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Comparative Biochemistry and Physiology Part A 130 (2001) 689–699

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Gas exchange and energy metabolism of the ostrich (*Struthio camelus*) embryo

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Received 6 December 2000; accepted 3 July 2001

Abstract

We measured oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide emission ($\dot{V}_{CO_2}$) rates, air-cell gas partial pressures of oxygen (P_AO_2) and CO_2 (P_ACO_2), eggshell water vapour conductance and energy content of the ostrich (*Struthio camelus*) egg, 'true hatchling' and residual yolk, and calculated RQ and total oxygen consumption ($\dot{V}_{O_2tot}$) for ostrich eggs incubated at 36.5°C and 25% relative humidity. The $\dot{V}_{O_2}$ pattern showed a drop of approximately 5% before internal pipping. $\dot{V}_{O_2}$ just prior to internal pipping agrees with allometric calculations. Despite the higher incubation temperature compared to other studies, and the resultant shorter incubation duration (42 days), $\dot{V}_{O_2tot}$ (91.7 l kg^{-1}) was similar to a previously reported value. RQ values during the second half of incubation (approx. 0.68) were lower than expected for lipid catabolism. Prior to internal pipping, P_AO_2 and P_ACO_2 were 98 and 48.3 torr (13.1 and 6.4 kPa), respectively. The growth pattern of the ostrich embryo is different from the typical precocial pattern, showing a time delay in the rapid growth phase. As a result, the lowered overall energy expenditure for tissue maintenance, as compared to other species, is reflected in the low yolk utilization and high residual yolk fraction of the whole hatchling dry mass. These could also result from the relatively short incubation period of the ostrich egg, thereby evading desiccation by excess water loss.

Keywords: Ostrich; Ratites; Incubation; Embryonic development; Energy metabolism; Gas exchange; Eggshell conductance; Incubation

1. Introduction

The ostrich (*Struthio camelus*), a member of the Ratitae, is the largest living bird, weighing up to 150 kg and reaching a height of 2.75 m. The average mass of the ostrich egg, 1461 ± 163 g (Ar et al., 1996), makes it the largest egg among existing avian species. Natural incubation duration averages 42 days (Deeming, 1997).

Human interest in ostrich products dates back to 3000 BC, but its farming developed rapidly in South Africa only in the 19th century (Laufer, 1926). Ostrich farming has spread worldwide recently, and the growing interest in the ostrich as a farmed bird has led to more extensive studies of its biology (Deeming, 1996; Huchzermeyer, 1998). Nevertheless, the embryonic development, which is a major aspect of both ostrich biology and successful ostrich farming, is poorly studied (Deeming, 1997).

The ontogeny of the avian embryonic oxygen consumption rate ($\dot{V}_{O_2}$) differs between embryos of the different maturity types (Vleck et al., 1979). It was suggested that the typical pattern of altri-

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1095-6433/01/$ - see front matter
PII: S1095-6433(01)00401-9

cial embryonic $\dot{V}_{O_2}$ is characterized by a continuous exponential increase with time, while that of precocials shows a 'plateau' stage in $\dot{V}_{O_2}$ after approximately 80–85% of the incubation (Vleck and Vleck, 1987). Other authors have suggested that the difference between maturity types is not the absence of the 'plateau' stage in altricial embryos, but rather its duration (Prinzinger and Dietz, 1995; Prinzinger et al., 1995). Ratites, however, show a unique embryonic $\dot{V}_{O_2}$ vs. time pattern, with a decrease of up to 25% between the maximal recorded value (peak $\dot{V}_{O_2}$) and the one recorded prior to internal pipping ($_{Pre\text{-}IP}\dot{V}_{O_2}$) (Hoyt et al., 1978; Vleck et al., 1980; Meir and Ar, 1990; Reiner and Dzapo, 1995). This resembles the pattern seen in some reptile eggs (Thompson, 1989), which was shown to vary with incubation temperature (Leshem et al., 1991).

The need to further investigate the ostrich embryo energy metabolism arises as a result of the better understanding of the meaning of optimal incubation temperature, which may differ from actual egg temperature. Initially, incubation temperatures for the ostrich were determined using measurements of naturally incubated eggs (Swart and Rahn, 1988). Since then, it has been appreciated that in nature there is a temperature gradient within the egg, a different situation from that experienced by artificially incubated eggs (Turner, 1991). Eventually, common hatchery practice has shown that incubator air temperature of approximately 36.5°C results in favourable hatchability in artificial incubation of ostrich eggs. Hence, this temperature may also represent an averaged natural incubation temperature, as it results in similar incubation duration to those seen in natural nests (Ar, 1996; Deeming, 1997).

The objective of this study is to describe the ontogeny of the ostrich embryonic $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$, at what are the currently accepted as optimal incubation conditions of incubation temperature and egg water loss (Ar et al., 1996). The study includes observations on the embryo's growth pattern, since this is directly linked to the embryonic gas exchange pattern.

