Gas exchange of the ostrich embryo during peak metabolism in relation to incubator design

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Abstract

Oxygen (O_2) consumption and carbon dioxide (CO_2) excretion of ostrich embryos were studied on 45 ostrich eggs in various stages of development. A closed respirometry system was used for eggs subjected to ≤ 10 days of incubation, while an open flow system was used for older eggs. A total of 102 measurements were made and repeat measurements on the same egg were treated as independent during statistical analysis. The O_2 consumption and CO_2 excretion of ostrich embryos increased exponentially during the first 70% of incubation, reaching a maximum between day 31 and 38 of incubation. During peak metabolism about 180 ml/h of O_2 was consumed and 120 ml/h carbon dioxide (CO_2) was excreted. This stage was followed by a decline in metabolic rate to approximately 75 % of the peak value. The gas exchange of ostrich eggs incubated in this study at 36 °C was compared with studies where incubation temperatures of 35, 35.5 and 36.3 °C were used. Although the time of hatch differed between these studies (41, 44.6 and 47 days) in contrast to the 42 day incubation period in the present study, the general trends in O_2 consumption and CO_2 excretion were broadly similar, although there were slight differences in the plateau phases. From the data on O_2 consumption and CO_2 excretion during peak embryo metabolism (*ca.* 32 to 37 days of incubation), it was calculated that an airflow of 54.2 l/egg hour^{-1} is needed to prevent a decline in O_2 levels to below 21% and an increase in CO_2 concentration to levels exceeding 0.3% in single stage incubators. This airflow is less than that required for chicken eggs incubated in the same single stage incubator. Results of this study enable incubator operators incubating ostrich eggs to adjust ventilation rates to accommodate embryonic age and metabolism and to avoid costly heat loss because of excessive ventilation.

Keywords: Artificial incubation, carbon dioxide, ostrich eggs, oxygen
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Introduction

Oxygen (O_2) consumption of domestic chicken embryos (*Gallus gallus*) was described by Wangensteen & Rahn (1970). Similar data are available for many other avian species (Lewis *et al*., 1965; Hoyt *et al*., 1979; Rahn, 1981; Burton & Tullett, 1983; Meir *et al*., 1984). It is known that O_2 consumption may differ between altricial and precocial birds (Vleck *et al*., 1979), while O_2 consumption patterns may also vary with egg size and incubation period (Wangensteen *et al*., 1970). Studies on the O_2 consumption of ratite embryos suggest different patterns with age (Hoytt *et al*., 1978). Eggs of the common rhea (*Rhea americana*) and the emu (*Dromiceius novaehollandiae*) are approximately the same size, but the two species differ significantly in incubation period and egg-shell conductance (Hoyt *et al*., 1978; Vleck *et al*., 1980; Meir & Ar, 1990). Decuypre *et al*. (1979) demonstrated that the metabolic rate of chicken embryos increases when exposed to four different temperatures between 35.9 and 38.8 °C. The same phenomenon was observed in studies with ostrich eggs (Hoyt *et al*., 1978; Meir & Ar, 1990; Reiner & Dzapo, 1995) where temperatures of 35 °C, 35.5 °C and 36.3 °C were used, respectively. Incubation periods varied considerably between these studies (41, 44.6 and 47 days, respectively) as opposed to the typical 40 to 44 days with an average of 42 days under natural conditions (Sauer & Sauer, 1966; Leuthold, 1970; Swart, 1988). These temperatures differ from the 36 °C incubation temperature used in the present study, which is typical for artificially incubated ostrich eggs in South Africa (Van Schalkwyk, 1998).

Proper air circulation in an incubator is important to provide an even temperature distribution throughout the incubator environment. Ventilation maintains a high O_2 concentration and a low carbon dioxide (CO_2) concentration, in spite of the O_2 uptake and CO_2 production of developing embryos. It also prevents accumulation of water vapour and limits undesirable increases in incubator temperature as a result of the metabolic heat production of the embryos.

Optimum gaseous conditions for the incubation of chicken eggs have been discussed in early studies (Romanoff, 1930; Barott, 1937; Wilgus & Sadler, 1954), for example, Riddle (1924) suggested that young chicken embryos are sensitive to high O_2 concentrations while older embryos are more sensitive to lower O_2

concentrations. Chicken embryos exhibited an increased tolerance to higher CO_2 concentrations during the second half of incubation (Wilgus & Sadler, 1954). However, little is known about optimum gaseous conditions for ostrich eggs; in part because information on ostrich embryonic metabolism has only recently become available.

