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DIRECT AND INDIRECT CALORIMETRY OF THE RESTING METABOLISM OF JAPANESE QUAIL (COTURNIX COTURNIX JAPONICA)

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SUMMARY

The energy metabolism of Japanese quail (Coturnix coturnix japonica) was examined with an experimental apparatus comprising a modified camping cold box as calorimeter. The resting metabolic rate, measured in the dark by indirect calorimetry, amounted to 8.1 W/kg in males and 8.8 W/kg in females. The rates of metabolic heat production H and of negative heat storage S summed up to 9.1 W/kg (H: 89%, S: 11%), the rate of total heat loss equalled 9.6 W/kg (nonevaporative heat losses: 95%, evaporative heat loss: 5%).

INTRODUCTION

The simultaneous determination of the metabolic heat production of an animal by indirect calorimetry and of its heat losses by direct calorimetry should lead to the same results, unless a part of the heat produced by the animal has originated from anaerobic metabolic processes. Both methods of measurement have been employed in the past for metabolic examinations of medium sized animals (ref. 1). Recently, however, indirect calorimetric methods have been used more often because the available direct calorimeters are technically complex and expensive. With the advent of paramagnetic oxygen analyzers, a convenient and exact method for the measurement of the gaseous exchange of larger animals has been brought into use. Up to now, measurements of the energy metabolism have usually been taken either by direct or by indirect calorimetry only. A simultaneous application of both methods has the advantage that both heat production and heat losses of an animal can be registered, resulting in a complete heat balance. During metabolic measurements on human subjects, differences between the results of direct and indirect calorimetry sometimes occurred that could not be explained by experimental errors (ref. 2).

The energy metabolism of birds has been examined by indirect calorimetry since the beginning of this century (e.g. refs. 3, 4). Nearly all of the constantly increasing number of metabolic mea-

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surements on birds employed indirect calorimetric methods; only in a few cases the heat loss of birds has been determined by direct calorimetry (refs. 5, 6).

The Japanese quail (Coturnix coturnix japonica) is frequently used as experimental animal since about 30 years. Several papers concerning the energy metabolism of the quail dealt with the resting metabolic rate and the ontogenetic development of thermoregulation (refs. 7-9), the influence of testosterone on the energy metabolism (refs. 10, 11), shivering (ref. 12), and the energy metabolism in quail during exercise (ref. 13). The central goal of the present investigation was to establish a heat balance for the Japanese quail by simultaneously measuring the metabolic heat production by indirect calorimetry, the nonevaporative heat loss by direct calorimetry, the evaporative heat loss by observing the atmospheric humidity, and the heat storage of the animals by the determination of their body temperature.

MATERIAL AND METHODS

Seven male and six female adult Japanese quails (Coturnix coturnix japonica), that had been purchased from a poultry breeder, were used several times in these investigations, leading to a total of 46 experiments. The birds were kept in individual cages on sand at a temperature between 20°C and 23°C and a light-dark cycle of 12:12 h. They had access to water and dry feed ad libitum.

The experimental setup consisted of the calorimeter chamber with additional instruments for the measurement of temperatures, partial pressure of oxygen, and relative humidity and a system for air supply with a pump, a flowmeter, and an absorber tube (Fig. 1). The calorimeter was a partially rebuilt plastic cold box as used for camping (Sunny Cool; Quelle). The bottom of the box contains a Peltier element which can be utilized as a heat flow meter to transduce a heat flow into a measurable voltage (ref. 14). A polarographic oxygen sensor (Transoxode; Dräger), a humidity sensor (DLFM 32; Reinhardt), the sensor of a digital thermometer (Oriel), and two NiCr-Ni thermocouples had been installed into the lid of the box. During the experiments, the box was placed into a larger wood-styropore container for better insulation. An airflow was drawn through a heat exchanger coil for temperature equilibration before entering the calorimeter and could afterwards be pumped through a carbon dioxide absorption tube. Under experimental con-