2. Materials and methods

Seventy-four fertile ostrich (*S.c. var. domesticus*) eggs were obtained from the commercial hatchery of Zemach Ostriches Ltd. in Kibbutz Ha'on, Israel. Thirty-four eggs were used for egg gas exchange measurements, which were carried out in the hatchery. The use of multi-stage incubators (eggs are set once a week) allowed us to sample eggs of various ages on the same measuring day. Incubation conditions there were approximately 36.5°C and relative humidity (RH) of approximately 25%. Eggs were placed in the incubator trays horizontally for the first 2 weeks, and then vertically with their air cell up for the remainder of the incubation (Smith et al., 1995). The eggs were turned automatically once an hour to $\pm 45°$.

An additional 40 fresh fertile eggs were incubated at the Tel Aviv University, and used for measurements that required killing the embryos at various stages for determination of embryonic dry mass and morphological description (Gefen and Ar, 2001). Upon arrival, eggs were weighed to the nearest 0.01 g (Sartorius, Model 1518 MP8), and placed in forced draught incubators (Victoria, V-34) set to similar incubation conditions as in the commercial hatchery (36.5 ± 0.1°C; 25 ± 2% RH; 1 turn/h). Eggs incubated for more than 14 days were weighed again in order to calculate the specific water vapour conductance of the shell ($_{sp}GH_2O$) as described by Meir and Ar (1987). Eggs with $_{sp}GH_2O > 130$ mg kg^{-1} torr^{-1} day^{-1} were transferred to a higher humidity incubator ($RH = 40 \pm 2\%$) for the rest of the incubation in order to maintain normal incubation water loss.

2.1. Egg gas exchange

Eggs were stored for 1–7 days at 16°C before being set in the incubators. Calculations of water loss based on measured $_{sp}GH_2O$, together with known storage and incubation humidities and durations, allowed an estimation of the eggs' initial mass.

The gas exchange of each egg was measured 4–6 times as follows: on days 8–13 (daily measurements of 2 eggs); 14–20 (4); 21–26 (4); 27–31, 32–37 and 38–41 (8 eggs in each group) of the incubation. Two embryos from the 32–37 day age group failed to hatch, and their measured values were discarded. Measurements on day 41 were conducted on embryos that had not externally pipped. The measuring scheme did not allow us to track individual eggs throughout incubation. However, it better represents the egg population's gas exchange.

Gas exchange measurements on days 21–41 were carried out in an 'open' system. Individual eggs were placed in 16.5-cm diameter pyrex desiccators. The total volume of the desiccators was approximately 2.75 l, which resulted in a space of approximately 1.25 l between the inserted egg and the desiccator's inner walls. The desiccators were placed in incubators (Curfew electric observation incubator) set to 36.5°C. Air temperature cycle outside the desiccator, due to the thermostat insensitivity, was 5–10 min in duration and 0.8°C in amplitude. Together with an egg cooling constant of 1.01×10^{-4} °C °C^{-1} s^{-1} (Meir and Ar, 1990), this resulted in egg temperature fluctuations of less than ± 0.025°C.

Dry air flow through the desiccators was regulated by calibrated flow meters (Brooks, model 1357) according to the age and the expected metabolic needs of the embryos. These were estimated from previous measurements (Hoyt et al., 1978; Meir and Ar, 1990). Thus, the oxygen concentration decrease in the desiccators' atmosphere did not exceed 0.5%, corresponding to the permissible 0.4% CO_2 concentration increase in commercial incubators. The air flowing through the desiccator first passed through a spiral copper tube placed in the incubator, in order to avoid temperature differences between the desiccator and the incubator. Water vapour was removed from the excurrent air by passing it through a silica gel column, before reaching the O_2 and CO_2 analyzers (S-3A, Ametek; and 1400B, Servomex, respectively — both accurate to $\pm 0.01\%$). Instruments were calibrated, each at two points, with certified gas mixtures using dry, CO_2 free air and a mixture of 0.91% CO_2; 19.50% O_2 balanced by N_2. Data were recorded using PC-LabCard application software (Model PCLS-702).

The $\dot{V}_{O_2}$ measured in the 'open' system was corrected for changes in concentration due to RQ effect using the following equation (after Hill, 1972):

$$ {}_{sp}\dot{V}_{O_2} = \dot{V}_I \cdot M^{-1} \cdot \left[F_IO_2 - F_EO_2 \cdot \left((1 - F_IO_2 - F_ICO_2) \cdot (1 - F_EO_2 - F_ECO_2)^{-1} \right) \right] \quad (1) $$

where:

- ${}_{sp}\dot{V}_{O_2}$ = Specific oxygen consumption rate ($ml_{[STPD]}$ kg^{-1} h^{-1}).
- $\dot{V}_I$ = Inlet air flow rate ($ml_{[STPD]}$ h^{-1}).
- F_IO_2 = Oxygen fraction in dry inflowing air.
- F_EO_2 = Oxygen fraction in dry outflowing air.
- F_ICO_2 = Carbon dioxide fraction in dry inflowing air
- F_ECO_2 = Carbon dioxide fraction in dry outflowing air.
- M = Initial egg mass (kg).

The specific carbon dioxide emission (${}_{sp}\dot{V}_{CO_2}$, in $ml_{[STPD]}$ kg^{-1} h^{-1}) were similarly corrected using the following equation:

$$ {}_{sp}\dot{V}_{CO_2} = \dot{V}_I \cdot M^{-1} \cdot \left[(F_ECO_2 \cdot (1 - F_IO_2 - F_ICO_2) \cdot \left((1 - F_EO_2 - F_ECO_2)^{-1} \right) - F_ICO_2 \right] \quad (2) $$

The $\dot{V}_{O_2}$ of each egg was measured daily, while $\dot{V}_{CO_2}$ was measured every second day. These values allowed calculation of the RQ values of the developing embryo during the course of incubation.

