

# VARIATION IN THE RESPIRATORY QUOTIENT OF BIRDS AND IMPLICATIONS FOR INDIRECT CALORIMETRY USING MEASUREMENTS OF CARBON DIOXIDE PRODUCTION

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## Summary

Determination of animal power consumption by indirect calorimetry relies upon accurate estimation of the thermal equivalent of oxygen consumed or carbon dioxide produced. This estimate is typically based upon measurement or assumption of the respiratory quotient (RQ), the ratio of CO<sub>2</sub> produced to O<sub>2</sub> consumed. This ratio is used to indicate the mixture of lipids, carbohydrates and proteins in the metabolic substrate. In this analysis, we report the RQ for two bird species, *Passer domesticus* and *Auriparus flaviceps*, under several dietary and fasting

regimes. RQ commonly differed substantially from those typically assumed in studies of energy metabolism and often included values below those explainable by current knowledge. Errors that could result from these unexpected RQ values can be large and could present the primary limit to the accuracy of power consumption estimates based upon measurement of carbon dioxide production.

Key words: carbon dioxide production, indirect calorimetry, respiratory quotient, *Passer domesticus*, *Auriparus flaviceps*.

## Introduction

Indirect calorimetry is a commonly used method for estimating an animal's metabolic energy expenditure based on rates of oxygen consumption or carbon dioxide production. An important complication in such analyses is that the relationship between respiratory gas exchange and power consumption is variable; the thermal equivalent of gas consumed or produced varies with the metabolic pathways used and the substrate catabolized. Workers must therefore deduce or assume what mixture of materials constitutes the metabolic substrate. The index that is customarily used in this deduction is the animal's respiratory quotient (RQ) of CO<sub>2</sub> produced to O<sub>2</sub> consumed. (Although RQ is formally the exchange ratio of these gases at the cellular level, we here follow the convention for intact vertebrates and assume that it equals the gas exchange ratio at the lungs.) For example, an RQ of 1.00 characterizes carbohydrate catabolism. An RQ of 0.71 is the minimum ratio expected and is taken as indicating reliance on lipid substrates. For animals catabolizing only lipids and carbohydrates, the ratio of these materials in the substrate used can be calculated from values of RQ between 0.71 and 1.00 (Kleiber, 1961). Possible catabolism of proteins presents difficulties because of the variable effects of nitrogen excretion. Therefore, in animals such as birds and mammals, whose dominant nitrogenous wastes are urates or urea, respectively, the thermal equivalents of CO<sub>2</sub> production or O<sub>2</sub> consumption differ, as do the RQ values associated with protein consumption.

Historically, indirect calorimetry has relied largely upon

measurements of oxygen consumption. This was convenient, because errors in assumptions regarding the substrate catabolized do not lead to large errors in estimating energy expenditure from oxygen consumption. For example, the thermal equivalent of consumed oxygen differs by only 12% between pure carbohydrate (20.9 kJ l<sup>-1</sup>) and pure lipid (18.7 kJ l<sup>-1</sup>) (Table 1). Assuming an intermediate value, therefore, is unlikely to produce errors of more than a few per cent.

In recent years, however, much more emphasis has been placed on measurements of carbon dioxide production. One reason is the availability of improved instrumentation. Current CO<sub>2</sub> analysers are approximately 100 times more sensitive than typical O<sub>2</sub> analysers. In addition, isotopically labeled water (either <sup>2</sup>H<sub>2</sub>O<sup>18</sup> or <sup>3</sup>H<sub>2</sub>O<sup>18</sup>) is being used more frequently to estimate carbon dioxide production by free-living animals in ecologically interesting situations. Validation tests of this technique on mammals, birds and reptiles indicate that it estimates CO<sub>2</sub> production with an error in the range of ±7% (Nagy, 1989). Estimating metabolic power consumption from measurements of carbon dioxide production is, however, a separate problem that may lead to substantially larger errors than are likely from measurements of oxygen consumption. The thermal equivalent of carbon dioxide produced varies much more with substrate type than does the thermal equivalent of oxygen consumed. For example, thermal equivalents for CO<sub>2</sub> production are typically 33% lower for

Table 1. *Typical respiratory quotients and thermal equivalents of metabolic substrates*

