Development of a metabolic calorimeter system to measure heat production of domestic avian embryos during incubation

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Segura, J.C., Ouellette C., Feddes J.J.R., Fasenko G.M. and Zuidhof M.J. 2006. **Development of a metabolic calorimeter system to measure heat production of domestic avian embryos during incubation.** Canadian Biosystems Engineering/Le génie des biosystèmes au Canada **48**: 4.1 - 4.6**.** A metabolic calorimeter system was developed to facilitate collection of metabolic data from 24 embryos within an incubator. The system was designed to monitor carbon dioxide (CO_2), oxygen, and water vapour over a 2.5-minute period every hour from each metabolic chamber during both the incubation (18 days) and the hatching stage (21 days). The system can be conveniently calibrated by adjusting individual calorimeter airflow rates with a volumetric flow meter. An experiment was conducted to test the system's ability to measure embryonic heat production calculated from CO_2 production data and an assumed respiratory quotient (RQ) value of 0.84 to calculate oxygen consumption. Two pre-incubation egg storage duration treatments (4 or 15 days) were used to test the system for reliability and ability to measure heat production indirectly. The system satisfactorily measured the CO_2 production of chicken embryos during the 18 day incubation period. At 0 and 18 days, the production of CO_2 ranged from 3 to 480 mL/d, respectively, while calculated heat production values ranged from 1 to 137 mW, respectively. **Keywords**: avian embryo, calorimeter, incubation, heat production.

Un calorimètre métabolique a été développé pour permettre la cueillette de données métaboliques sur 24 embryons placés dans un incubateur. Le système a été conçu pour mesurer le dioxyde de carbone (CO_2), l'oxygène et la vapeur d'eau sur une période de 2,5 minutes à chaque heure dans chacune des chambres métaboliques durant la période d'incubation (18 jours) et celle d'éclosion (21 jours). Le système peut être facilement calibré en ajustant les débits individuels des calorimètres à l'aide d'un débitmètre volumétrique. Une expérience a été réalisée pour tester la capacité du système à évaluer la production de chaleur d'un embryon calculée à partir des données de production de CO_2 et d'une valeur de quotient respiratoire (QR) fixée à 0,84 pour ainsi calculer la consommation d'oxygène. Deux traitements de durée d'entreposage de pré-incubation pour les oeufs (4 ou 15 jours) ont été utilisés pour tester le système en termes de fiabilité et de sa capacité à mesurer indirectement la production de chaleur. Le système a mesuré de manière satisfaisante la production de CO_2 des embryons de poulet durant la période d'incubation de 18 jours. À 0 et 18 jours, la production de CO_2 atteignait respectivement 3 et 480 mL/jour pour une production de chaleur calculée variant entre 1 et 137 mW, respectivement.

INTRODUCTION

Although in the past 30 years, there have been tremendous changes in the genetic growth potential of broiler chickens, egg incubation methods during that time have remained relatively the same. Recent research has shown that the viability of embryos from different broiler genetic strains is influenced by different incubation temperatures (Lourens and van Middelkoop 2000; Hulet et al. 2003). This indicates that embryonic metabolism may be different between different genetic strains. Other research has shown that long-term egg storage prior to incubation (Fasenko et al. 1992) reduces broiler embryonic metabolic rate. Other factors such as parent flock age and maternal nutrition and investment into the egg may also influence embryonic metabolism of the offspring. It may be possible to tailor incubator conditions to optimize domestic avian embryonic metabolism, growth, and survival. However, the effect of each of the factors on the baseline metabolic rates of embryos needs to be determined.

During the three weeks of chicken embryonic development (egg incubation), yolk lipids are metabolized by the embryo via β-oxidation. Oxygen (O_2) is obtained by the embryo for this metabolic process via diffusion through microscopic pores in the shell. A byproduct of lipid metabolism is carbon dioxide (CO_2), which diffuses out of the egg through the same eggshell pores. If only CO_2 is measured, Eq. 1 can be used to calculate oxygen O_2 consumption of the embryo.

$$O_2\ consumed = \frac{CO_2\ produced}{RQ} \quad (1)$$

where: RQ = respiratory quotient.