Respiratory measurements carried out on young eggs required a long time to reach steady state conditions, due to the low flow rates. Therefore, eggs incubated for 8–20 days were measured in a 'closed' system. The eggs were placed in desiccators, which were then flushed with dry air at 1 l min^{-1} before being sealed for a given period of time which allowed an oxygen concentration decrease of up to 0.5%. Subsequently, the inlets and outlets were opened, and the gaseous environment within the desiccator was washed out at a known flow rate, dried using silica gel, and analyzed for O_2 and CO_2 concentrations. The amounts of O_2 and CO_2 exchanged were determined by integrating the area below/above the concentration vs. time curves, for which the initial and final dry air values served as a baseline. During the washout, the oxygen concentration decrease in the outflow, due to embryonic oxygen consumption, never exceeded 0.1% of the oxygen fraction in the inflow, and therefore was ignored. The $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were determined by dividing the integrated values, corrected to standard conditions, by the total time of desiccator closure.

2.2. Air cell gas partial pressures

Air cell gas partial pressures of O_2 (P_AO_2) and CO_2 (P_ACO_2) were measured on eggs incubated for 26–40 days ($n = 14$), using the air cell equilibrium method (Tazawa et al., 1980). A single measurement was carried out on each egg before opening the eggs for morphological description of the embryo (Gefen and Ar, 2001). A 4-mm hole was drilled in the shell above the air cell, and a sawn needle hub was glued around it with epoxy glue. This constructed syringe adapter was sealed with a plastic plug until the day before measurement, when a plastic syringe with a shortened tip, containing 20-ml room air, was attached to it. During the following day the contents of the syringe reached equilibrium with the air cell atmosphere (Meir and Ar, 1990). Then the contents were injected into a precalibrated blood gas analyzer (BMS Mk2, Radiometer). From the P_AO_2 values obtained, together with $\dot{V}_{O_2}$ measured as described above, the shell's O_2 conductance was calculated using the following equation:

$$ {}_{sp}G_{O_2} = {}_{sp}\dot{V}_{O_2} \cdot (P_IO_2 - P_AO_2)^{-1} \quad (3) $$

where: ${}_{sp}G_{O_2}$ = specific eggshell O_2 conductance (mg kg^{-1} $torr^{-1}$ day^{-1}), and P_IO_2 = ambient air oxygen partial pressure (torr). P_IO_2 was calculated as 20.95% of the dry ambient air pressure.

2.3. Mass and energy content of the egg, embryo and hatchling

The initial mass (± 0.01 g) of six infertile eggs was determined after injecting distilled water to their air cells, thus accounting for evaporative water loss. The egg contents were then removed, homogenized and dried at 60°C to a constant mass. Three pellets of dry, homogenized contents from each egg were used to determine their energy content by bomb calorimetry (ballistic bomb calorimeter, CB-370. Gallenkamp, calibrated with benzoic acid pellets). Results were used to calculate energy value of the initial egg contents.

Fourteen embryos of different ages were weighed and homogenized. Samples of the homogenates were weighed and dried at 60°C to a constant mass. Embryonic growth rates were calculated between day 24 and 40 of incubation every second day by dividing the difference in average dry mass of a preceding (e.g. day 30) and the succeeding developmental day of sampling (e.g. day 34) by the time interval between them (4 days). The value was assigned to the in-between sampling day (e.g. day 32).

Six hatchlings were killed immediately after hatching in the commercial hatchery, for measurements of hatchling and residual yolk mass. The residual yolks and 'true hatchlings' (hatchlings with their residual yolk removed) were homogenized, dried, and weighed to the nearest 0.01 g as above. Triplicates of the residual yolks were used for calorimetry, as above. Representative samples for determination of energy content of the hatchlings could not be obtained due to technical problems.

2.4. Statistical analysis

STATISTICA for WINDOWS version 5.0 software (STATSOFT® Ltd.) was used for the statistical analyses throughout. *t*-Tests were carried out after confirming that the relevant variables, including ratios, were normally distributed.

3. Results

The mean initial egg mass of eggs measured for gas exchange was 1440.6 ± 157.5 g (mean $\pm$ S.D.; $n = 34$), and their daily water loss during incubation was 0.27–0.36% of initial egg mass, which resulted in a calculated diffusive mass loss of 11.3–15.1% for a full incubation course (42 days). The average initial mass of eggs incubated in our laboratory was 1498 ± 124 g ($n = 40$). Their mean eggshell specific diffusive conductance for water vapour was 118.6 ± 23.0 mg kg^{-1} $torr^{-1}$ day^{-1}. This indicates that the eggs used for this study were within the normal range for mass and water loss of ostrich egg (Ar et al., 1996).