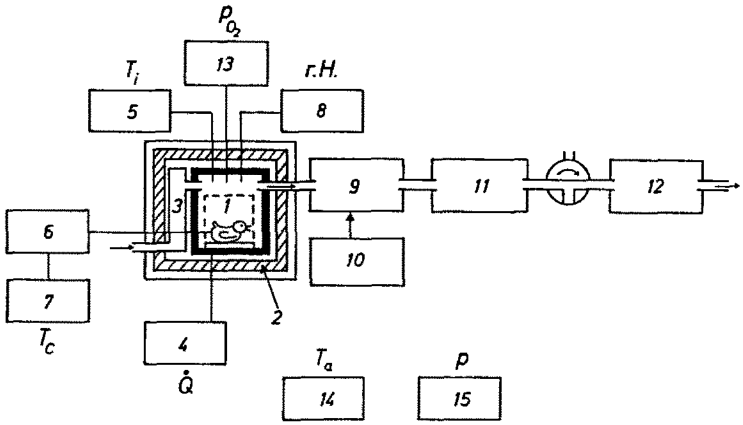

Fig. 1. Schematic representation of the experimental setup. 1: calorimetric box (Sunny Cool), 2: styropore box, 3: heat exchanger, 4: calorimeter recorder, 5: digital thermometer, 6: temperature transducer, 7: digital voltmeter, 8: hygrometer, 9: pump, 10: adjustable transformer, 11: flowmeter, 12: CO_2 absorber, 13: oxygen sensor, 14: mercury thermometer, 15: barometer. The direction of airflow is indicated by arrows. Measurements of the heat flow ($\dot{Q}$) were taken with the calorimeter; in addition, the colonic temperature (T_c) of the quail and the air temperature (T_i), the partial pressure of oxygen (p_{O2}), and the relative humidity (r.H.) inside the calorimeter chamber could be determined. On the outside, air temperature (T_a) and atmospheric pressure (p) were registered.

ditions, with a constant airflow of 9.9 l/h drawn through the calorimeter chamber and a calibration source of heat situated in a small experimental cage with plastic bottom inside the box, the sensitivity of the calorimeter amounted to 8.0 mV/W and its time constant to 41 min.

All measurements took place between 1 and 5 p.m. at external temperatures ranging from 20°C to 23°C. Before an experiment, the air supply of the measuring system was turned on until the base line of the calorimeter had stabilized. The experimental animal was weighed, and one of the thermocouples was inserted 3 cm into the cloaca of the quail and fixed to the tailfeathers. The bird was then confined to the experimental cage and placed into the dark calorimeter chamber. When after about one hour and a half the readings of the calorimeter and of the oxygen sensor remained constant, the quail was released from the calorimeter box and weighed again. The measurements were continued until the readings of the calorimeter had reached the initial base line values.

The average heat flow dissipated by a quail, representing the rate of nonevaporative heat loss, was determined from the difference between the calorimeter recording towards the end of an experiment and the base line. The rate of oxygen consumption was calculated from the airflow rate reduced to standard temperature and pressure and the difference between the partial pressures of oxygen inside the calorimeter first without the quail before the experiment and again with the quail towards the end of the experiment. The rate of carbon dioxide production of a quail was calculated from the quantity of carbon dioxide that had been absorbed in a solution of barium hydroxide during an exactly determined time interval; the absorbing liquid had been titrated with oxalic acid. For each experiment, the respiratory quotient (RQ), the caloric equivalent (assuming a metabolism of carbohydrates and fats only; ref. 24), and the rate of metabolic heat production could be calculated from the measuring data. The relative humidities inside the calorimeter, measured without the quail before the experiment and with the quail towards the end of the experiment, were converted to absolute humidities, and the rate of evaporative heat loss from the skin and from the respiratory tract of the quail was calculated with a heat of vaporization equalling 2.4 J/mg H_2O at 40°C (ref. 15). The rate of heat storage S was estimated from the difference of colonic temperatures ΔT_c at the end and at the beginning of an experiment, the duration Δt of the experiment, and the average specific heat of body tissue ($c = 3.5$ kJ $kg^{-1} \cdot {}^{o}C^{-1}$; ref. 16):

