Measurement of avian embryo respiration by an indirect calorimetric system

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Hamidu, J.A., J.J.R. Feddes, C.A. Ouellette and G.M. Fasenko. 2010. **Measurement of avian embryo respiration by an indirect calorimetric system**. Canadian Biosystems Engineering/Le génie des biosystèmes au Canada. 52: 4.9–4.14. Accurate measurement of embryonic respiration can be used to determine the viability of the chicken embryo and the chick upon hatching. This measurement is based on both the rate of oxygen (O_2) consumption and carbon dioxide (CO_2) production. The respiratory quotient (RQ), which is the ratio of the CO_2 production to O_2 consumption, indicates the energy source being metabolized. The metabolism of embryos under different environmental conditions can be evaluated from heat production and RQ values. In this study, an embryo indirect calorimeter was evaluated for accuracy and reliability for use over an entire incubation period. The method of evaluation was a simulated control respiration source using the combustion of butane drawn into the system at three different flow rates. The difference between the measured RQ values (mean = 0.64) and the theoretical RQ value of butane value (0.62) was of interest (3% > than theoretical). Results from the butane combustion study indicated that the current calorimetry system designed for measurement of gas exchange rates in avian species can be reliably used to monitor O_2 and CO_2 exchange rates, system flow rates and RQ. **Keywords:** avian embryo, CO_2 analyzer, differential O_2 analyzer, respiratory quotient.

La mesure précise de respiration embryonnaire peut être utilisée pour déterminer la viabilité de l'embryon de poulet et le poussin sur la hachure. Cette mesure est basée tant sur le taux de la consommation d'oxygène (O_2) que sur la production de dioxyde de carbone (CO_2). Le quotient respiratoire (QR), qui est la proportion de la production CO_2 à la consommation O_2, indique la source d'énergie étant métabolisée. Le métabolisme d'embryons dans des conditions environnementales différentes peut être évalué de la production de chaleur et des valeurs de QR. Dans cette étude, un calorimètre indirect d'embryon a été évalué pour l'exactitude et la fiabilité pour l'utilisation sur une période d'incubation entière. La méthode d'évaluation était une source de respiration de contrôle simulée utilisant la combustion de butane entraîné le système à trois débits différents. La proximité des valeurs de QR mesurées (avec la valeur moyenne de 0.64) avec la valeur de QR théorique de valeur de butane (0.62) avait d'intérêt (3% > que théorique). Les resultats de l'étude de combustion de butane a indiqué que le système actuel de calorimétrie conçu pour la mesure de taux de change du gaz dans l'espèce aviaire peut être utilisé de façon fiable pour contrôler les taux de change de O_2 et CO_2, des débits de système et QR. **Mots clés:** embryon aviaire, analyseur de CO_2, analyseur différentiel de O_2, quotient respiratoire.

INTRODUCTION

Accurate measurement of embryonic metabolism and respiratory quotient (RQ) of birds has been a subject of interest spanning over decades (Pearson et al. 2002). Indirect calorimetry utilizes the oxygen (O_2) consumption and the carbon dioxide (CO_2) production values to calculate heat production and RQ (Kleiber 1987; Hamidu et al. 2007). The RQ, which is the ratio of CO_2 production to O_2 consumption (McClave et al. 2003), indicates the nutrients (carbohydrates, proteins or lipids) being metabolized (Gefen and Ar 2001). The avian egg and the embryo developing within represent a closed system in which the entire supply of nutrients, including vitamins and minerals necessary to support complete embryonic development, is deposited by the hen into the egg at the time of its formation (Richards 1996). Although the avian egg has been described as a closed system in other studies (Berg et al. 1999) it can exchange gases with its environment. However, the embryo occupies a defined environment where there is little outside environmental influence on the embryo's respiration. The eggshell dictates the rate of exchange of gases between the egg and its environment (Rahn et al. 1979). It is very important that any experimental apparatus that is used to study the energetics of the embryo does not have a negative effect on the embryo.

A number of researchers have attempted to measure the process of gaseous exchange in avian embryos, along with the accuracy of the methods (Monge et al. 2000; Mortola and Labbe 2005; Christensen et al. 2007). In most of the methods, only a single egg was monitored at a time, and the number of measurements that could be obtained during the experimental period was limited (Sato et al. 2006). Also, some of the methods could not monitor the embryo up until the end of incubation (Carey et al. 1989). Because some of the methods involved the handling of eggs throughout the experiment, accurate measurements of the O_2 consumption and CO_2 production rates were difficult to report. In addition, measurements were also tedious as the researchers had to constantly observe the operation of the respiration equipment (Vleck and Kenagy 1980). In the last decade, indirect calorimetry has been used to continuously measure embryo CO_2 production during the incubation period. With this method, embryonic heat production was calculated from the measured CO_2 production and reported RQ values (Vleck et al.