The respiratory quotient value, which depends upon the type of nutrients being metabolized, has been reported to range from 0.74 to 0.84 (Rahn et al. 1979; Romanoff 1967). Airflow through the metabolic calorimeter, along with incoming and exhaust CO_2 and O_2 concentration, are used to calculate O_2 consumption and CO_2 production by the avian embryo.

Previous investigators have developed methods to measure embryonic metabolism in chickens (Romijn and Lockhorst 1960; Rahn and Poaganelli 1979) and other avian species (Hoyt et al. 1978; Vleck et al. 1979; Vleck and Kenagay 1980). However, these techniques are limited to a maximum of two measurements per day and necessitate a great deal of egg handling.

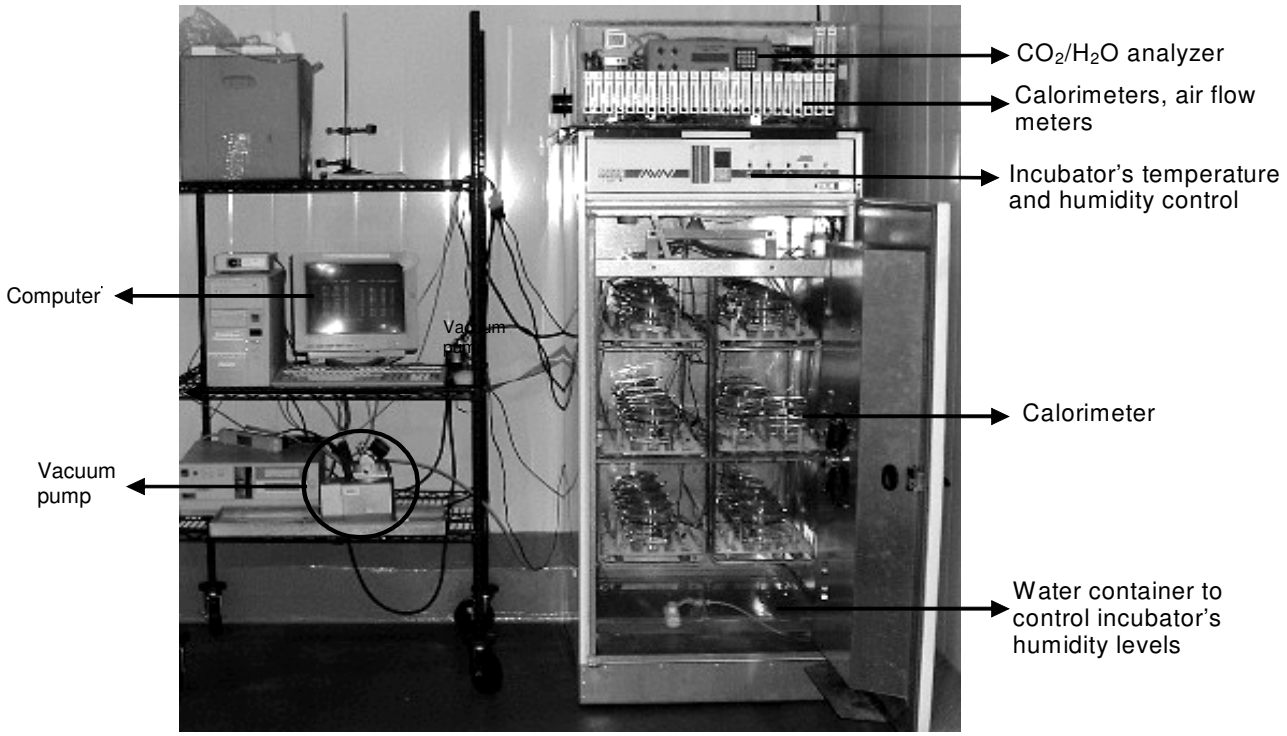

Fig. 1 View of equipment to measure heat production of ebryos.