$$S = c \quad \Delta T_c / \Delta t \tag{1}$$

RESULTS

The result of a typical experiment is shown in Fig. 2. About 90 min after the quail had been placed into the calorimeter, the calorimeter signal had stabilized and corresponded to a heat flow of 1.4 W; the partial pressure of oxygen had dropped to a stable value of 137 Torr. The external temperature remained nearly constant during the experiment, whereas the calorimeter temperature increased steadily up to a value 2.9°C above the external temperature. The massive increase in humidity was due to a defecation of the quail; consequently, the evaporative heat loss could not be evaluated for this experiment.

The male quails had a mean body weight of 136 ± 10 g (mean ±

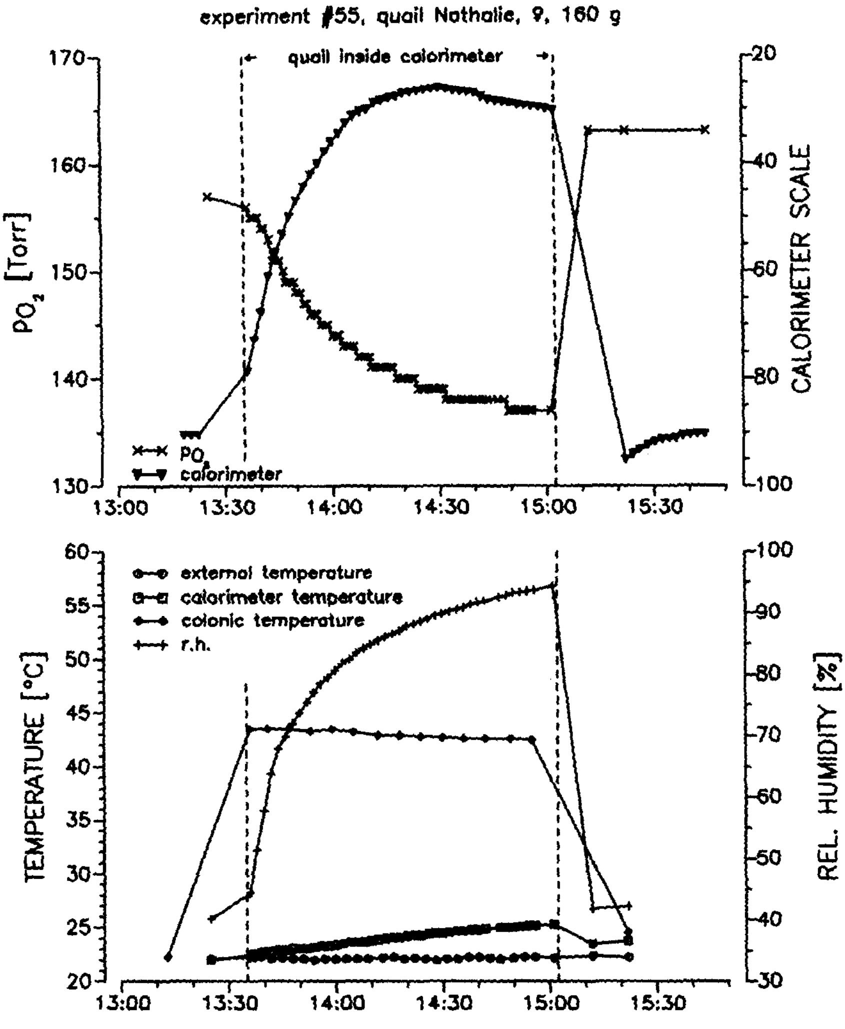

Fig. 2. Measuring data in the course of a typical experiment. The quail stayed in the calorimeter chamber during the time interval indicated by dashed lines. Partial pressure of oxygen (p_{O2}) and relative humidity (r.H.) inside the calorimeter, the readings of the calorimeter recorder, air temperatures on the outside and inside the calorimeter, and the colonic temperature of the quail are shown.