1980; O'Dea et al. 2004; Segura et al. 2006). From the CO_2 production values and RQ values, the O_2 consumption values were calculated (O'Dea et al. 2004). A method developed by Segura et al. (2006) involved incubating chicken eggs individually in 24 1-L metabolic chambers inside an existing incubator. Each chamber was sampled for 2.5 min every 1 h for CO_2 production.

Sato et al. (2006) measured a chicken embryo's O_2 consumption and CO_2 production but could only do so after day 12 of incubation because concentrations of these gases are very small during the early periods of incubation. They also reported an RQ value of 0.71 over the incubation period studied. Because the RQ of lipids is 0.7 (Krogh and Lindhard 1920) the RQ value reported by Sato et al. (2006) suggested that chicken embryos were metabolizing almost exclusively lipids after 12 days of incubation. Hamidu et al. (2007) reported different RQ values during a 21-d incubation period of chicken eggs. They reported that the RQ of chicken embryos during the first 10 days was approximately 1.00 suggesting that the embryos were metabolizing carbohydrate substrates. Afterwards, the RQ value began to decrease; by day 21 the RQ value was 0.63, which suggested that the embryo was actively involved in lipid metabolism. In order to accurately obtain RQ data, both the O_2 and CO_2 concentrations must be measured frequently over the incubation period. The objective of this study was to determine the accuracy of a calorimetric system over the entire incubation period of an avian embryo. This included the performance of the gas monitoring equipment and the airflow rate measuring devices. The hypothesis of the study was that the RQ values obtained from this system would not be significantly different from the theoretical RQ value of a burning butane source at three metabolic chamber air flow rates.

MATERIALS and METHODS

Embryo metabolism system

The details of the current metabolism system have been previously described (Hamidu et al. 2007). An indirect calorimetric system for incubating avian embryos as described by O'Dea et al. (2004) and Segura et al. (2006) was modified in this study with the addition of a differential O_2 analyzer (DOX) (Qubit Systems Inc., Kingston, ON, Canada). To ensure that the CO_2 (LI-6262 CO_2/H_2O analyzer, LI-COR, Lincoln, NE, USA) and the differential O_2 analyzers were providing accurate measurements, butane, which has a theoretical RQ value of 0.62 (Nunn et al. 1989), was used as a source of combustion to simulate metabolic respiration of an avian embryo (Fig. 1). The differential O_2 and CO_2 concentrations were measured between inlet air to the chamber from the commercial incubator and the exhaust air from each of the 24 metabolic chambers (Fig. 2). Air flow through each of the 24 metabolic chambers was maintained by individual airflow meters (Fig. 1; Fig. 2). Each flow meter was calibrated every 24 h with a standardized external flow meter (Dry Cal® DC Lite-ML, Bois International Corporation, Butler, NJ, USA). Each flow meter was connected to a solenoid-activated valve (Fig. 2) (ASCO Valve Canada, Brantford, ON, Canada). The valves were