3.1. Egg gas exchange

The ${}_{sp}\dot{V}_{O_2}$ and ${}_{sp}\dot{V}_{CO_2}$ increased continuously until reaching maximum values (peak $\dot{V}_{O_2}$) on days 34–35 (Figs. 1 and 4, Table 1), which were followed by a 4.7% decline in ${}_{sp}\dot{V}_{O_2}$ until just prior to the internal pipping (${}_{Pre\text{-}IP}\dot{V}_{O_2}$). After the initiation of internal pipping, ${}_{sp}\dot{V}_{O_2}$ and ${}_{sp}\dot{V}_{CO_2}$ rose again, and by days 40–41 these values exceeded previous peak values (Fig. 1).

Table 1
Mass-specific O_2 consumption and CO_2 emission rates of the ostrich egg, incubated at 36.5°C

	Period	
	'Peak'	Pre-IP
Stage	Days 34–35	Day 38
Egg mass (g)	1387 ± 217	1487 ± 155
${}_{sp}\dot{V}_{O_2}$ ± S.D. ($ml_{[STPD]}\ kg^{-1}\ h^{-1}$)	164.2 ± 11.4 ($n = 6$)	156.5 ± 16.3 ($n = 7$)
${}_{sp}\dot{V}_{CO_2}$ ± S.D. ($ml_{[STPD]}\ kg^{-1}\ h^{-1}$)	111.6 ± 5.3 ($n = 3$)	102.3 ± 17.5 ($n = 4$)

The total amount of O_2 consumed throughout incubation ($\dot{V}_{O_2 tot}$) was 91.7 l, which is 63.7 l O_2 per kg initial egg mass (${}_{sp}\dot{V}_{O_2 tot}$). This value results from interpolation and summation of the measurements carried out during days 8–41. The $\dot{V}_{O_2}$ during the first 7 days of incubation was considered to be insignificant for this summation (Fig. 1). The rates of CO_2 emission (Fig. 1) allowed calculation of apparent RQ values throughout the incubation (Fig. 2).

RQ values declined continuously from day 8 (RQ > 1) and throughout the first half of the incubation period, with the lowest values recorded between days 22–28 (RQ = 0.65 ± 0.01). Slightly higher RQ values were observed for the remainder of the incubation, with the mean ± S.D. RQ for days 30–41 being 0.68 ± 0.02 (Fig. 2).

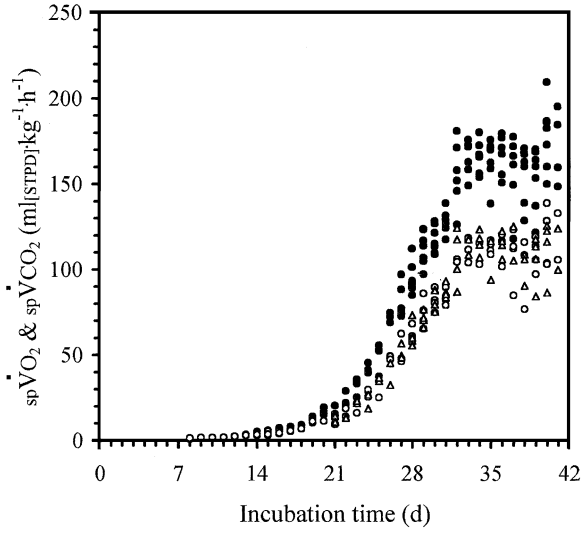

Fig. 1. Rates of mass-specific oxygen consumption (${}_{sp}\dot{V}_{O_2}$- ●) and carbon dioxide production (${}_{sp}\dot{V}_{CO_2}$- ○ indicates measured values, and Δ indicates values calculated from $\dot{V}_{O_2}$ and daily average RQ) as a function of incubation time in ostrich eggs incubated and measured at 36.5°C.

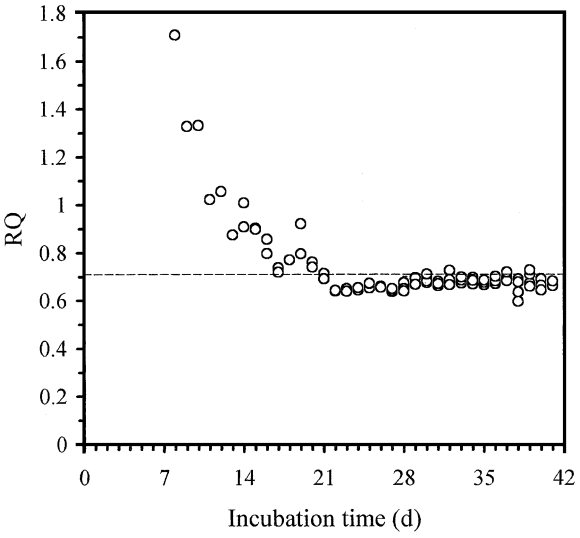

Fig. 2. Respiratory quotient (RQ) values as a function of incubation time in the ostrich egg. Values were calculated from measured $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$. The dashed line indicates RQ value of 0.71, as expected for lipid catabolism.

3.2. Air cell gas partial pressures

Between days 26 and 38, measured P_AO_2 showed a continuous decline, with a concurrent rise in P_ACO_2 (Fig. 3). The lowest P_AO_2 and highest P_ACO_2 values, 98 and 48.3 torr (13.1 and 6.4 kPa, $n = 2$), respectively, were recorded on day 38, just prior to internal pipping, and were followed by a noticeable rise in P_AO_2 and a decline in P_ACO_2 on day 40 (Fig. 3).