SD), the females were significantly heavier with 175 ± 11 g (t-test: p << 0.001; ref. 17). Both the rate of oxygen consumption and the heat flow increased with the body weight of the quails (Fig. 3, 4). Regression analyses of the logarithmically transforme

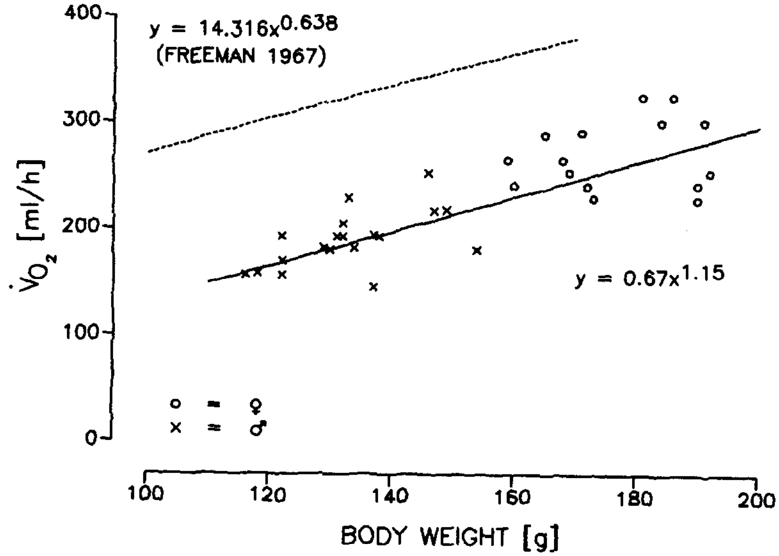

Fig. 3. Rate of oxygen consumption ($\dot{V}_{O2}$) of the quails as a function of their body weight at mean calorimeter temperatures between 21°C and 24°C from all experiments with $0.7 \leq RQ \leq 1$. A calculated regression line is plotted through the experimental data, the dashed line is a similar regression function for quail given by FREEMAN (ref. 7).

data according to the method of least squares (ref. 17) resulted in the following allometric equations:

$$\lg \dot{V}_{O2} = -0.171 + 1.15 \lg w \quad (n = 34;\ r = 0.84) \tag{2}$$

$$\lg \dot{Q} = -1.904 + 0.94 \lg w \quad (n = 46;\ r = 0.84) \tag{3}$$

where $\dot{V}_{O2}$ is the rate of oxygen consumption in ml O_2/h, $\dot{Q}$ is the heat flow in W, and w is the body weight in g. The weight specific rates of metabolic heat production calculated from the results of indirect calorimetry amounted to 8.09 ± 0.89 W/kg (mean ± SD; n = 19) for males and to 8.79 ± 1.03 W/kg (n = 16) for females; the value for females was significantly larger (t-test: $p < 0.05$). The rates of nonevaporative heat losses measured by direct calorimetry were the same for both sexes: 9.27 ± 0.77 W/kg for males (n = 23) and 9.27 ± 0.99 W/kg for females (n = 23). The evaporative heat loss could be determined in a few cases only because the quails defecated frequently during the experiments; the average rate of evaporative heat loss was 0.46 ± 0.10 W/kg. The colonic temperature, measured at the end of the experiments, amounted to 41.15 ± 0.39°C