Substrate	RQ	Gas volume produced or consumed (l g <sup>-1</sup> substrate)		Catabolized energy		
		CO <sub>2</sub>	O <sub>2</sub>	(kJ g <sup>-1</sup> substrate)	(kJ l <sup>-1</sup> CO <sub>2</sub> )	(kJ l <sup>-1</sup> O <sub>2</sub> )
Lipid	0.71	1.43 <sup>a</sup>	2.01 <sup>b</sup>	39.7 <sup>c</sup>	27.8	19.8
Carbohydrate	1.00	0.80 <sup>d</sup>	0.80 <sup>d</sup>	16.7 <sup>c</sup>	20.9	20.9
Protein <sup>e</sup>	0.74	0.70	0.95	17.8	25.4	18.7

<sup>a</sup>Brouwer (1957); <sup>b</sup>Brody (1945); <sup>c</sup>Kleiber (1961); <sup>d</sup>Average value computed by Gessaman and Nagy (1988); <sup>e</sup>King (1957), for uricotelic species.

pure carbohydrate substrates than for pure lipid substrates, producing a range of possible error almost three times greater than that derived from oxygen consumption measurements (Table 1). Accurate knowledge of the metabolic substrate is therefore critical. In addition, rates of carbon dioxide release are more likely to be influenced by factors such as non-respiratory excretion or short-term storage in body tissues and fluids.

Such considerations make clear that the overall limit on the accuracy of indirect calorimetry using measurements of carbon dioxide production may well lie in the estimation of the thermal equivalent of the CO<sub>2</sub> produced. This, in turn, will depend on the accuracy of assumptions regarding the substrate being catabolized and its associated RQ value. As an initial step in assessing this problem, we explore interspecific variation in the respiratory quotient of two bird species and also investigate variation associated with contrasting dietary regimes and periods of fasting.

### Materials and methods

Two species were used. House sparrows (*Passer domesticus* Linnaeus) are small (about 23 g) birds that consume both insects and plant material. Verdins (*Auriparus flaviceps* Sundevall) are very small (about 6.5 g) and insectivorous. Sparrows were captured in Tempe, Arizona, and maintained in an outdoor aviary during November and December. Prevailing air temperatures ranged from daytime minima of 5–9 °C to maxima of 21–27 °C. Sparrows were maintained on a mixed seed diet and given water *ad libitum*. Verdins were captured in the Superstition Mountains, Arizona, in December and held in an environmental chamber at an air temperature that cycled daily between 12 and 24 °C, and at a photoperiod of 11 h:13 h L:D. Verdins were maintained on a diet of mealworms (*Tenebrio molitor* larvae), grapes and water given *ad libitum*.

The effects of widely differing diets and time spent fasting during daylight hours on house sparrows were tested after feeding birds either mealworms or millet. Expected values of the RQ for each diet are 0.93 for millet and 0.75 for mealworms, as calculated by Nagy (1983) based on their compositions and assuming that the nitrogenous waste is

urate. Animals were supplied with only one type of food, *ad libitum*, commencing 24 h before measurements. On the day measurements were made, birds were removed from the food and placed individually and unrestrained within 600 ml metabolic chambers 1 h after sunrise. Birds were held in darkness and rested quietly. Sparrows had commenced feeding approximately 25 min prior to sunrise, giving them about 85 min to feed. Measurements were made on four birds each day, starting 30 min after removal from food and lasting until 6.5 h after removal from food. Values reported are averages over 3 min. All measurements were made at 25±1 °C.

Parallel measurements were made for verdins, except that birds were maintained at 15±1 °C and measurements were made only for birds fed mealworms. Data collection commenced 90 min after feeding started in the morning and continued at 30 min intervals for 5 h.

For both species, measurements were also made during the nocturnal fast following a day in which they were supplied with their normal maintenance diet and allowed to feed throughout daylight hours. Measurements commenced 30 min after darkness prevented feeding.

Oxygen consumption and carbon dioxide production were measured simultaneously. Air flowed to the metabolic chambers at 400 ml min<sup>-1</sup> (*Passer domesticus*) or 200 ml min<sup>-1</sup> (*Auriparus flaviceps*), after being dried and scrubbed of CO<sub>2</sub> by a Puregas model CDA 1112 air dryer/CO<sub>2</sub> absorber system. Air flow was measured with Omega FL3402G-HRV rotameters, calibrated to ±1% with soap-bubble flow meters. These flow rates allowed the entire respiratory apparatus to equilibrate in 6.9 min and 13.8 min, following the calculations of Lasiewski *et al.* (1966).