3.3. Mass and energy content of the egg and hatchling

The mean mass of the six infertile eggs used for analysis of their contents was 1261 ± 122 g. The eggshell mass was 271 ± 23 g (21.4 ± 0.6% of initial egg mass). Water consisted 77.5% of the fresh egg contents, and energy content of the dry mass was 30.19 ± 0.64 kJ g^{-1}.

The five eggs used for hatchling and residual yolk analysis had an initial mass of 1491 ± 206 g. Mean whole hatchling mass was 976 ± 96 g (65.5% of initial egg mass). The residual yolk fresh mass was 286 ± 54 g (dry mass 137 ± 26 g), and the 'true hatchling' mass was 690 ± 109 g (dry mass 109 ± 7 g). The energy content of the dry residual yolk was 32.15 ± 0.87 kJ g^{-1}.

From measurements of embryonic dry mass,

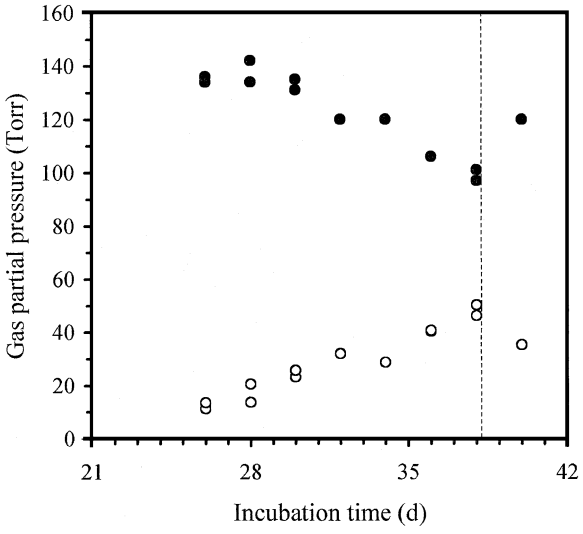

Fig. 3. Oxygen (●) and carbon dioxide (○) partial pressures in the ostrich egg air cell as a function of incubation time. A single measurement per egg was made ($n = 14$). Measurements on Day 38 were carried out before the embryos had internally pipped. The dashed line indicates internal pipping time.

embryonic growth rates for the second half of incubation were calculated. Growth rates increased until day 34, and again on day 40.

4. Discussion

4.1. Egg gas exchange

The $_{sp}\dot{V}_{O_2}$ vs. time curve obtained in our study (Fig. 1) has a similar shape to that described in previous studies (Fig. 4; Hoyt et al., 1978; Meir and Ar, 1990; Reiner and Dzapo, 1995). In general, the first 80% of the incubation period is characterized by an exponential rise almost until peak $\dot{V}_{O_2}$ is reached. This is followed by a drop in $\dot{V}_{O_2}$ values until just prior to internal pipping ($_{Pre\text{-}IP}\dot{V}_{O_2}$), after which $\dot{V}_{O_2}$ increases again.

However, the higher incubation temperature used in this study resulted in a shorter incubation period, and thus a shift to the left in the $_{sp}\dot{V}_{O_2}$ vs. time curve was observed (Fig. 4). Reiner and Dzapo (1995) report $\dot{V}_{O_2}$ values, which were measured in a 'closed' system at incubation temperature of 36.3°C. The reduced $\dot{V}_{O_2}$ values they report for the last 14 days of incubation (Fig. 4) may result from their measuring method, which allowed an average oxygen decrease of 10% from the initial oxygen content, without absorbing the CO_2 emitted by the eggs.

The peak $\dot{V}_{O_2}$ value was not significantly different from the one reported by Hoyt et al. (1978) (t-test; $P = 0.477$), but $_{Pre\text{-}IP}\dot{V}_{O_2}$ was significantly higher than the value reported by the same authors ($P = 0.006$), Meir and Ar (1990) and Reiner and Dzapo (1995). Unlike these reported values, $_{Pre\text{-}IP}\dot{V}_{O_2}$ measured in this study did not differ significantly (t-test, $P = 0.071$) from the value predicted by allometry (Hoyt and Rahn, 1980), for an egg of 1487 g and incubation period of 42 days (170 $ml_{[STPD]}\ kg^{-1}\ h^{-1}$). The $_{sp}\dot{V}_{O_2}$ values recorded in this study show a 5% decrease following peak $\dot{V}_{O_2}$. This is a moderate drop compared with those of up to 25% reported previously (Hoyt et al., 1978). It should be noted that a certain decrease in $\dot{V}_{O_2}$ after reaching peak $\dot{V}_{O_2}$ has been reported for other avian species, including the domestic fowl (Romijn and Lokhorst, 1960; Prinzinger et al., 1995). As Hoyt et al. (1978) have predicted, this $_{sp}\dot{V}_{O_2}$ decrease is concurrent with the apparent drop in the embryonic growth rate (Table 2; Fig. 5 ostrich data).