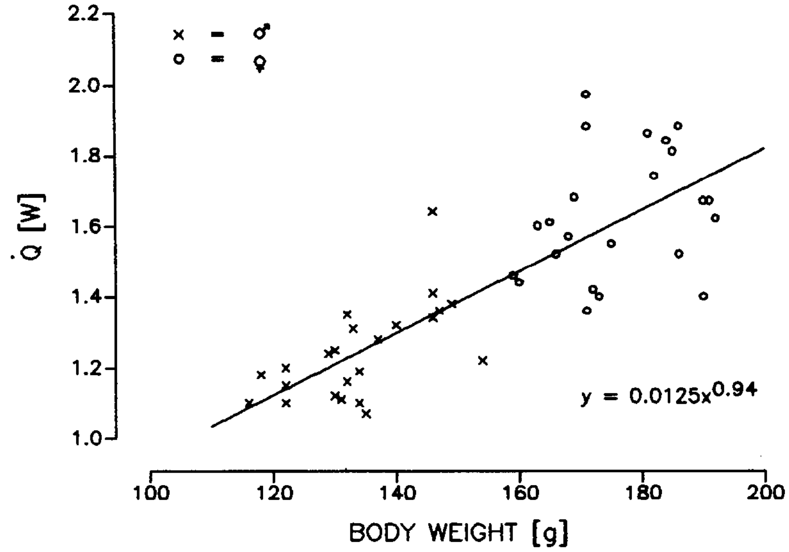

Fig. 4. Heat flow ($\dot{Q}$) emitted by the quails as a function of their body weight at mean calorimeter temperatures between 21°C and 24°C. The solid line represents a regression function calculated from the experimental data.

for the males (n = 14) and to 41.72 ± 0.73°C for the females (n = 6) with a significant sex difference (t-test: $p < 0.05$). In the course of each experiment, the colonic temperatures decreased regularly by 1.3°C on average, so that the heat storage of the quails became negative.

The heat balances of four experiments are shown in Fig. 5. In a steady state, heat production and heat losses must be balanced, i.e.

$$H = E + R + C + K + S \qquad (4)$$

where H is the metabolic heat production as measured by indirect calorimetry, E is the evaporative heat loss, R, C, and K are the heat losses by radiation, convection, and conduction, respectively, as measured by direct calorimetry, and S is the heat storage (ref. 16). The metabolic heat production H and the negative heat storage S totalled to 9.1 W/kg on average; H accounts for 89% and S for 11% of the sum. The average total heat loss of the quail, the sum of evaporative heat loss E and nonevaporative heat losses (R + C + K) amounted to 9.6 W/kg, with (R + C + K) accounting for 95% and E for 5% of the total.

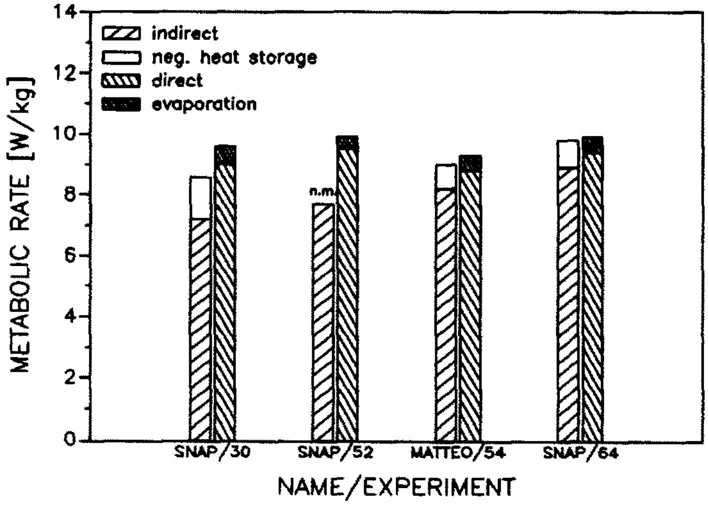

Fig. 5. Heat balances of the resting quail from four single experiments. For each experiment, the metabolic rate measured by indirect calorimetry (= metabolic heat production), the (negative) heat storage (not measured in experiment # 52), the metabolic rate measured by direct calorimetry (= nonevaporative heat loss), and the evaporative heat loss are specified.