The O<sub>2</sub> concentration of air entering and leaving the chamber was determined using a 50 ml min<sup>-1</sup> sample of efflux air from the chamber, using an Applied Electrochemistry S3a oxygen analyser. The standard method of calibrating this instrument merely calls for the adjustment of instrument reading to a single point using the fractional composition of oxygen in dry air ( $F_{O_2}=0.2094$ ) as a calibration gas. However, this procedure does not test the accuracy of the response to changes in oxygen concentration. This was determined by altering the partial pressure of oxygen in the sample cell. The

cell's efflux of sample air was vented under 18 equally spaced depths of a 0.5 m column of distilled water. Under prevailing conditions (barometric pressure about 97 kPa), this was equivalent to a change in  $P_{O_2}$  equal to that which would be produced by changing the fractional oxygen concentration from 0.1993 to 0.2093. This equates to the maximum range normally experienced in metabolic measurements. This procedure revealed that the oxygen analyser was highly linear in its response ( $r^2=0.9998$ ), but exhibited a response sensitivity 7% greater than expected based upon changes in  $P_{O_2}$ . Calculations of oxygen consumption corrected for this error.

An approximately  $50 \text{ ml min}^{-1}$  subsample of gas was dried with anhydrous calcium sulphate and passed to a LiCor model 6252 carbon dioxide analyser that determined  $\text{CO}_2$  concentration to  $\pm 2 \text{ p.p.m.}$  The gas analyser was calibrated daily using both  $\text{CO}_2$ -free air and a calibration gas known to contain 0.284%  $\text{CO}_2$ . Both the resolution (1 p.p.m.) and full-scale range of this instrument are much smaller than that typical of oxygen analysers. Depressions in oxygen concentration are approximately similar to elevations in carbon dioxide concentration; that is, RQ is expected to be approximately 0.7–1.0. To match the response range of the  $\text{O}_2$  and  $\text{CO}_2$  analysis systems better, the sample of air from the metabolic chamber flowing to the  $\text{CO}_2$  analyser was diluted approximately 4:1 with  $\text{CO}_2$ -free, dry air. The sample dilution was maintained constant with FL3441ST-HRV (diluent) and FL3445ST-HRV (sample) precision rotameters. Thus, the calibration sequence for the carbon dioxide analyser was as follows. (1) Instrument zero was set using  $\text{CO}_2$ -free air. (2) Instrument span was set using a calibration gas containing 0.284%  $\text{CO}_2$ . (3) Sample gas was diluted with  $\text{CO}_2$ -free air. The exact dilution was determined by observing the depression of the calibration gas. Sample flow rates to the oxygen and carbon dioxide analysers were adjusted so that the analysers responded simultaneously to changes in sample gas concentration.

Instrument signals were recorded on a Campbell CR21x datalogger, which collected data 56 times per minute and averaged values over 1 min. To compensate for signal drift in the oxygen analyser, the fractional concentration of oxygen in incurrent air to the chambers was also measured at 30 min intervals. The carbon dioxide analyser did not exhibit measurable signal drift.  $\text{O}_2$  consumption was calculated using equation 2 of Hill (1972). The basic equation for carbon dioxide production ( $\dot{V}_{\text{CO}_2}$ ) is:

$$\dot{V}_{\text{CO}_2} = \dot{V}_E F_{\text{ECO}_2} - \dot{V}_I F_{\text{ICO}_2}, \quad (1)$$

where,  $\dot{V}_E$  is the volume of excurrent air from the chamber,  $F_{\text{ECO}_2}$  is the fractional concentration of carbon dioxide in the excurrent air,  $\dot{V}_I$  is the volume of incurrent air to the chamber, and  $F_{\text{ICO}_2}$  is the fractional concentration of carbon dioxide in the incurrent air. Because oxygen consumption ( $\dot{V}_{\text{O}_2}$ ) was measured, the need to measure  $\dot{V}_E$  was obviated by the relationship:

$$\dot{V}_E = \dot{V}_I - \dot{V}_{\text{O}_2} + \dot{V}_{\text{CO}_2}. \quad (2)$$

Substituting equation 2 into equation 1 and rearranging yields the equation used to calculate  $\text{CO}_2$  production:

$$\dot{V}_{\text{CO}_2} = (\dot{V}_I F_{\text{ECO}_2} - \dot{V}_{\text{O}_2} F_{\text{ECO}_2} - \dot{V}_I F_{\text{ICO}_2}) / (1 - F_{\text{ECO}_2}). \quad (3)$$

All values were corrected to STP ( $0^\circ\text{C}$ , 101 kPa).