The aim of this study was to establish if O_2 uptake and CO_2 excretion patterns on the average commercial incubation temperature of 36 °C would match those obtained in previous studies (Hoyt *et al*., 1978; Meir & Ar, 1990; Reiner & Dzapo, 1995). Because little attention has been paid to proper incubator ventilation in the past, guidelines based on the findings of this study are presented to assist in optimising incubator operation for ostrich eggs.

Materials and methods

Eggs were collected from the experimental ostrich flock at the Klein Karoo Agricultural Development Centre (ADC) near Oudtshoorn. Details regarding the management of breeding stock and the collection and incubation of eggs were reported by Van Schalkwyk *et al*. (1996; 2000). Eggs used for this investigation were collected early in the morning, fumigated with formalin, and stored at 17 °C and 75% relative humidity. Eggs were set weekly in a Buckeye® electronic incubator, which was set to turn eggs hourly through an angle of 60°. A wooden incubator, of the type described by Van Schalkwyk *et al*. (1999) and used by 70% of ostrich farmers in the Klein Karoo region, was set up at Rhodes University in Grahamstown. Eggs from the Klein Karoo ADC were transferred at various stages of development less than 27 days in cool boxes maintained at a constant temperature of 25 °C to the incubator in Grahamstown where they were incubated at 36 °C and a RH of 28%. Eggs measured between 14 and 27 days were candled at 14 days, as described by Van Schalkwyk *et al*. (2000), to ensure that eggs developing normally, were used. Turning in the wooden incubators was done manually, twice daily, at 08:00 and 16:00. Eggs measured during the first 14 day of incubation were taken as fresh eggs and incubated on site.

Oxygen consumption (VO_2) and CO_2 excretion (VCO_2) of embryos less than 10 days old were measured in a closed respirometry system at 36 °C and VO_2 and VCO_2 of older embryos were measured using an open-flow respirometry system.

In the closed respirometry system, each egg was weighed and its volume measured by water displacement before being placed inside a gas-tight plastic chamber of predetermined volume. Chamber lids contained an inlet with a stopcock valve, and an outlet with a 3-way valve. Chambers were maintained at a constant temperature in the incubator.

After being placed in the chambers, egg temperatures were allowed to restabilise to incubator temperature for at least 15 min. before being sealed. A 60 ml gas-tight syringe, with its plunger drawn back to the 50 ml mark, was attached to the 3-way outlet valve of the chamber. After the initial 15 min. the chambers were sealed and the inlet stopcock closed. Eggs were left in the respirometer for a period that depended on their age (30 min. for eggs at 42 days of developmental age to 180 min. for eggs at five days of developmental age). The syringe was then pumped several times to ensure thorough mixing of the air in it with that in the respirometer. A 50 ml gas sample was subsequently drawn up. The 3-way valve was then sealed and the syringe and valve disconnected from the outlet. Barometric pressure was measured after each run.

The gas sample was analysed for O_2 and CO_2 as described by Lighton (1991). Baseline (atmospheric) air was pulled through plastic tubing connected to the outside of the building past an injection port (3-way valve) and a gas analysis system by an Applied Electrochemistry R1 flow controller. The air-stream passed through a silica gel tube to remove water vapour before entering an Applied Electrochemistry Model CD-3A CO_2 analyser. The air was subsequently scrubbed of both water and CO_2 by a carbosorb/silica gel tube before entering an Applied Electrochemistry Model S-3A/1 O_2 analyser. The gas analysers were set assuming concentrations of O_2 of 20.95 % and CO_2 concentrations of 0.03% in the baseline air-stream. Flow rate through the system was measured using a bubble flow meter and converted to STPD [standard absolute temperature (°K); gas tension (mm Hg) and diffusion coefficient (cm^2/sec)], as described by Levy (1964) and Rahn (1981).