The objective of this research project was to develop a metabolic calorimeter system that could indirectly monitor embryonic metabolism from 24 calorimeters every hour, with minimal embryo disturbance throughout the three-week period of embryo incubation. To ensure that each calorimeter had the same temperature and incoming air quality, the system was located within the same incubator airspace. A calorimeter system is described in this paper in which airflow is individually controlled and gases can be individually monitored, ie, CO_2, O_2, and water vapour. To check the reliability of the system, an experiment was conducted to test the hypothesis that eggs stored 15 days versus eggs stored for 4 days prior to incubation reduces broiler embryonic metabolic rate.

MATERIALS and METHODS

Egg metabolic calorimeter system

Figures 1 and 2 illustrate the components of the calorimeter system. Each egg can be placed in a cylindrical airtight 1-L metabolic calorimeter (Fig. 3). Each metabolic calorimeter has a 4-mm opening on the side towards the bottom of the calorimeter that serves as the air inlet. The exhaust port for each metabolic calorimeter is located on the opposite side of the the metabolic calorimeter inlet. A 2-m length of tubing of 1.6 mm ID is used to connect the exhaust port to the manifold.

Four metabolic calorimeters are fixed to a tray that slides into an incubator rack (Jamesway Incubator Company Inc., Cambridge, ON). This enables the chambers to move in unison as they are turned left or right 45 degrees to the horizontal within the incubator (Fig. 1). Within each metabolic calorimeter, the egg is held in place by a plastic egg holder cutout from an incubator egg flat that is fastened to the bottom of each metabolic calorimeter (Fig. 3). Experiments normally end on day 18 before the chicks hatch; however, from day 18 to time of hatching, a hatching tray can be simulated by removing the individual egg holder, laying the egg flat, and not allowing the turning mechanism to operate.

Control system

The exhaust port from each metabolic calorimeter is connected to a three-way solenoid activated valve (ASCO Valve Canada, Brantford, ON) that directs the airflow to a vacuum manifold or to the gas analyzers (Fig. 2). Only a CO_2/H_2O analyzer (LI-6262, LI-COR Inc., Lincoln, NE) monitored the exhaust air; however, when available, an oxygen analyzer can be readily connected to the system. When a solenoid activated valve is "ON", the air from an individual metabolic calorimeter is directed to the gas analyzers (Fig. 2). A constant flow-sampling pump (Gilian BDX II Abatement Air Sampler, Sensidyne, Clearwater, FL) is used to draw air through the gas analyzers (Fig. 2). When a solenoid activated valve is "OFF", the exhaust air is directed to the vacuum manifold. A vacuum/compressor pump (Gast Manufacturing, Benton Harbor, MI) draws air through the vacuum manifold to maintain a similar air exchange rate. The tubing from the metabolic calorimeters to the solenoid activated valves is Tygon (Labcor Inc., Concord, ON) lab tubing 1.6 mm ID. The same tubing is used between the solenoid activated valves and the gas analyzers.

For either airflow direction, the airflow through the metabolic calorimeters is adjusted by flow meters (RMA – 12, Dwyer Instruments Inc, Michigan City, IN). The flow was maintained at 0.3 ± 0.012 L/min (Fig. 1). A volumetric airflow meter (Dry Cal DC Lite-ML, Bios International Corporation, Butler, NJ), with an accuracy of ± 0.003 L/min, was available