Values are presented as means  $\pm 95\%$  confidence limits. Sample sizes are eight in all cases, except for the case of verdins during their nocturnal fast. Here,  $N=7$  because data from one animal were excluded from analyses. During eight of 10 measurement periods, values for this animal differed from the mean for the other seven by 7–32 standard deviations. We cannot explain this variance, but exclude the animal as a statistical outlier (Zar, 1984). Mean values were compared with expected values using a one-tailed Mann–Whitney  $U$ -test (Zar, 1984). Statistical significance was accepted at  $P < 0.05$ .

## Results

### *Effects of diet and diurnal fasting*

When fed mealworms, house sparrows initially exhibited respiratory quotients near what was expected (0.75) from their diet composition (Fig. 1). The RQ slowly declined, however, and mean values dropped below 0.71 within 3 h after removal from food. For the subsequent 3.5 h of measurements, the RQ averaged 0.66 and was significantly ( $P=0.013$ – $0.043$ ) below the theoretically expected minimum of 0.71, which should occur when the animal relies entirely upon mobilization of lipid stores.

When fed millet, the RQ of house sparrows averaged 0.88 within 30 min after removal from food – a value significantly lower than the value of 0.93 which would be expected on the basis of diet composition ( $P=0.043$ ; Fig. 1). During the first 2 h post-feeding, the respiratory quotient dropped rapidly. As with a mealworm diet, the RQ stabilized near 0.66 during the latter portion of the measurement period; this is significantly below the expected minimum of 0.71 ( $P=0.0019$ – $0.040$ ).

Verdins fasting after a mealworm diet initially exhibited substantial individual variance in the RQ, with a mean value of 0.68 30 min after removal of food (Fig. 1). Within 1 h of fasting, however, the RQ stabilized at 0.73–0.76 and remained in this range for most of the 5 h of fasting. Except for the first and last measurement periods, RQ was always significantly above 0.71 ( $P=0.0027$ – $0.036$ ).

### *Effects of the nocturnal fast*

While undergoing their normal nocturnal fast, the respiratory quotient of house sparrows dropped progressively for about 8 h from the start of the measurement period (Fig. 2). The RQ was significantly greater than 0.71 for the first 6 h of fasting ( $P=0.012$ – $0.047$ ). For some of the latter portion of the nocturnal fast, RQ declined and it averaged 0.69 from 9.5–10.5 h post-feeding. In contrast, verdins during their nocturnal fast exhibited mean RQ values of 0.75–0.79, differing significantly ( $P=0.0081$ – $0.041$ ) from 0.71 during all

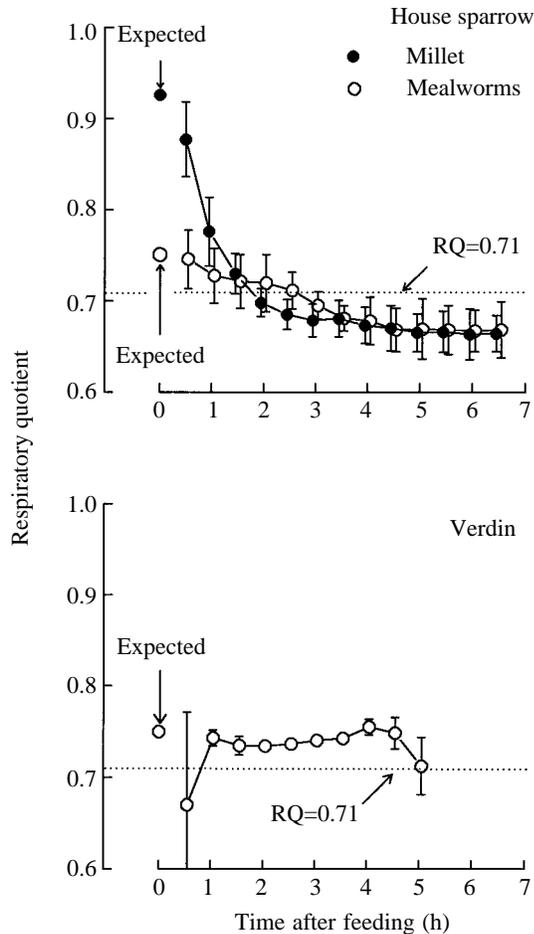


Fig. 1. Respiratory quotients (RQ) of birds fasting during daylight hours. Verdins (*Auriparus flaviceps*) were fed mealworms. House sparrows (*Passer domesticus*) were fed either mealworms or millet. Values are means  $\pm$ 95% confidence limits,  $N=8$ . Also shown are the expected RQ values for each diet and the value of the RQ (0.71) anticipated for fasting birds relying on stored lipids.

periods except at 30 min post-feeding. Calculated over the entire fast, the RQ averaged 0.76.