Oxygen and CO_2 concentrations of the samples were recorded directly onto a microcomputer using Datacan V data acquisition software (Sable Systems Inc., Salt Lake City). The syringe containing the gas sample was inserted into the injection port and the port valve opened. After recording a 30-point baseline, the syringe valve was opened and the gas sample injected as a bolus into the airflow. Percentages of O_2 and CO_2 were recorded at 1-second intervals until they returned to baseline levels. After running a second 30-point baseline, recording was terminated. The fractional volumes of O_2 depletion and CO_2 enrichment were calculated by integrating areas

under the curves, using equations 6 and 7, as described by Lighton (1991). These values were substituted into equations 1 and 2 below to calculate VO_2 and VCO_2.

$$VO_2 \text{ (ml/h)} = (VC-VE)/VS * (VI/t) * 60 \quad (1)$$

Where:

VC = respirometer chamber volume (cm^3), VE = egg volume (cm^3), VS = sample volume (cm^3), VI = fractional volume of O_2 depleted (cm^3) and t = time the egg was in the chamber (min.).

$$VCO_2 \text{ (ml/h)} = (VC-VE)/VS * (VI/t) * 60 \quad (2)$$

Where:

VI is the fractional volume of CO_2 produced (cm^3) and other variables are the same as above.

In the open-flow respirometry system, eggs were weighed to 0.01 g and placed in an airtight Perspex chamber (33x17x20 cm) in a constant temperature cabinet. The temperature in the chamber (T_a) was set at 36 °C and was monitored by a thermocouple inserted through a rubber stopper into the chamber. Dry, CO_2-free air was pumped through a mass flow meter (Aalborg GFM 1700) at a flow rate of 750-850 ml/min. before entering the chamber. Air leaving the chamber passed through a tube of silica gel, an Applied Electrochemistry CD-3A CO_2 analyser and another tube of carbosorb and silica gel before entering an Applied Electrochemistry S-3A/1 O_2 analyser. Eggs were left for approximately 30 min. prior to measurement of VO_2 and VCO_2. Flow rate, the percentages of CO_2 and O_2, and chamber temperature were then recorded every 20 seconds throughout the run on a computer using Datacan V data acquisition software. Each experimental run lasted 2 - 3 h. VO_2 was calculated for each run from the lowest stable period of metabolism without activity using equation 4a of Withers (1977).

The age of the embryo concerned was recorded for each measurement. At least two eggs were analysed per day of development. A total of 102 measurements on 45 eggs was recorded. Some eggs were sampled repeatedly at different stages of incubation, but no eggs were used for more than three measurements. For the purpose of this investigation, repeated measurements on the same egg were assumed to be independent. Oxygen consumption and CO_2 excretion were recorded for each measurement and the ratio of CO_2 produced to O_2 consumed (Respiratory Quotient) over the 42-day incubation period was calculated.

Standard non-linear regression techniques were used to describe the rate of change in O_2 consumption and CO_2 excretion over the first 31 days of incubation (see Figure 1). Maximum levels of O_2 consumption and CO_2 excretion were used to calculate airflow rates through incubators that would be sufficient not to impair embryonic growth. The non-linear regression of CO_2 concentration on airflow rate in an incubator was computed to determine the airflow rate required for the maintenance of CO_2 concentrations at acceptable levels.

Results

Oxygen consumption and CO_2 excretion increased exponentially over the first 31 days of incubation (Figure 1). Pooled data of 102 measurements on 45 eggs resulted in the following regression (± s.e.) for O_2 consumption:

$$VO_2 = 0.434 \pm 0.096e^{0.189 \pm 0.007t} \ (R^2 = 0.96)$$

Where:

VO_2 = O_2 consumption (ml/h), and t = day of incubation.

The corresponding equation for CO_2 excretion was:

$$VCO_2 = 0.225 \pm 0.52e^{0.198 \pm 0.008t} \ (R^2 = 0.97)$$

Where:

VCO_2 = CO_2 excretion (ml/h) and t = day of incubation.

This exponential increase was followed by a plateau phase, during which O_2 consumption and CO_2 excretion stabilised at approximately 180 and 120 ml/h respectively. This was followed by a suggestion of a reduction in O_2 consumption during the period from 38 to 40 days of incubation to 140 ml/h O_2 consumption. The last two days of incubation were characterised by a return of O_2 consumption to plateau level. Both O_2 consumption and CO_2 excretion were extremely variable on the 42nd day of incubation, presumably reflecting different levels of activity of embryos just prior to hatching.