DISCUSSION

The measured metabolic heat production of 8.1 W/kg in male quails and 8.8 W/kg in females is corresponding to their resting metabolic rate according to the definition by BLIGH & JOHNSON (ref. 18). The metabolic rate and the body temperature of C. c. japonica are subject to a marked circadian rhythm that is closely correlated to the light-dark cycle (ref. 11). The body temperature of quails that are exposed to an artificial light-dark cycle of 3:3 h follows the new rhythm; within one hour after the onset of the dark phase, the body temperature decreases by 0.4°C to a value typical for the resting phase of the birds (ref. 19). Since the present experiments took place in the dark, the measured resting metabolic rate of the quails is relatively low compared to the results of previous studies with metabolic rates of Japanese quail resting in the light ranging from 12.2 to 13.9 W/kg (refs. 8, 11, 13). The related bobwhite (Colinus virginianus) with a mean body weight of 180 g has a metabolic rate of 8.7 W/kg when resting in the light at 25°C (ref. 20).

The rates of oxygen consumption given in the literature for Coturnix c. japonica resting in the dark at temperatures between 20°C and 25°C range from 1.71 ml O_2 g^{-1} h^{-1} (corresponding to 9.5

W/kg assuming a caloric equivalent of 20.1 J/ml O_2; ref. 10) to about 2.4 ml O_2 g^{-1} h^{-1} (13.4 W/kg; ref. 12). The high rate of oxygen consumption given by FREEMAN (ref. 7; Fig. 3) for Japanese quail is probably due to his use of rather small, possibly still subadult quails and to the conditions of measurement in the light. The large exponent of the allometric equation found in the present experiments, indicating an increase in the weight specific rate of oxygen consumption with the body weight of the quail, is caused by the sex specifically higher metabolic rate of the heavier females that is in contrast with FREEMAN's results. The metabolic rate of laying quail hens, however, is reported to be at least 20% higher than the metabolic rate of non-laying hens (ref. 22); this increased metabolic activity during egg production might be the reason for the observed sex differences in weight specific metabolic rates.

Since the relative humidity inside the calorimeter chamber increased beyond 70% during the present experiments, the measured evaporative heat loss of the quail reached only 0.46 W/kg. Previously published rates of evaporation in C. c. japonica range from 1 to 4 mg H_2O g^{-1} h^{-1} at 30°C to 32°C (ref. 22), corresponding to evaporative heat losses between 0.7 and 2.7 W/kg. In bobwhite (Colinus virginanus), the evaporative heat loss amounted to 1.2 W/kg at 25°C and at relative humidities below 50% (ref. 20).

The observed sex difference in colonic temperatures of Japanese quail is in agreement with former investigations (refs. 10, 23). The decrease of the colonic temperatures during the experiments, resulting in a negative heat storage, ought to be due both to the influence of darkness inside the calorimeter and to the abatement of the handling effect that had been caused by the preparation of the birds for measurement; when handled, the body temperature of C. c. japonica can increase within 8 min and return to normal 16 min later (ref. 19).

The sum of metabolic heat production and negative heat storage agreed with the measured total heat losses within 5%, the approximate accuracy of measurement. The apparatus used in the experiments has the disadvantage of substantial deviations during the measurements of several environmental parameters like calorimeter temperature, oxygen content and carbon dioxide content of the air, and relative humidity inside the calorimeter chamber. An increase of the airflow rate through the calorimeter is not possible because of thermal disturbances of the calorimeter function in spite of the heat exchanger coil. The apparatus, nevertheless, seems suited for relatively easy and inexpensive determinations of the metabolic rates and heat balances of medium sized animals or groups of smaller animals like mice by direct and indirect calorimetry.

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