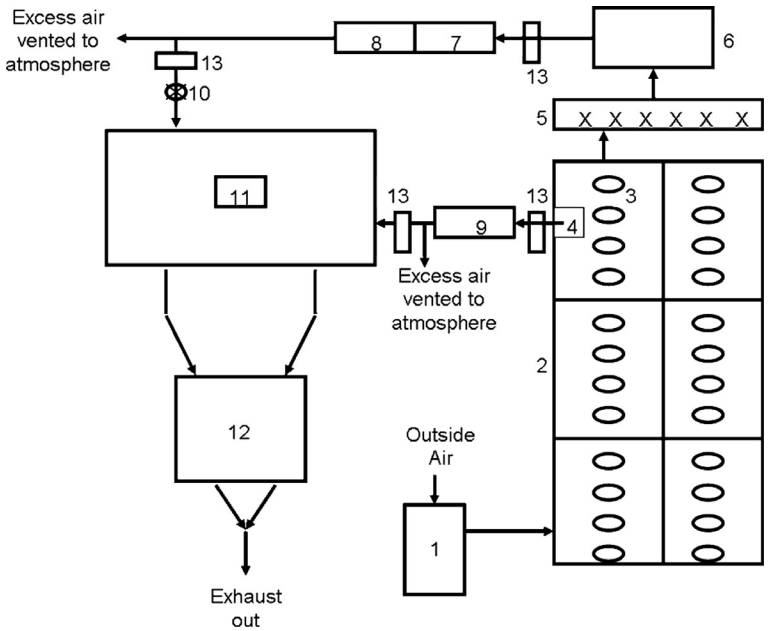

Fig. 1. The avian embryonic metabolism system use to measure embryonic oxygen consumption and carbon dioxide production. The avian embryo metabolism system of: (1) premixing air chamber, (2) incubator, (3) metabolic chambers, (4) reference air from incubator, (5) air sampling valves, (6) carbon dioxide analyzer, (7) sampling air pump, (8) sampling air pump controller, (9) reference air pump, (10) needle valve to balance differential pressure between sample (S) and reference (R) O_2 sensors, (11) differential oxygen analyzer, (12) dual flow pumps draw air through the DOX at a rate of 25 mL/min, (13) moisture absorbing magnesium perchlorate.

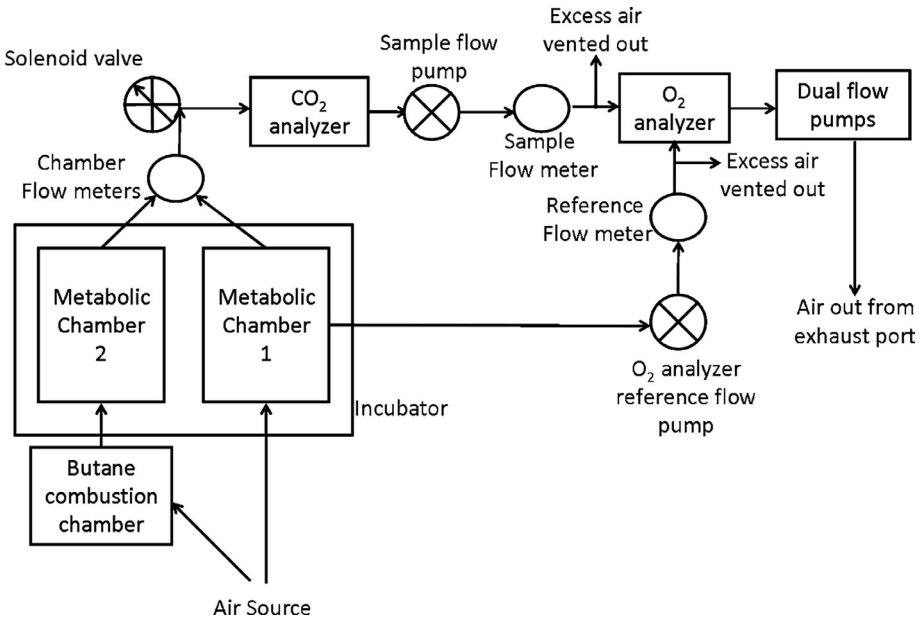

Fig. 2. Schematic diagram representing the calorimetric system using butane combustion gases to validate the accuracy of O_2 and CO_2 analyzers and chamber flowmeters.

operated by computer software to facilitate sequential sampling of the metabolic chambers for gas analysis. An open solenoid valve allowed the air sample to be directed to the CO_2 analyzer and the DOX. When the valve was closed the airflow from the chamber was directed to the incubator exhaust port. The DOX was set up in a differential mode such that the O_2 concentration was based on the electronic difference between O_2 partial pressures between the sample [$(PO_2)_S$, kPa] and a reference [$(PO_2)_R$, kPa] sensor and the prevailing atmospheric pressure (Atm, kPa), as shown in Eq. 1 below. The differential O_2 pressure (kPa) was expressed as parts per million (ppm).

$$\text{Diff } O_2 \text{ (ppm)} = \frac{(PO_2)_S - (PO_2)_R}{\text{Atm}} \times 10^6 \qquad (1)$$

The CO_2 production rate was based on the flow rate and the difference in CO_2 concentration between the sampling chamber and the incubator due to the embryo respiration. The incubator CO_2 and O_2 concentrations were assumed to be the same as the exhaust concentration from an empty metabolic chamber and the incoming concentrations to each metabolic chamber.