The $\dot{V}_{O_2}$ decrease was thought to be part of a mechanism that enables hatching synchronization in both reptiles and avian species (Cannon et al., 1986; Thompson, 1989), but a previous study has

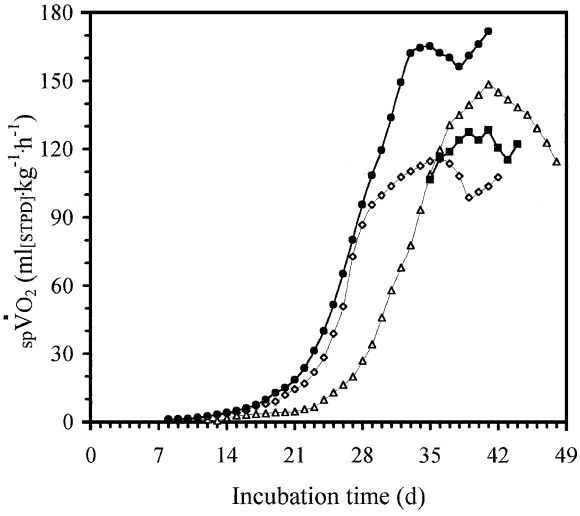

Fig. 4. A comparison of daily average $_{sp}\dot{V}_{O_2}$ values of ostrich eggs during embryonic development at different incubation temperatures, as reported by different authors. This study — 36.5°C, open measuring system (●); (Hoyt et al., 1978) — 35.0°C, closed system (Δ); (Meir and Ar, 1990) — 35.5°C, closed system with CO_2 absorption (■); (Reiner and Dzapo, 1995) — 36.3°C, closed system without CO_2 absorption (◇).

Table 2
Average dry mass, calculated growth rates (g day^{-1}) and percent of final dry mass for ostrich embryos incubated in 36.5°C for 24–40 days

Incubation (day)	Growth rate (g day^{-1})	Dry mass (g)	Relative mass (% of hatchling)	*n*
22		0.31	0.3	1
24	1.78	2.85	3	1
28	1.87	10.96	10	1
30	6.38	14.07	13	2
32	6.92	36.49	33	2
34	7.60	41.74	38	3
36	5.99	66.90	61	2
38	7.77	65.71	60	2
40	10.82	97.98	90	1
Hatchling (42)		109	100	5

For growth rate calculations details see Section 2.

Table 3
Comparison between ostrich and chicken eggs: V_{O_2tot} and $_{sp}\dot{V}_{O_2tot}$ values calculated for initial egg mass and egg content mass

	Ostrich[a]	Ostrich	Chicken
Fresh egg mass (kg)	1.440	1.450[e]	0.058[b]
Shell fraction (%)	21.4	18.1	10.7
Egg content mass (kg)	1.132	1.188[d]	0.0518[b]
Amount of O_2 consumed			
$l_{[STPD]}$	91.7	87.9[e]	4.62[c]
$l_{[STPD]}$ $kg\ egg^{-1}$	63.7	60.6	79.7
$l_{[STPD]}$ $kg\ contents^{-1}$	81.0	74.0	89.2

[a] Data from: This study.
[b] Romanoff and Romanoff, 1949.
[c] Romanoff, 1967.
[d] Ar et al., 1987.
[e] Vleck and Vleck, 1987.

failed to show that such synchronization occurs in ostrich eggs (Bertram, 1992).

The relationship between incubation duration and incubation temperature in ostriches is hyperbolic (Ar et al., 1996). However, the total amount of O_2 consumed during the entire incubation period per kg fresh egg mass ($_{sp}\dot{V}_{O_2tot}$) was 63.7 l kg^{-1}, similar to the reported value of 65 l kg^{-1} (Hoyt and Rahn, 1980), despite the difference in incubation temperatures and duration. The above $_{sp}\dot{V}_{O_2tot}$ of ostrich eggs is significantly lower ($P <$ 0.001) than the average for 33 avian species, which is 103.0 ± 19.8 l kg^{-1} (Hoyt and Rahn, 1980). However, the magnitude of the difference could be misleading, as it could be influenced by the eggshell fraction of the initial egg mass, which does not participate in the energy metabolism (Table 3). In order to eliminate the influence of eggshell mass, $_{sp}\dot{V}_{O_2tot}$ was calculated as the amount of O_2 consumed per mass unit of fresh egg contents. The eggshell fraction of total egg mass was calculated in this study using eggs that were relatively small (see Section 3), and therefore the following calculation is based on previously reported values (Table 3). We find, using literature values (Ar et al., 1987; Vleck and Vleck, 1987), a difference of approximately 20% between $_{sp}\dot{V}_{O_2tot}$ of the ostrich and chicken embryos compared to 31% when calculation is based on initial egg mass. Such interspecific comparison is valid despite the difference in egg masses, since the slope of log $_{sp}\dot{V}_{O_2tot}$ on log M is not significantly different from zero ($P = 0.25$, $n = 34$; data from Hoyt and Rahn, 1980). This indicates that $_{sp}\dot{V}_{O_2tot}$ in bird eggs is independent of egg mass.