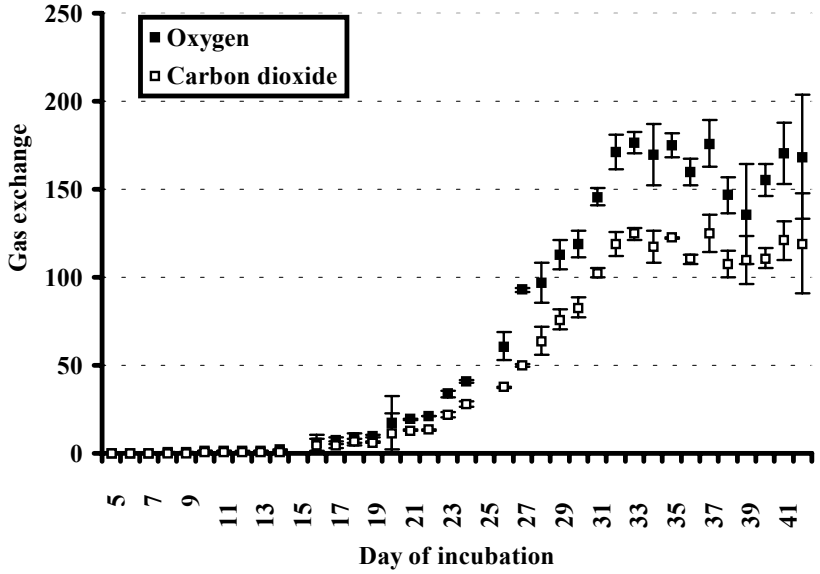

Figure 1 Oxygen consumption and carbon dioxide excretion of ostrich embryos over 42 days when incubated at 36 °C. Vertical lines above and below points represent standard deviations

The respiratory quotient (RQ) averaged 0.68 until day 7 of incubation, whereafter it declined to reach its lowest level (0.55) at 10 days of incubation (Figure 2). From day 10 it gradually increased until day 21, after which it averaged 0.72.

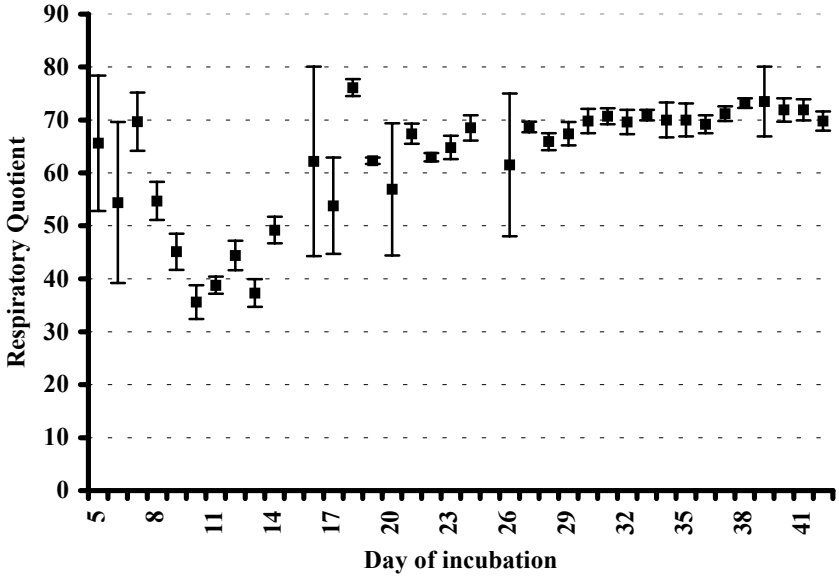

Figure 2 The respiratory quotient (RQ) of ostrich eggs incubated at 36 °C. Vertical lines above and below points represent standard deviations

Discussion and conclusions

In eggs of chickens and ducks, the rate of O_2 consumption increases nearly exponentially during the first 80% of incubation and remains relatively constant during the remaining plateau phase (Rahn *et al*., 1974). The plateau phase is followed by a rise in O_2 consumed before the pipping of the shell hours later. Visschedijk (1968) suggested that the rise at the end of the plateau phase occurs when the embryo penetrates the air shell. This pattern is typical of precocial species of birds (Vleck *et al*., 1979).