Two of the 24 metabolic chambers were used to test the reliability of the CO_2 analyzer and the DOX (Fig. 2). The air sampled from chamber 1 was drawn from an available air source and used as the reference air, while, the sample air from chamber 2 was drawn from a butane combustion chamber located outside the incubator. The air for combustion was provided by the same air source to maintain the butane flame. Both the reference air from chamber 1 and butane sampling air from chamber 2 were drawn at flow rates ranging from 75 to 300 mL/min that were typical to that required for an avian embryo over a 21-day incubation period.

Over a 5-min period, the air was sampled from chamber 1 at a flow rate of 75, 100 or 300 mL/min. The volume of the tubing from each metabolic chamber to the solenoid valve before the air is directed to the analyzers was approximately 5.54 mL (Fig. 2). At these three airflow rates (75, 100 and 300 mL/min), the sample air reached the analyzers in approximately 4, 3 and 1 s, respectively. As the metabolic chambers were being sampled, the DOX reference sensor sampled the reference O_2 air from the incubator at the same flow rates, respectively (Fig. 2). As shown in Fig. 1 and 2, the sample air was directed through the CO_2 analyzer, however, only 25 mL/min was sampled by the DOX sample and reference sensors. The remaining air was vented away to atmosphere (Fig. 2). The pressure differential of air between the sample and the reference sensors of the DOX was adjusted to be negligible. The O_2 and CO_2 readings were automatically averaged during the last 10 s of the 5-min period for each metabolic chamber.

$$\text{Gas exchange rate } (\mu\text{L/h}) = \frac{O_2 \text{ or } CO_2 \text{ concentration (ppm)} \times \text{flow rate } (\mu\text{L/h})}{10^6} \qquad (2)$$

A butane torch was used to burn gas at a level that produced a good analyzer response at airflow rates of either 75, 100 or 300 mL/through the metabolic chambers. The combustion air was drawn from the combustion chamber into metabolic chamber 2. As shown in Fig. 2, the sample air from metabolic chamber 1 was considered to be the background concentrations prior to combustion. The chamber flow rates were used to calculate the O_2 consumption and the CO_2 production rates (μL/h) based on the O_2 and CO_2 differential concentrations (Eq. 2). The

accuracy of the flow rates calibrated on the flow meters was ± 5 mL/min.

The RQ was calculated as the ratio of the CO_2 production rate to O_2 consumption rate. These values were computed and compared to the theoretical RQ of butane (RQ = 0.62) and analyzed by the generalized linear model of SAS ($P \leq 0.05$) (SAS 2003).

RESULTS and DISCUSSION

The combustion of butane is the following: $C_4H_{10} + 6.5\ O_2 = 4\ CO_2 + 5\ H_2O$; 1 mol of butane is equivalent to 6.5 mol of O_2 and the RQ is 0.62 (Nunn et al. 1989). The mean RQ using the current calorimetric system was 0.64 ± 0.004 (Table 1). The calculated RQ was 3% higher than the theoretical RQ of butane and the standard deviation was 0.019 ($n = 30$). However, there was a small but significant difference (0.06) in RQ ($P < 0.05$) between the theoretical and the stoichiometrical calculations (RQ = 0.64). Though the standard error and the standard deviation of the mean were very small, the actual mean difference between the theoretical and current value was also small. Since the two RQ values were only 3% different from each other, the performance of the calorimetry system is acceptable. These results are consistent with those of a similar study where butane combustion resulted in RQ measurement 5% lower than the theoretical RQ value of butane (Forsberg et al. 1986). The minimum RQ value measured (0.62) and the maximum RQ value (0.68) differed by 0.06. While the real difference is small, the standard deviation and standard errors were also very small. Table 1 also shows that the real time flow rate measurements were within the expected values of 75, 100 and 300 mL/min (± 5 mL/min). The metabolism system (Fig. 1) can thus be used to measure accurate O_2 and CO_2 concentrations (or O_2 consumption and CO_2 production rates) during avian embryo respiration.

Accurate O_2 consumption and CO_2 production rates data that rely on reported RQ have been a subject of concern, especially when small exchange rates occur during the first 7 days in the development of a chicken

Table 1. Respiratory quotient result from individual butane combustion measurements.