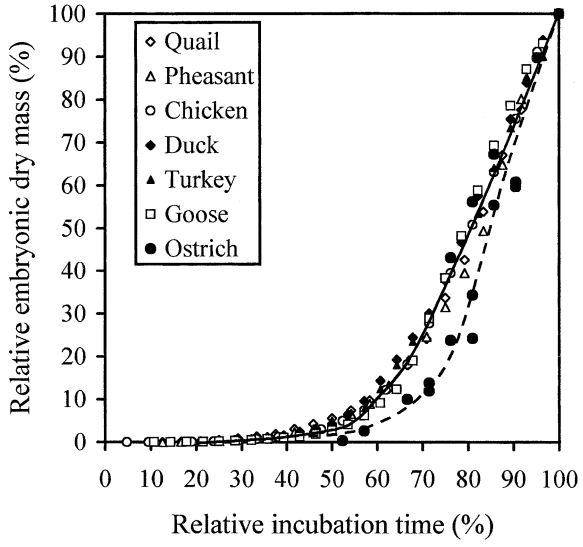

Fig. 5. Relative embryonic dry mass (% of the 'true hatchling' dry mass) of the ostrich and different domesticated precocial species, as a function of relative incubation time. Curves for the ostrich and chicken are fitted by eye. Values for the six precocial species are calculated after Romanoff (1967).

The relative growth pattern (% mass gain/% incubation time) of the ostrich embryo is unique compared to other precocial domesticated species (Fig. 5). The stage of rapid embryonic mass gain in the ostrich is delayed in relative terms. This delay is compensated for later in development, when the ostrich embryo's relative growth far exceeds that of the other species (Fig. 5). The difference in relative embryonic dry mass is most distinct between 50 and 80% of the incubation

period. The area under the curves represents the relative total amount of tissues, which have to be energetically maintained throughout the incubation. A comparison between the ostrich embryo and the other species in Fig. 5 shows that the integrated daily relative dry mass of the ostrich throughout incubation is approximately 20% lower than that of the other species. This value corresponds to the difference in ${}_{sp}\dot{V}_{O_2 tot}$ between the ostrich and the chicken, based on their egg content mass (Table 3). The unique growth pattern of the ostrich embryo (Fig. 5) corresponds also to the approximately 20% lower than expected total cost of development per unit of 'true hatchling' dry mass (calculated from Vleck and Vleck, 1996). Therefore, the difference in the amount of oxygen consumed throughout the embryonic development seems to be a result of the relatively lower tissue mass and thus decreased energy expenditure by the ostrich embryo for maintenance. Our method of averaging daily growth rates tends to smooth out the actual growth rates, and therefore these values should be addressed with caution. However, calculated growth rates for incubation days 34–38, based on 10 embryos, may suggest at least a certain pause in the increasing growth rate (Table 2), which correlates with the 'plateau' in ${}_{sp}\dot{V}_{O_2}$ (Fig. 1).

The high RQ values (>1) early in the incubation period (Fig. 2) cannot be explained by catabolism, and may result from emission of CO_2 initially stored in the fresh egg (Mueller, 1958). Subsequent values (7–21 days) of $1 > RQ > 0.7$ may also be explained by catabolism of combinations of proteins, carbohydrates and lipids. During the second half of the incubation period the apparent RQ value is just under 0.7. RQ of approximately 0.7 is the expected value based on lipid catabolism (Romanoff, 1967). Nevertheless, during days 22–28, RQ values averaged 0.65, which is significantly lower than 0.7 ($n = 16$, $P < 0.001$). We could not quantitatively account for these low values, which have also been reported previously for other avian species (Barott, 1937; Ancel and Visschedijk, 1993). Factors such as soluble CO_2 accumulation in egg contents, carbonate fraction in bones, changes in unsaturated fatty acid contents and the difference between the molar volumes of CO_2 and O_2 may all contribute to the low RQ values.

Table 4
The ostrich eggshell gas conductance (G_{gas}) for oxygen and water vapour ($n = 9$)

	Mean	S.D.
GO_2 ($ml_{[STPD]}\ day^{-1}\ torr^{-1}$)	169	55
GH_2O ($ml_{[STPD]}\ day^{-1}\ torr^{-1}$)	204	30
GO_2/GH_2O	0.82	0.2
$DO_2, N_2/DH_2O, N_2$	0.85[a]	

GH_2O has been converted from mg to $ml_{[STPD]}$ according to Paganelli et al. (1978).
[a] Paganelli et al. (1978).

4.2. Air cell gas partial pressures

The lowest P_AO_2 and highest P_ACO_2 values (Fig. 3) agree with previously reported pre-internal pipping values, both for the ostrich and for other avian species (Rahn et al., 1974; Meir and Ar, 1990). The ratio of the calculated GO_2 to measured GH_2O, agrees (t-test, $P = 0.66$) with the ratio of the respective diffusion coefficients of the two gases in nitrogen (Table 4). This suggests that O_2 and water vapour follow the same route through the eggshell, i.e. the gas filled pores (Paganelli et al., 1978). However, Meir and Ar (1990) reported a calculated GO_2/GH_2O ratio of 0.585, which is significantly lower than the ratio of the diffusion coefficients of the respective gases in nitrogen. They explained the discrepancy by the higher temperature (and water vapour pressure) of the egg contents surface area, compared to that of the incubator atmosphere in a still-air incubator. This could limit GO_2 and increase GH_2O due to condensation of water vapour in the shell membranes.

Egg surface temperatures were not measured in this study, but both the 'open' measuring system (see Section 2) and the use of force-draught incubator should limit the effect of thermal boundary layers, and thus minimize any temperature difference between the egg and its surrounding atmosphere.