### Discussion

#### *Deviations of the respiratory quotient from expected patterns*

Our results disagree with the two common assumptions made by workers regarding respiratory quotients in birds during indirect calorimetry. These are (1) that, for the period soon after feeding, the RQ should be that expected on the basis of the composition of the diet, and (2) that the RQ of birds fasting for extended periods is that expected for lipid catabolism. Rather, we observed the following.

First, in extended fasts, the RQ differed substantially from that expected for exclusive reliance upon stored lipids and included values not currently explainable. The RQ in fasting verdins was significantly above 0.71, while in house sparrows it was commonly below the theoretically expected minimum

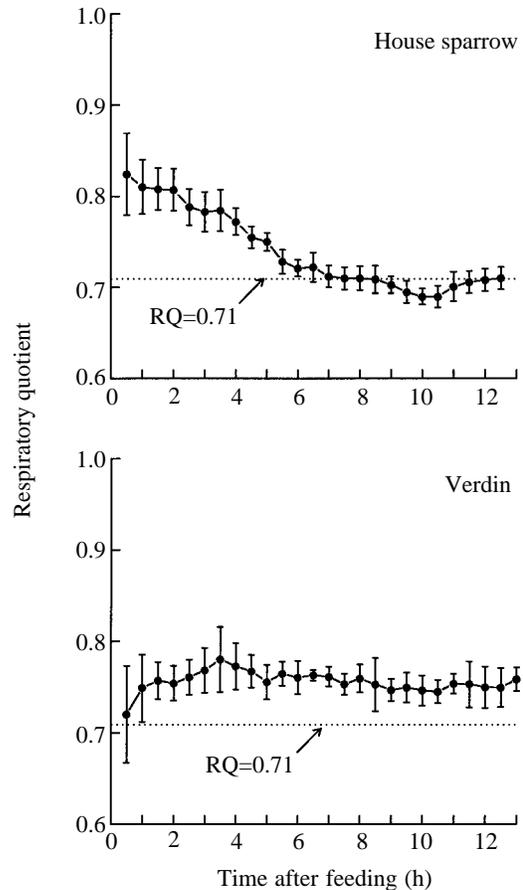


Fig. 2. Respiratory quotients of birds during their nocturnal fast. Values are means  $\pm$ 95% confidence limits. For sparrows,  $N=8$ , for verdins,  $N=7$ . Also shown is the value of RQ=0.71 anticipated for fasting birds relying on stored lipids.

of 0.71. It is notable, however, that although textbooks (e.g. Schmidt-Nielsen, 1975; Gordon *et al.* 1977; Hill and Wyse, 1988) commonly describe the 'normal' scope for the RQ as 0.71–1.00, there is ample precedent for values beyond this range. Although birds synthesizing fat may exhibit RQ values above 1.00 (King, 1957; Blaxter, 1989), the occurrence of values below 0.7 has rarely been discussed and is more controversial. For example, Freeman (1983) states, without elaboration, that such low RQ values simply result from experimental errors. In contrast, King (1957) reviewed the problem and concluded that RQ values of 0.6–0.7 are probably normal in birds and *not* merely the result of measurement error. He could not, however, offer an explanation for their origin. Although the oxidation of certain amino acids (e.g. glycine) yields respiratory quotients well below 0.7, this is not the case for any likely mixture of amino acids (King, 1957). Possible explanations for the low RQ values we observed included incomplete oxidation of fat and non-pulmonary loss of  $\text{CO}_2$ . Possible non-respiratory sinks for  $\text{CO}_2$  include loss by storage or excretion of bicarbonate ions. In at least some other taxa, such bicarbonate excretion can be an important mode of carbon

dioxide excretion. In *Alligator mississippiensis*, for example, about 15% of metabolically produced CO<sub>2</sub> is excreted in this fashion (Coulson and Hernandez, 1983). We have no data to evaluate such possibilities for birds.

Second, RQ exhibited rapid changes during fasting. For *Passer domesticus* on a millet diet, for example, the mean RQ dropped from 0.89 to 0.71 in 1 h during the diurnal fast. In contrast, the RQ dropped slowly in this species during the nocturnal fast and reached the value expected for reliance upon fat reserves only after 7 h.