The rate of O_2 consumption for ostrich eggs also increased exponentially during the first part of incubation as was demonstrated by Hoyt *et al*. (1978) and Reiner & Dzapo (1995). In the present study, the steepest rate of incline of the non-linear curve was between days 26 and 31 of incubation. Studies by Reiner & Dzapo (1995) similarly found that increased embryonic metabolic rate starts at day 25 and lasted until day 36 with the sharpest rise of 30% per day prior to day 26. Hoyt *et al*. (1978) found that an increase in metabolic rate only started at day 30 and reached a peak at 41 days in ostrich eggs incubated at 35 °C. This delay is probably due to the lower incubation temperature employed. The steep exponential phase in the studies of Hoyt *et al*. (1978) and Reiner & Dzapo (1995) was followed by a decline of about 25% during the following four to six days. In our study, the steep exponential phase was also followed by a more definite plateau phase for the next six days until day 38 when O_2 consumption and CO_2 excretion decreased by about 22% and 10% to 140 and 110 ml/h respectively. The reason for the differences in the plateau phase may be related to the fact that the same egg was sampled in succession. A plateau phase followed by a decline was also observed in studies with rhea and emu embryos. Vleck *et al*. (1979) suggested that the decline in growth rate and O_2 consumption late in incubation may indicate that development is essentially complete several days prior to pipping, which enables synchrony of hatching. Finally, the rate of O_2 consumption increased just before pipping. In the present study this increase coincided with the 42nd day of incubation. In the studies of Hoyt *et al*. (1978) and Reiner & Dzapo (1995) it occurred after respectively 44 and 40 days of incubation. This increase in metabolic rate has been suggested to coincide with internal pipping and the beginning of the transition from chorio-allantoic to pulmonary respiration (Ar, 1996).

Slight differences in incubation temperatures in the different studies on ostrich embryonic metabolism appear to have relatively little effect on the overall pattern. It seems as if increased incubation temperatures lead to a shift of the curve to the left. Decreased temperatures led to a flattening of the steep increases in metabolism observed in the present study in the middle period of incubation and a delay in hatching.

The average RQ calculated from data in the present study was 0.72, which was slightly lower than the RQ of 0.79 measured by Meir & Ar (1990). The RQ of 0.72 is indicative of predominantly lipid catabolism and is typical of avian embryos (Meir & Ar, 1990). The reduction in RQ to low levels between seven and 10 days of incubation is unusual. To the best of our knowledge, this has not previously been reported for avian embryos. Such patterns have, however, been reported for animals entering torpor or hibernation as well as for poikilotherms during changes in body temperature. They are indicative of a period of CO_2 storage or change in acid-base status (Stinner & Wardle, 1988). The pattern in ostrich embryos is, however, not readily explicable and may warrant further investigation. The possibility that it may be related to the change in technique used to measure metabolism over this period also cannot be disregarded.

Bird nests in which eggs are incubated naturally are periodically ventilated. Under conditions of artificial incubation, however, ventilation conditions generally remain relatively constant and gas concentrations in the incubator may change as embryonic metabolism increases, especially in the wooden incubators that predominate in the Klein Karoo area (Van Schalkwyk *et al*., 1999). Ar (1996) suggested that the partial pressure of O_2 around the ostrich egg is important in the determination of hatchability. Oxygen (as well as CO_2 and water vapour) crosses the eggshell by diffusion (Rahn *et al*., 1974). The diffusion of gases is dependent on gas concentration gradients inside and outside the eggshell. As development proceeds the rising demand for O_2 and increasing production of CO_2 produce changes in the concentrations of the respiratory gases within the egg. Taylor *et al*. (1971) suggested that eggshells with a low conductance result in an accumulation of CO_2 causing hypercapnia and hypoxia. On the other hand, the excessive loss of CO_2 from the egg may lead to a loss of blood buffering capacity, which can change the acid base balance of the embryo. However, hypocapnia appears not to significantly affect the O_2 consumption, growth or hatchability of avian embryos. Landauer (1967) suggested that a good hatchability in chicken eggs can be obtained between concentrations of 18 and 50% O_2 in the incubator, while CO_2 concentrations should be maintained at below 0.5%. The maximum hatchability has been reported when O_2 concentrations were maintained at 21%.

Wilgus & Sadler (1954) observed the greatest hatchability in chicken eggs at CO_2 concentrations of 0.5%, and they suggested that CO_2 may stimulate embryonic development within certain limits. In contrast, Gildersleeve & Boeschen (1983) reported a better hatchability of turkey eggs when incubator CO_2 concentrations were not more than 0.3%. Romanoff (1930) and Barott (1937) found that CO_2 concentrations above 1% resulted in slow growth, a high incidence of abnormalities and early embryonic deaths in chickens.

Synergistic interactions between CO_2 and O_2 concentrations were found to be present in incubator air. When levels of CO_2 were high (> 0.5%) and the concentrations of O_2 in the incubator air were maintained at normal atmospheric levels of 21%, hatchability of fowl eggs was significantly improved (Wilgus & Sadler, 1954). Conversely, hatchability was reduced when CO_2 levels were high (> 0.5%) and O_2 levels (< 19%) were low. A

reduction in hatchability was observed also when both O_2 and CO_2 concentrations were high (Taylor *et al*., 1956; Taylor *et al*., 1971).