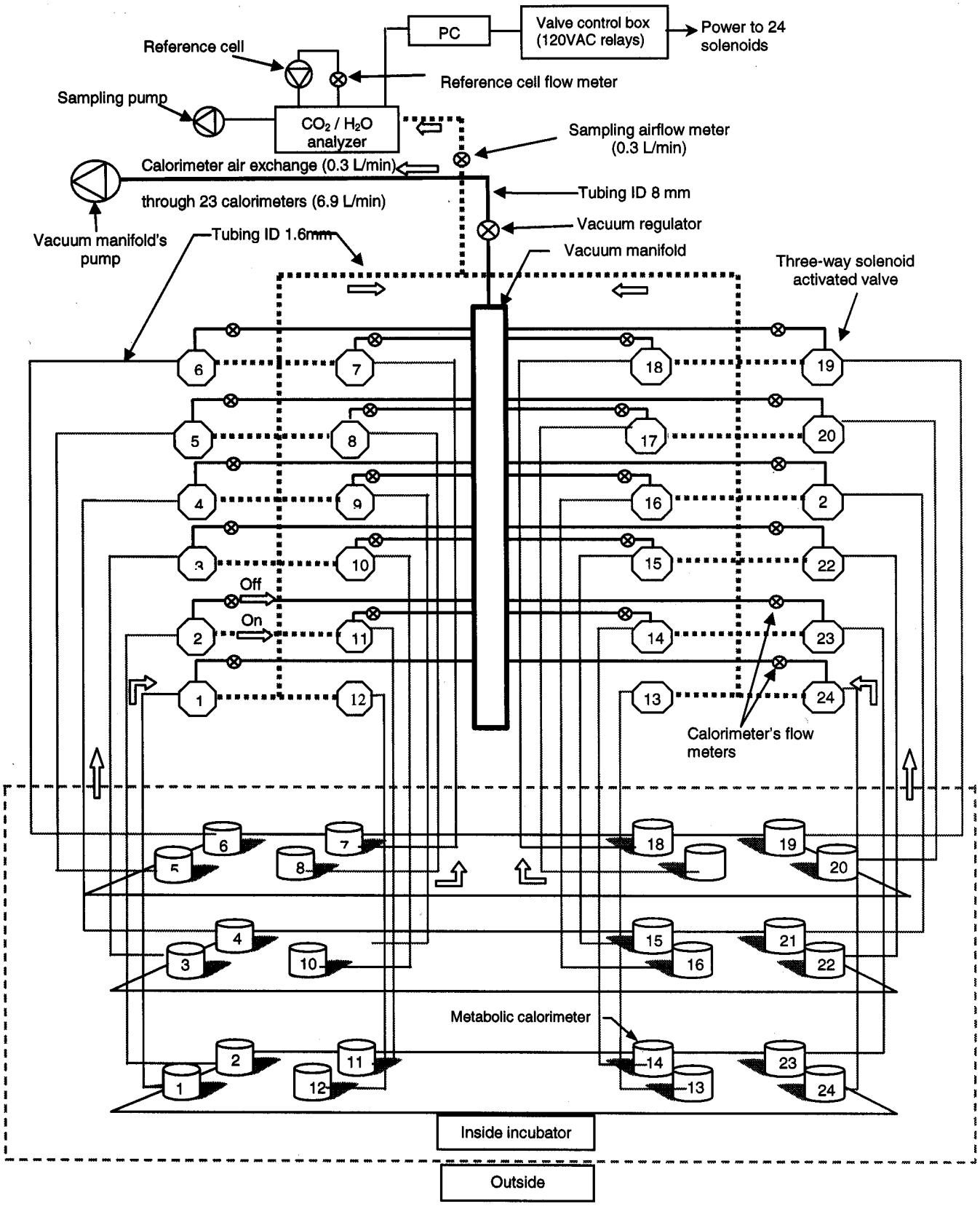

Fig. 2. Metabolic calorimeters, valves, flow meters, vacuum manifold, and instrumentation.