#### Implications for indirect calorimetry

Rapid changes in the RQ and significant deviations from expected values can create substantial difficulties in the confident use of carbon dioxide production to estimate power consumption. Although quantitative data do not exist to predict such effects adequately, their nature is likely to depend upon a complex suite of factors that may include, for example, the length of fasting, the composition and quantity of food consumed and how rapidly crop reserves are expended. Each of these could well depend upon secondary determinants, such as thermostatic demands imposed upon the animal as well as the nature of productive processes (e.g. fat deposition, feather growth, egg synthesis) in which it is engaged. Our data for *Passer domesticus* and *Auriparus flaviceps* cannot, therefore, be generalized to other species or even to these species in other situations.

In addition, the existence of very low RQ values makes it unclear how to estimate reliably the thermal equivalent of the CO<sub>2</sub> produced. Such equivalents are typically based upon assumptions of a particular ratio of carbohydrate and lipid in the substrate metabolized, as indicated by the respiratory quotient, or are calculated from the mix of lipid, carbohydrate and protein in the diet and the assumption that the animal catabolizes material in direct proportion to its occurrence in the food ingested. RQ values substantially below 0.71, however, indicate that unidentified processes affect the fate of carbon dioxide, oxygen or both.

The potential error in estimates of animal power consumption using erroneous assumptions of the substrate metabolized is variable and can be large. Even a simple error analysis is problematic, because it requires comparison of expected values that are based upon assumed metabolic substrates (e.g. the diet consumed prior to initial periods of the fast or fat reserves during later periods) with the actual value for the calorific equivalent of produced CO<sub>2</sub>. Even if the RQ is measured, standard equations do not extend to RQ values below that of lipids (0.71). It is, however, worth exploring three initial estimates of the errors that can be associated with an incorrect assumption of the respiratory quotient.

#### Potential error associated with rapid changes in RQ

Here, we use data for house sparrows fasting during daylight hours after eating millet. We use data only for the first 1.5 h of fasting, the period for which workers are most likely to assume that the animal remains in an absorptive state and therefore

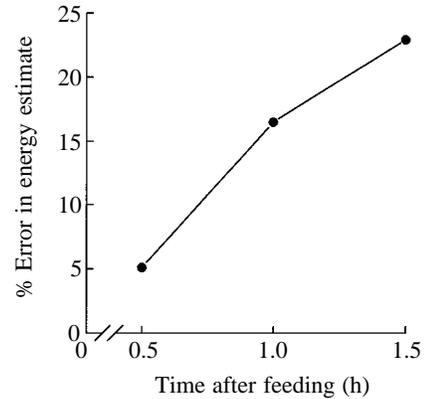


Fig. 3. Potential error associated with rapid changes in the respiratory quotient in house sparrows (*Passer domesticus*) fasting after consuming millet. Estimate based on (1) the expectation that a worker assumes the bird is in an absorptive state and power consumption is therefore estimated using the RQ and thermal equivalent of CO<sub>2</sub> expected from the composition of millet (21.9 kJ l<sup>-1</sup>; Nagy, 1983), and (2) that the animal is actually catabolizing a mix of carbohydrate and lipid as indicated by the measured respiratory quotient, with the actual thermal equivalent of the CO<sub>2</sub> produced equal to that calculated from the equation of Kleiber (1961). Positive error values indicate that the actual power consumption is below the estimated values.

heat production is calculated using the thermal equivalent of CO<sub>2</sub> expected from the composition of millet (21.9 kJ l<sup>-1</sup>; Nagy, 1983). We calculate the error by assuming that the animal is conserving protein and that the catabolized substrate consists of lipid and carbohydrate. The thermal equivalent of the CO<sub>2</sub> produced is, therefore, calculated from the equation of Kleiber (1961).

This analysis indicates that rapid changes, such as those observed in the respiratory metabolism of *Passer domesticus*, can be associated with large errors in estimates of power consumption (Fig. 3). The expected error reaches 16% within 1 h after the end of feeding and 23% within 1.5 h.

#### Potential error associated with unexpectedly high RQ values at night

Here, we estimate the error that could result from the common assumption that birds rely almost exclusively on stored lipids during their nocturnal fast. We use our data for verdins, which exhibited an average RQ of 0.76 rather than the value of 0.71 expected for the use of lipid stores.