In ostriches the peak rates of O_2 consumption and CO_2 excretion during the plateau phase are unlikely to exceed approximately 200 ml/egg hour^{-1} and 150 ml/egg hour^{-1}, respectively. Assuming these maxima, and an O_2 concentration of approximately 21% in atmospheric air, incubator air quality could be calculated accordingly for a single egg (Figure 3). In order to maintain CO_2 concentrations below 0.3%, a function fitting the CO_2 curve was developed. The non-linear function that fitted the data best was the following hyperbola:

$$CO_2 \text{ concentration in incubator air (\%)} = 1/(0.046 + 0.0607 * \text{Flow}) \qquad (R^2 = 0.9993)$$

Where:

Flow is the airflow rate through the incubator in litres/h.

By substitution, it can be calculated from this equation that an ostrich embryo at peak metabolic rate requires an airflow of 54.15 litres/h. Ar (1996) suggested that the reduction in O_2 partial pressure in the incubator environment should not change by more than three Torr (0.4%) at sea level. The ventilation needed at various stages of incubation to maintain O_2 uptake for ostrich eggs amounts to about 47 litres/h per egg of 1.4 kg. This result is consistent with our estimates. Extrapolation of this result to the incubator situation indicates that at least 54200 litres of air should pass through a 1000 egg single-stage incubator per hour at times of peak embryonic metabolism (approximately 34 to 37 days of incubation). This airflow requirement may be reduced substantially if a multi-stage incubator is used, since all eggs are not at the same stage of embryonic development. Multi-stage incubators commonly accommodate approximately five batches of eggs set in subsequent weeks in the Klein Karoo area.

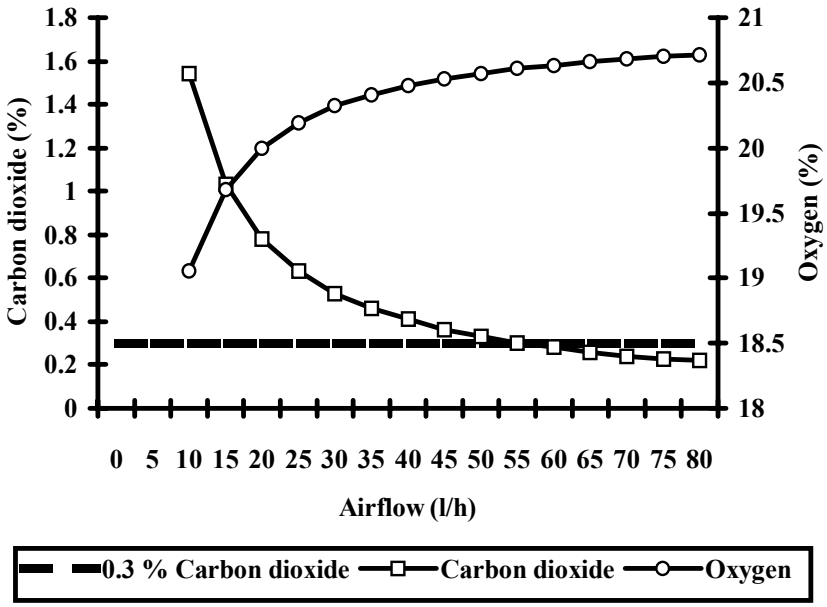

Figure 3 Oxygen and carbon dioxide concentrations in a single stage incubator for the determination of optimal ventilation rates

Most electronic incubators presently used for ostrich egg incubation are chicken incubators with converted trolleys. A standard commercial Buckeye® incubator for 19000 chicken eggs accommodates only 1000 ostrich eggs when converted. The ventilation rate required for maximum removal of CO_2 for chicken eggs is approximately 3400 litre/1000 sixty gram eggs over 21 days (Owen, 1991). Therefore, the incubator ventilation rate needed for optimal hatchability of 1000 ostrich eggs is slightly lower (54200 *vs*. 64600 litre/h) than the same incubator filled with chicken eggs. No adjustment is normally made to reduce ventilation rate when ostrich eggs are incubated in incubators designed for chicken eggs, which may result in the loss of costly heat energy because of overventilation (Ar, 1996). Poor embryonic development can be prevented by simply either altering the fan speed or by the opening of the buffers to purge excess CO_2 (Owen, 1991). Our results present incubator operators with a means to regulate ventilation during times of low embryonic metabolism in such a way that CO_2

can be purged on a daily basis, while heat loss can be reduced at the same time.

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