to calibrate the inlet airflow to each metabolic calorimeter. The CO_2 analyzer is calibrated with certified CO_2 gas (2790-ppm ±2%, balance nitrogen). All the solenoid activated valves are operated by relays located in a valve control box (Fig. 2). Computer software written in the QBASIC (Microsoft Corporation, Redmond, WA) programming language, running on an IBM compatible computer, operates the solenoid activated valves in a serial sequence. The valve control box is operated by a 24-bit digital I/O card PO-12 (Keithley Metrabyte, Tauton, MA) installed in the computer. Every 2.5 min, the computer displays and stores gas measurements from an individual metabolic calorimeter, then switches the valves. The gas analyzer transmits the data via an RS-232 serial port interface to the computer. Thus, the exhaust air from each metabolic calorimeter is directed to the gas analyzers once per hour.

System performance

The objective of this research project was to develop a metabolic calorimeter system that could indirectly monitor embryonic metabolism from 24 calorimeters every hour, with minimal embryo disturbance throughout the three-week period of embryo incubation. An experiment was carried out to test the design specifications of the metabolic calorimeter system to indirectly measure egg heat production. This experiment tested the hypothesis that eggs stored 15 days versus eggs stored for 4 days prior to incubation reduces broiler embryonic metabolic rate. Fertile eggs were stored in a cooling room for either 4 days (50 eggs) or 15 days (50 eggs) prior to incubation. The experiment was replicated, in which ten eggs from each storage treatment were placed randomly in the metabolic calorimeters. At 3-4 days of incubation, all eggs inside the individual metabolic calorimeters were candled to establish if they contained viable embryos. If a viable embryo was not present, the egg was replaced with an alternate viable egg from the same storage treatment group incubated simultaneously in an adjacent incubator.

The units of the CO_2 readings were μmol/mol or parts per million (ppm). The water vapour (H_2O) data from this experiment were not used.

The CO_2 production rate for each egg was determined as:

$$CO_2\ (mL/min) = \frac{Airflow\,(mL/min) * (CO_2\ exhaust - CO_s\ inlet)\ ppm}{10^6} \quad (2)$$

The production rate of CO_2 was expressed as mL/min and the airflow throughout each metabolic calorimeter was 300 mL/min. The ambient or inlet CO_2 concentration was based on the CO_2 concentration of the air exhausted from the empty calorimeters. This value was subtracted from the measured CO_2 concentration of the exhaust air from the calorimeter containing an embryo. The empty first and 13th calorimeter were used to provide inlet concentration data. The CO_2 concentration of the incubator space was found to be stable over a 30-min period.

Since an oxygen analyzer was not available, the oxygen consumption was calculated by dividing the CO_2 production by the assumed respiratory quotient value of 0.84 (Eq. 1). The embryo heat production is calculated by Eq. 3 (Kleiber 1987).

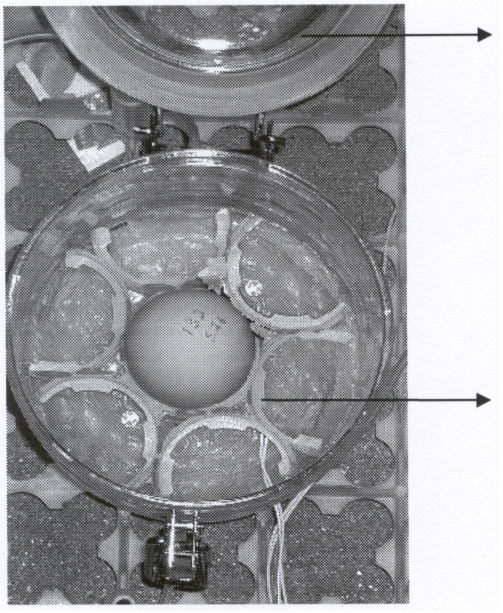

Fig. 3. Egg inside of a metabolic calorimeter.

$$Heat\ production\ (mW) = 187.6A + 57.86B \quad (3)$$

where:

A = calculated embryo oxygen uptake (L/d) and
B = embryo carbon dioxide output (L/d).