Given the high protein diet of verdins, the possibility of substantial reliance upon protein as a metabolic substrate during portions of the nocturnal fast cannot be dismissed. This complicates analyses, as the thermal equivalent of CO<sub>2</sub> at a given respiratory quotient depends upon the ratio of lipid:carbohydrate:protein that constitutes the substrate, and the observed average RQ of 0.76 can be produced by various ratios of these materials. However, the ratios of any two of these substrate classes (e.g. carbohydrate and protein) can be calculated as follows if a particular fraction of the substrate is

assumed to be composed of the third class (e.g. lipid). The overall respiratory quotient (RQ) equals:

$$\text{RQ} = (V_{\text{CO}_2}/V_{\text{O}_2}) \\ = (V_{\text{CCO}_2} + V_{\text{LCO}_2} + V_{\text{PCO}_2})/(V_{\text{CO}_2} + V_{\text{LO}_2} + V_{\text{PO}_2}), \quad (4)$$

where  $V$  represents the volume of the gas species produced ( $\text{CO}_2$ ) or consumed ( $\text{O}_2$ ) per unit of substrate mass. The letters C, L and P designate the metabolic substrate as carbohydrate, lipid or protein, respectively. Each such volume quantity equals the fraction ( $F$ ) of the substrate composed of a substrate class multiplied by the volume ( $K$ ) of the gas produced or consumed per unit mass of that substrate class:

$$V = KF. \quad (5)$$

Substituting equation 5 into equation 4 yields:

$$\text{RQ} = [(K_{\text{CCO}_2}FC) + (K_{\text{LCO}_2}FL) + (K_{\text{PCO}_2}FP)] / \\ [(K_{\text{CO}_2}FC) + (K_{\text{LO}_2}FL) + (K_{\text{PO}_2}FP)]. \quad (6)$$

By definition:

$$FC + FP = 1 - FL. \quad (7)$$

For any given value of  $FL$ , the values of  $FC$  and  $FP$  that produce a particular RQ may be determined by substituting equation 7 into equation 6, and rearranging the terms:

$$FC = [FL(K_{\text{LCO}_2} - \text{RQ}K_{\text{LCO}_2} + \text{RQ}K_{\text{PO}_2} - \\ \text{RQ}K_{\text{PCO}_2}) - \text{RQ}K_{\text{PO}_2} + K_{\text{PCO}_2}] / (\text{RQ}K_{\text{CO}_2} - \\ K_{\text{CCO}_2} - \text{RQ}K_{\text{PO}_2} + K_{\text{PCO}_2}). \quad (8)$$

Our calculations with this equation use the values presented in Table 1 for the volume of  $\text{CO}_2$  produced or oxygen consumed for each substrate class. Equation 8 yields the fraction of the substrate composed of carbohydrate if a given fraction is

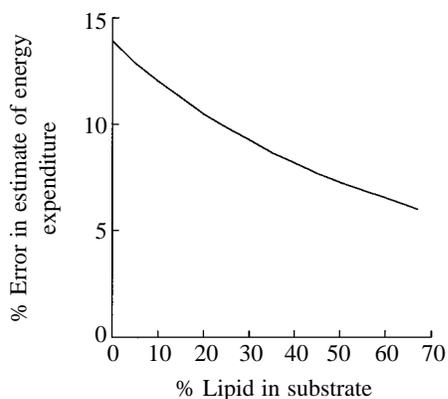


Fig. 4. Potential error associated with unexpectedly high RQ values at night. The estimates are based on (1) the expectation that a worker assumes that a bird is in a postabsorptive state and power consumption is therefore calculated using the thermal equivalent of carbon dioxide expected for stored lipids ( $27.8 \text{ kJ l}^{-1} \text{ CO}_2$ ; Table 1), and (2) that the animal is actually catabolizing some mixture of protein, carbohydrate and lipid that produces the average measured RQ of 0.76. Various ratios of the three types of substrates can yield the observed RQ value of 0.76 and would produce the range of errors shown. Positive error values indicate that the actual power consumption is below the estimated values.

composed of lipid. The remaining fraction ( $1 - FC - FL$ ) is protein. These values for the fractional representation of substrate classes were then used to derive the net thermal equivalent of  $\text{CO}_2$  produced, based upon thermal equivalents for each substrate class (Table 1).

This analysis indicates that the unexpectedly high RQ values characteristic of verdins fasting at night would produce errors of 6–14% if workers assumed that the catabolic substrate was entirely lipid (Fig. 4). Errors are smallest when the actual substrate consists of lipid and carbohydrate only (i.e. no protein) and are largest when the substrate consists entirely of carbohydrate and protein (i.e. no lipid).