ID	Flow rate* (mL/min)	CO_2 production rate** (μL/h)	O_2 consumption rate** (μlLh)	Respiratory quotient†
1	73	5920	8720	0.68
2	74	5880	8790	0.67
3	75	5910	8740	0.68
4	75	5750	8980	0.64
5	76	5770	8880	0.65
6	76	5820	9010	0.65
7	95	5670	8900	0.64
8	95	5720	8960	0.64
9	95	5670	9200	0.62
10	95	5760	8840	0.65
11	95	5720	9030	0.63
12	95	5740	9040	0.64
13	95	5810	8560	0.68
14	95	5710	9110	0.63
15	96	5730	9050	0.63
16	96	5700	9240	0.62
17	96	5700	9240	0.62
18	96	5810	8700	0.67
19	96	5800	8700	0.67
20	96	5780	8950	0.65
21	96	5830	8790	0.66
22	96	5790	9100	0.64
23	96	5740	9230	0.62
24	96	5740	9240	0.62
25	298	5780	9120	0.63
26	299	5770	9190	0.63
27	295	5860	8850	0.66
28	301	5890	8900	0.66
29	296	5840	8970	0.65
30	300	5850	8900	0.66

*Flow rate monitored at three different levels (75, 100 and 300 mL/min).
**Gas exchange rate = O_2 or CO_2 concentration × sample air flow rate/10^6.
†Respiratory quotient = CO_2 exchange rate/O_2 exchange rate.
P value < 0.05 (statistically, theoretical value of butane is lower than measured value).

embryo (Hamidu et al. 2007). Our ability to measure different concentrations of O_2 and CO_2 during butane combustion, which resulted in consistent gas exchange rates have given enough confidence in the use of the calorimetry system to measure avian O_2 consumption and CO_2 production during the first week of incubation. The data confirm that the results obtained reported in previous studies using this system are reliable (Hamidu et al. 2007). The consumption and production data presented in Table 1 were similar to those observed in previous experiments. In our current study, the O_2 exchange rates varied between 8720 and 9240 μL/h, whereas, CO_2 exchange rate values ranged between 5700 and 5920 μL/h. In turkey eggs, embryonic CO_2 exchange reaches between 5000 and 7000 μL/h by day 15 of incubation and reached over 43 000 μL/h by 28 days of incubation when the embryos hatch (Hamidu, J.A. 2009, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB. Personal Communication). In the same manner, embryonic O_2 exchange rate peaked at about 8000μL/h in 15 days and reached over 60 000 μL/h by 28 days of incubation, whereas, in chicken embryos, similar embryonic CO_2 ($\sim$6000 μL/d) and O_2 ($\sim$8000 μL/d) exchange rates were reached at day 12 of incubation. By 21 days of incubation, when the embryos were hatching, CO_2 and O_2 exchange rates reached about 34 000 μL/h and 50 000 μL/h, respectively (Hamidu et al. 2007).

In recent years, emphasis has been placed on measurements of CO_2 production in the estimation of embryonic heat production and RQ (O'Dea et al. 2004; Segura et al. 2006). One reason is the availability of improved instrumentation, with current CO_2 analyzers being approximately 100 times more sensitive than typical O_2 analyzers (Walsberg and Wolf 1995). However, estimating metabolic energy production from CO_2 measurement alone may lead to substantially larger errors than those obtained from measurements of both O_2 consumption and CO_2 production. This could be due to a number of assumptions, including the substrate that an embryo is metabolizing as well as the RQ assumed to calculate O_2 consumption from CO_2 production data (Walsberg and Wolf 1995). As such the validation of the current calorimetric system, which has an O_2 analyzer that has an accuracy varying within $\pm$10 ppm will produce reliable gas exchange and heat production data.

SUMMARY and CONCLUSIONS

Calibration of the CO_2 and O_2 analyzers and the flow meters is essential to develop confidence in measuring embryo gas exchange and energy production. In this study, butane combustion was found to simulate the respiration of an organism inside a metabolic chamber with O_2 and CO_2 exchange rates that were typical of avian embryo CO_2 production and O_2 consumption rates. By validating the performance of the CO_2 and O_2 analyzers with butane (theoretical RQ value of 0.62) it has been confirmed that the current avian calorimetric system as previously used, measures O_2 and CO_2 concentrations reliably. The calculated RQ value (0.64) was 3% higher than the theoretical RQ value of butane (0.62). We conclude that O_2 consumption and CO_2 production rates or exchange rates, and flow rates measured in our previous and ongoing studies with chicken and turkey embryos were measured with sufficient accuracy.

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