The air exhausted from each calorimeter was monitored for CO_2 once every hour over an 18-day period. The experiment ended on day 18. The experiment was approved by the Faculty of Agriculture, Forestry and Home Economics Animal Policy and Welfare Committee at the University of Alberta, in accordance with the principles and guidelines set out by the Canadian Council on Animal Care (CCAC 1993).

Statistical analysis

The daily CO_2 production data were analyzed using the mixed procedure of SAS (SAS Institute 2001). The experimental treatments (eggs stored 15 days versus eggs stored for 4 days prior to incubation) were considered fixed effects and time was considered a random effect. To account for the heterogeneous variation in CO_2 production with time, with day as the random effect and calorimeter as subject, a heterogeneous first order autoregressive [ARH (1)] covariance structure was used. There was not a significant trial effect, so data were pooled. Significant treatment differences in CO_2 production were determined using pairwise differences. Differences were reported as significant at the $P<0.05$ level.

RESULTS and DISCUSSION

The effects of treatment and day were significant as well as the interaction between treatment and day ($P<0.05$). Embryos stored 4 days before incubation produced (118.2 mL CO_2/d) significantly more ($P<0.05$) than embryos stored 15 days before incubation (108.7 mL CO_2/d) over the 18-day period

Carbon dioxide production dramatically increased over time ($P<0.05$), reflecting a fast embryo growth rate. The treatment x day interaction ($P<0.05$) reflected a difference in CO_2 production during the first 9 days of incubation, after which no

Table 1. Daily average embryo CO_2 production for two pre-storage treatments.

	Eggs pre-stored 4 days		Eggs pre-stored 15 days	
Day	mL/d	CV[1](%)	mL/d	CV[1](%)
1	3.9a	3.6	2.7b	5.2
2	2.4a	9.2	1.7b	11.2
3	3.5a	5.7	2.9b	5.2
4	7.8a	8.1	6.4a	6.1
5	14.2a	2.8	12.2b	4.1
6	18.6a	3.3	16.8b	2.9
7	19.4a	5.9	16.4b	5.9
8	27.8a	2.3	25.3b	2.0
9	36.5a	4.4	32.7b	2.2
10	43.2	7.0	37.5	4.9
11	64.4	6.2	58.1	3.0
12	90.9	6.3	80.8	2.9
13	129.9	4.2	117.1	3.2
14	190.2	6.1	170.1	3.0
15	231.2	6.5	203.7	5.1
16	353.6	3.6	326.3	2.4
17	424.4	2.7	396.1	2.1
18	465.3	1.9	449.9	2.2
Average		5.0		4.1

[1] Coefficient of variation

a,b Means within rows with no common letter are significantly different ($P<0.05$).

difference was detected. Embryos that were stored 4 days prior to incubation had significantly higher average CO_2 production than eggs that were stored for 15 days (Table 1). After day 11, the CO_2 production began to increase sharply until day 16 where the slope decreased prior to hatching, at which point the egg shell physically inhibits embryo growth (Fig. 4). This observation was consistent among the eggs for both storage durations and for the heat production curves provided (Rahn 1979; Rumanoff 1967) as shown in Fig. 4. Carbon dioxide production for broiler embryos ranged from 3 to 480 mL/d over an 18-day period. From the CO_2 data and an assumed RQ value of 0.84, the calculated heat production values are plotted in Fig. 5 (Eq. 2). The heat production from the embryos ranged from 1 to 137 mW over an 18-day period.

