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SHORT COMMUNICATION

ANAEROBIC HEAT PRODUCTION MEASUREMENTS: A NEW PERSPECTIVE

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Heat production measurements can be performed in two ways: direct and indirect calorimetry. Direct calorimetry is the measurement of heat loss, which under normal steady-state conditions equals the heat production (H). Indirect calorimetry is the calculation of heat production based on parameters related to the heat production, such as respiratory gas exchange (O_2 and CO_2) and nitrogen excretion (urea, uric acid or ammonia). A simplified form of indirect calorimetry is the calculation of heat production by means of an oxycalorific value. The calculation of this oxycalorific value is rather complex (Gnaiger, 1983*b*). Furthermore, it is only possible if the ratio of the amounts of the three substrates for oxidation (carbohydrate, fat and protein) is known. Both forms of indirect calorimetry are based on a standard composition of the substrates for oxidation.

Direct calorimetry is a difficult and expensive technique. Indirect calorimetry, which is a relatively simple technique, is therefore used more frequently. Direct calorimetry, however, is independent of aerobic or anaerobic conditions, whereas indirect calorimetry, based on respiratory gas exchange and nitrogen excretion, is restricted to aerobic conditions. Thus direct calorimetry is the only available method for both aerobic and anaerobic heat production measurements. However, if the endproducts of incomplete oxidation can be measured accurately, the indirect calorimetric formula for the calculation of heat production can be adjusted, as Brouwer (1965) did for the methane production of ruminants. It should be noted that the excreted protein-nitrogen, in the form of urea, uric acid or ammonia, is also an endproduct of incomplete oxidation; correction of the formula is made by a reduction of the caloric value of protein, depending on the nitrogen excretion product(s). The excreted nitrogen is used to calculate the amount of oxidized protein. It is obvious that the oxycalorific value is useless under anoxic conditions. Indirect calorimetry not only involves a formula for the calculation of heat production, but also formulae for the calculation of the amounts of oxidized carbohydrate and fat.

The aim of this paper is to describe general formulae for indirect calorimetry and to discuss their usefulness for anaerobic heat production measurements.

Key words: indirect calorimetry, heat production, anaerobic metabolism.

To avoid the lengthy process of deriving the formulae every time they have to be adjusted, it is useful to derive general formulae. From Table 1 a system of three equations with three unknown factors (C, F and H; X_I is treated as a known factor) can be derived. More than one endproduct (X_I) is possible: I from 1 up to N. The amounts of consumed O_2 and produced CO_2 and heat per gram of substrate are indicated by the symbols a to k_I. This system can be resolved into three formulae (Table 2). As the factors d_I, g_I and k_I are only used in the coefficients of the

Table 1. *The amounts of oxygen consumed and of carbon dioxide and heat produced on oxidation of carbohydrate, fat and protein*

Oxidation of	O_2 consumption (mol)	CO_2 production (mol)	H (heat production) (J)
C g carbohydrate	aC	aC	hC
F g fat	bF	eF	iF
P g protein	cP	fP	jP
X_I g endproduct	$-d_IX_I$	$-g_IX_I$	$-k_IX_I$

Three related equations can be derived from the above.

$$O_2 = aC + bF + cP - \sum_{I=1}^{N} d_IX_I$$

$$CO_2 = aC + eF + fP - \sum_{I=1}^{N} g_IX_I$$

$$H = hC + iF + jP - \sum_{I=1}^{N} k_IX_I$$

X_I is an endproduct of incomplete oxidation for which O_2 consumption, CO_2 production and heat production should be corrected (1 endproduct I = 1, more endproducts I = N).