RQ calculations based on simultaneous air cell gas pressure values (Fig. 3), assuming diffusive gas exchange, are in the range of measured values (Fig. 2). Thus, the GCO_2 should be related to GO_2 as: $GCO_2 = GO_2\ 0.78 = 132\ ml_{[STPD]}\ torr^{-1}\ day^{-1}$ (Wangensteen and Rahn, 1970/1971).

4.3. Mass and energy content of the egg and hatchling

The energy content of the ostrich egg's contents (30.19 ± 0.64 kJ g^{-1} dry mass) is not different from the average of other precocial species (29.89 ± 1.35 kJ g^{-1}, $n = 13$) reported by Ar et al. (1987). The ostrich 'true hatchling' dry mass (109 g), however, is only 56% of the mass predicted by allometry for an egg mass of 1491 g (Vleck and Vleck, 1987). Furthermore, the 'true hatchling' constitutes only 44% of the whole hatchling dry mass (with 56% residual yolk), compared to the chicken's 54.5% (Ar et al., 1987).

The initial yolk dry mass of an ostrich egg is approximately 180 g (Deeming, 1997). Thus, a residual yolk dry mass of 137 g means that only 24% of the initial yolk mass is utilized during the course of embryonic development. Prinzinger et al. (1997) report a similar value (22%) for another ratite, the common rhea (*Rhea americana*). These values are much lower than that of the brown kiwi (*Apteryx australis*), which utilizes approximately 60% of its initial dry yolk content. It is interesting to note that while the ostrich and the rhea have a relatively low ${}_{sp}\dot{V}_{O_2 tot}$, the value for the kiwi (also a ratite) is similar to the average for other avian species (Hoyt and Rahn, 1980). Other avian species, for which data are available, have a range of 44–88% yolk utilization (dry mass), regardless of maturity type (estimated from Ar et al., 1987; Sotherland and Rahn, 1987).

The incubation duration of ostrich eggs in the wild is 39–42 days (Sauer and Sauer, 1966; Siegfried and Frost, 1974; Swart et al., 1987; Bertram, 1992), whereas according to allometry (Rahn and Ar, 1974) the expected duration for the 1500 g ostrich egg is 59 (95% confidence limits: 48–73) days. Swart et al. (1987) report an ambient water vapour pressure of 11 torr (1.5 kPa) in natural nests, and assuming a saturated environment within the egg at the incubation temperature (45.8 torr, 6.1 kPa, at 36.5°C), a water vapour gradient of 34.8 torr (4.6 kPa) exists across the eggshell. Together with an eggshell water vapour conductance of 118.6 ± 23.0 mg kg^{-1} $torr^{-1}$ day^{-1}, this would result in a predicted 17.3% mass loss in 42 days of incubation, a value higher than the calculated average for avian eggs (approx. 15%; Rahn and Ar, 1974; Ar and Rahn, 1980). An incubation period of 59 days under these conditions would result in a water loss of approximately 24%. This extent of water loss was reported to decrease hatching success of ostrich eggs (Ar et al., 1996). Excessive water loss cannot be prevented by a reduced GH_2O without having a similar effect on GO_2, and such a decrease would interfere with the metabolic needs of the embryo towards the end of its development (Ar et al., 1974). Alternatively, low GO_2 (and GH_2O) could be compensated for by a longer incubation period (Rahn et al., 1974). The relatively short incubation period of the ostrich egg, compared to other avian species, corresponds to the relatively smaller 'true hatchling' mass, and may represent an adaptation to the arid conditions encountered by the embryo in its natural habitat. It seems that the ostrich embryo avoids desiccation by hatching 'prematurely' for what is considered to be a precocial species, and therefore exhibits low yolk utilization and a relatively low hatchling dry mass. These characteristics are in accordance with the unique growth pattern of the ostrich embryo (Fig. 5) and its low ${}_{sp}\dot{V}_{O_2 tot}$.

In conclusion, the results of this study suggest that the ontogeny of the ostrich embryo's $\dot{V}_{O_2}$, at 36.5°C, when measured in an open system, is similar to that of precocial avian species. The values of ostrich ${}_{Pre\text{-}IP}\dot{V}_{O_2}$ and eggshell diffusive conductance resemble those predicted by allometry. Both P_AO_2 and P_ACO_2 are similar to values measured for other avian species. Since for a given egg mass the product of eggshell water vapour conductance (and daily water loss at given incubation conditions) and incubation period is constant (Ar and Rahn, 1985), reduction in water loss is achieved by either lowering GH_2O or by decreased incubation period. The metabolic needs of the embryo towards internal pipping do not allow a lowered eggshell diffusive conductance as means of avoiding desiccation without affecting P_AO_2. It seems that in order to avoid desiccation in its natural arid habitat, the ostrich embryo has evolved in the direction of a relatively short incubation period.

Acknowledgements

We thank Mr Dani Campi, Mr Nati Aizik, Dr Ehud Ashash and the staff of Zemach Ostriches Ltd. at Kibbutz Ha'on for their assistance and cooperation, egg donation and hospitality. Special thanks to Ms Ann Belinsky for her help in the lab throughout this study.

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