#### Potential error associated with RQ values below 0.71

Unexpectedly low RQ values were typical for most measurements of diurnal fasting *Passer domesticus*. Such values were also observed in this species during nocturnal fasts and in *Auriparus flaviceps* during diurnal fasts. The prevalence of these low RQ values suggests at least two possible sources of error in analyses in which authors assume that fasting animals rely exclusively on lipid reserves. As noted previously, the existence of RQ values below 0.71 indicates the operation of unidentified processes. For our error analysis, we assume that the most likely mechanism is non-pulmonary loss of carbon dioxide. In addition, the catabolized substrate may not consist entirely of lipids.

For a given fraction of lipid in the substrate, we therefore computed from the data given in Table 1 the range of respiratory quotients and thermal equivalents of the  $\text{CO}_2$  produced that could result from possible ratios of carbohydrate:protein making up the remainder of the metabolic substrate. This yielded the range of energy consumption values that could actually have existed. We then calculated how much carbon dioxide would have had to be lost through non-pulmonary means to depress the respiratory quotient to 0.66 and the power consumption that would be estimated using the lower value of pulmonary  $\text{CO}_2$  release and the thermal equivalent expected for lipid catabolism (Table 1). Computing this error as a function of the fractional representation of lipids in the substrate (Fig. 5) defines a space bounded by two extreme cases. Errors are minimal if the substrate contains only lipid and protein (i.e. no carbohydrate catabolized) and these minima increase as the fractional representation of lipid in the substrate increases. Errors are maximal if the substrate contains only lipid and carbohydrate (i.e. no protein catabolized) and these maxima decrease as the fractional representation of lipid in the substrate increases. If the substrate actually is 100% lipid, as is often assumed, then the sole source of error is the 7% loss of carbon dioxide required to decrease the respiratory quotient from 0.71 to 0.66.

#### Concluding comments

It is important to recognize that the values described above may underestimate the errors produced in indirect calorimetry. RQ values of 0.66 suggest that extra-pulmonary loss of  $\text{CO}_2$ ,

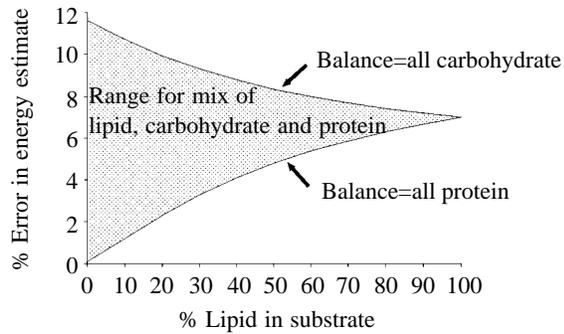


Fig. 5. Potential error associated with RQ values below 0.71. The estimate is based on (1) the expectation that a worker assumes the animal is in a postabsorptive state and power consumption is therefore calculated using the thermal equivalent of carbon dioxide expected for stored lipids, and (2) that the animal is actually catabolizing mixtures of protein, carbohydrate and lipid, and exhibits non-pulmonary losses of  $\text{CO}_2$  sufficient to produce the measured respiratory quotient of 0.66. Positive error values indicate that the actual power consumption is below the estimated values. The upper boundary of the error space represents the error that would be produced if the substrate consisted only of carbohydrate and lipids (no protein). The lower boundary of the space represents the error that would be produced if the substrate consisted only of protein and lipids (no carbohydrates).

at times, may be substantial. For verdins during diurnal fasts, this loss was estimated as 7% based upon the assumption that tissue-level RQ was 0.71. If this gas-exchange ratio was actually higher than 0.71 at the tissue level, then non-pulmonary  $\text{CO}_2$  loss must also have been higher. For measured RQ values above 0.71, it is similarly possible that non-pulmonary loss of carbon dioxide exists but is not suspected because the respiratory quotient falls in the range of values commonly expected. For example, a whole-animal RQ greater than 0.71 may, in fact, be produced by elevated values at the tissue level combined with non-pulmonary  $\text{CO}_2$  loss. Current data do not allow examination of this possibility. It is clear, however, that even with the error values we have estimated, inaccurate assumptions regarding the thermal equivalent of the  $\text{CO}_2$  produced can readily result in overwhelming errors in indirect calorimetry.

Finally, our analyses underscore the need for two types of fundamental physiological data. First, the prolonged maintenance of respiratory quotients near 0.66 suggests that current knowledge of the basic respiratory metabolism of birds contains significant gaps. Second, our data and the resulting uncertainties indicated in power consumption estimates demonstrate the need for simultaneous measurements of oxygen consumption, carbon dioxide production and heat

production by direct calorimetry of birds in a variety of nutritional and productive states.

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