool region at the top of each egg is due to the air cell, the size of which expands with the progression of incubation.

37.5 °C and 60% relative humidity, both monitored by a data logger (Hobo®, Onset Computer Corp., Bourne, MA), with progressive 90° rotation at least four times per day. Incubation started at midday (embryonic day E0).

The core measurements consisted in embryo's oxygen consumption ($\dot{V}_{O_2}$) and egg surface temperature (T_s) attributable to the metabolic processes (T*smetab*). For this latter measurement, fertile eggs were matched to sterile eggs of similar weight kept in the same incubator. The heat radiated by the sterile eggs represented the heat accumulated simply as the result of being in the incubator. Hence, the difference in T_s between pairs of fertile and sterile eggs was the T_s that resulted from the metabolic activity of the living tissues (T*smetab*), which included the embryo proper and the chorioallantoic membrane. Because it is impossible to establish the fertility or sterility of an egg at the onset of incubation, at E0 eggs were made 'sterile' either by puncturing the yolk membrane under transillumination with a 6-cm long needle through a pinhole made at the blunted end of the egg, or by exposure to pure CO_2 for 20 min. In the former case, the pinhole of the eggshell was later sealed with dental cement. The T_s data obtained in these two groups of sterile eggs were undistinguishable.

2.1. Oxygen consumption

Oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production were measured by an open flow methodology adapted to the chicken embryo (Mortola and Labbè, 2005). The egg was placed inside a respirometer, which consisted in a 120-ml plastic container maintained at the desired temperature (37.5 °C) by a circulating water bath. A flow of 100 ml/min, under the control of a precision flow-meter, continuously passed through two openings in the lid of the respirometer. The inflow and outflow O_2 and CO_2 concentrations were monitored by a calibrated gas analyzer (CA-1B CO_2 analyzer, Sable Systems Int., Las Vegas, NV) arranged in series, after the gas had passed through a drying column. The gas fractional concentrations were mathematically corrected for the error introduced by a respiratory exchange ratio different from unity (Mortola and Besterman, 2007); then, $\dot{V}_{O_2}$ was computed from the flow rate and inflow–outflow O_2 concentration difference. The $\dot{V}_{O_2}$ values, calculated at standard temperature, pressure and dry conditions, are presented in μl/min.

2.2. Egg surface temperature

In preliminary trials, thermographs were taken through the window with the egg sitting in the rotating rack of the incubator; they proved unsatisfactory for several reasons. The Plexiglas window caused unpredictable underestimate of T_s depending on the degree of rotation of the egg, the effect of curvature on emissivity (see below) and the radiant heat of the incubator, which was not uniform. Furthermore, the thermograph could only cover a small portion of the egg surface, the blunted end that protruded from the egg holder, which included the cool area of the air cell (Fig. 1). For all these reasons, the egg was removed from the incubator, paying attention not to touch its front part, and immediately transferred to a Styrofoam box positioned aside the incubator. The implication of the transfer time on T_s will be addressed below. The white Styrofoam box measured $30 \times 25 \times 22$ cm^3 (width, height and depth), 2.5 cm thick, and was open only to the lateral side that faced the thermo-camera. The egg support consisted of a 4×4 cm^2 polystyrene weigh dish with its base up; a circle of 2 cm in diameter had been cut out of the dish base to accommodate the blunted tip of the egg positioned vertically. In this way the egg effectively was suspended, the contact being less than 1% of its surface, so that heat loss by conduction must have been negligible. The temperature in the box, measured by a mercury thermometer, was steady at 24 °C. Thermographs were taken with a high-sensitivity infrared camera (Flir Systems Inc., i7 model, 144×102 pixels, < 0.1 °C thermal sensitivity) from a measured distance of 20 cm, immediately after positioning the egg in the box.

The relationship between the radiated energy W (Watts) and the temperature (K, in °K) of a surface A (cm^2) depends on the emissivity (ε, dimensionless) of the surface according to

$$W = \sigma A \varepsilon \, K^4 \qquad (2)$$

where σ is the Stefan–Boltzmann constant (5.6704×10^{-8} W K^{-4} m^{-2}). The emissivity ε is the ratio between the energy emitted by the surface and that of a perfectly black body. We assumed $\varepsilon = 0.95$, although there are indications that ε of the eggshell could be closer to 1 (Jiménez-Munoz and Sobrino, 2012). As apparent from Eq. (2), at a given W, higher values of ε result in a lower T_s. To evaluate the magnitude of our overestimates in T_s had ε been higher than 0.95 some thermographs were analyzed both at $\varepsilon = 0.95$ and $\varepsilon = 1$ (Fig. 2). At the most common T_s (38–39 °C), in the worse case of $\varepsilon = 1$, computations with $\varepsilon = 0.95$ would have overestimated T_s by ~0.8 °C. These overestimates applied similarly, but not quite equally, to both fertile and sterile eggs, because they depended on the absolute value of T_s, and could generate an error spanning from 0.09 °C at embryonic day 18 (E18) to ~0.003 °C at E6. These errors were one order of magnitude smaller than the T*smetab* values (see Section 3) and of no significance to the results.

Even a superficial look at the thermograph of an egg reveals regional differences in T_s (Fig. 3, top). Among these, the most peripheral regions of the egg silhouette appear cooler than the 'central' frontal regions of the egg image. We have measured the average T_s of the whole egg image and plotted it against the corresponding value of the central region for a group of sterile and of