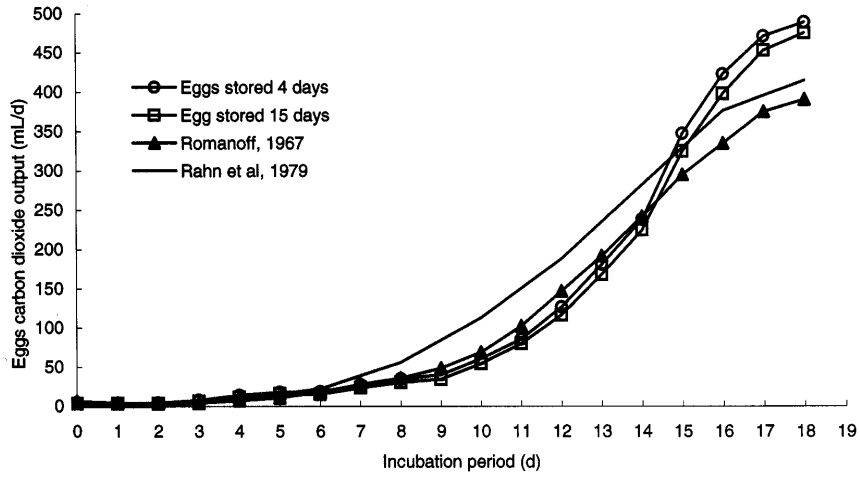

Fig. 4. Daily embryonic CO_2 production during incubation for eggs stored for 4 or 15 days before incubation (same broiler breeder flock) and embryo CO_2 production reported by Romanoff (1967) and Rahn et al. (1979).

The calorimeter system operated as designed. As shown in Table 1, the reliability of the system is expressed as a coefficient of variation (percentage). The mean percentage values were 5.0 and 4.1 for the 4 and 15 days before incubation, respectively (Table 1). The need to calibrate airflow rates and the CO_2 analyzer was minimal (once per week). Embryo mortality was similar to that occurring in a commercial incubator. Embryo mortality during incubation was 5% for eggs stored 4 days and 2.5% for eggs stored 15 days before incubation. Fasenko et al. (2001) reported embryo mortality values of 6.2 and 11.3% for eggs stored 4 and 14 days before incubation, respectively. The metabolic calorimeter system is currently used to study domestic avian embryonic metabolism (O'Dea et al. 2004).

CONCLUSIONS

1. The metabolic calorimeter system designed to measure the CO_2 production of embryos during incubation performed within the expected ranges reported in the literature.
2. The system was able to reliably detect the CO_2 concentration from individual embryos once an hour over an 18-day period.
3. The overall coefficient of variation for the calorimeter system was 4.5%. This suggests that this is a reliable methodology for measuring carbon dioxide production from avian embryos.

ACKNOWLEDGEMENT

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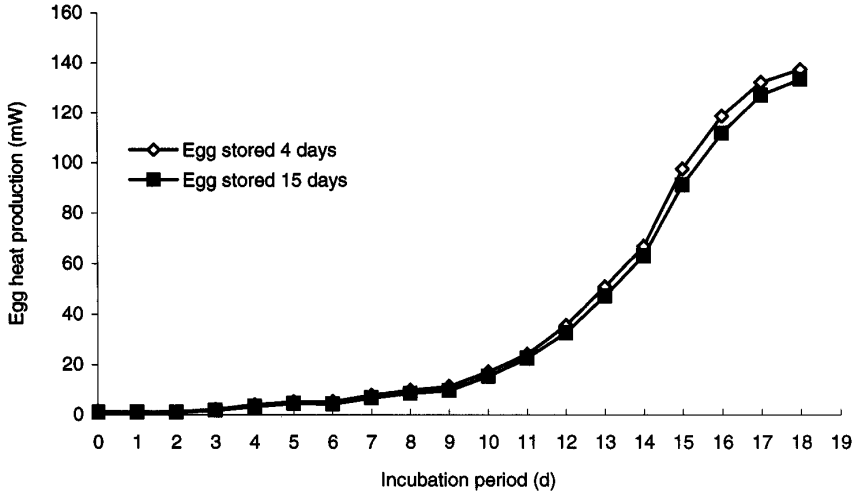

Fig. 5. Calculated heat production of incubated embryos stored for 4 and 15 days prior to incubation (Eq. 3).

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