Table 2. *The general formulae for the calculation of the amounts of oxidized carbohydrate (C) and fat (F) and the heat production (H), which can be derived from Table 1*

$$F = \underset{I=1}{m}O_2 - mCO_2 + nP + \sum^{N} o_IX_I \quad (g)$$

$$C = pO_2 + qCO_2 + rP + \sum_{I=1}^{N} s_IX_I \quad (g)$$

$$H = tO_2 + uCO_2 + vP + \sum_{I=1}^{N} w_IX_I \quad (J)$$

$m = 1/(b-e)$, $n = mf-mc$, $o_I = md_I-mg_I$, $p = -e/(ab-ae)$, $q = b/(ab-ae)$, $r = -pc-qf$ $s_I = pd_I+qg_I$, $t = (ia-he)/(ab-ae)$, $u = (hb-ia)/(ab-ae)$, $v = -tc-uf+j$, $w_I = td_I+ug_I-k_I$.
O_2, CO_2 in mol, P and X_I in g.

endproduct X_I, it can easily be seen that the formulae can also be used for aerobic situations ($X_I = 0$).

As an example, the formulae of Table 2 are adjusted for goldfish during anoxia. Goldfish under anoxic conditions produce ethanol as the major endproduct of incomplete oxidation (Shoubridge & Hochachka, 1980; Van den Thillart & Van Waarde, 1985). As this ethanol is excreted into the surrounding water it can be measured accurately. Lactic acid is a minor endproduct of anaerobic metabolism of goldfish. Lactic acid, as well as any other acidic endproduct, undergoes a side reaction with the buffers of the body fluids. Because of this neutralization reaction, the heat production is raised by approximately 20 kJ per mole of accumulated acids, such as lactic acid (Gnaiger, 1980, 1983*c*).

Based on the constants given by Brafield (1985) for carbohydrate (C), fat (F) and protein (P) oxidation of fish, the following indirect calorimetric formulae, adjusted for ethanol ($X_1 = E$), lactic acid ($X_2 = L$) and the neutralization reaction of lactic acid ($X_3 = N_L$) can be derived; Brafield (1985) only gave a formula for normoxic heat production (H) calculations:

$$F = 0{\cdot}0383O_2 - 0{\cdot}0383CO_2 - 0{\cdot}0728P + 0{\cdot}8314E + 0L + 0N_L \text{ (mg)},$$

$$C = -0{\cdot}0673O_2 + 0{\cdot}0947CO_2 - 1{\cdot}0865P - 0{\cdot}2645E + 0{\cdot}9148L + 0N_L \text{ (mg)},$$

$$H = 0{\cdot}3565O_2 + 0{\cdot}1160CO_2 - 1{\cdot}8631P - 1{\cdot}4083E + 0{\cdot}6352L + 0{\cdot}2222N_L \text{ (J)}.$$

O_2 and CO_2 are in μmol, P, E and L are in mg, and N_L is in mg L. For $X_1 = E$, d_1, g_1 and k_1 (Table 1) equals $65{\cdot}2\,\mu\text{mol}\,O_2\,\text{mg}^{-1}\,E$, $43{\cdot}5\,\mu\text{mol}\,CO_2\,\text{mg}^{-1}\,E$ and $29{\cdot}7\,\text{J}\,\text{mg}^{-1}\,E$, respectively. For $X_2 = L$, d_2, g_2 and k_2 equal $33{\cdot}3\,\mu\text{mol}\,O_2\,\text{mg}^{-1}\,L$, $33{\cdot}3\,\mu\text{mol}\,CO_2\,\text{mg}^{-1}\,L$ and $15{\cdot}1\,\text{J}\,\text{mg}^{-1}\,L$, respectively. For $X_3 = N_L$, d_3, g_3 and k_3 equal $0\,\mu\text{mol}\,O_2\,\text{mg}^{-1}\,L$, $0\,\mu\text{mol}\,CO_2\,\text{mg}^{-1}\,L$ and $-0{\cdot}2222\,\text{J}\,\text{mg}^{-1}\,L$, respectively.

So if respiration and ethanol and lactic acid production can be measured accurately, the anaerobic heat production and fat and carbohydrate oxidation of goldfish can be calculated with these formulae. They can also be used for goldfish during normoxia with E, L and $N_L = 0$.

The indirect calorimetric formulae, which are adjusted for the endproducts of anaerobic metabolism, can only be used if those endproducts can be measured accurately. For the ethanol production of goldfish in the example, this means that the ethanol accumulation in the fish should reach a stable concentration after which all the produced ethanol is excreted into the surrounding water. Because lactic acid is not excreted into the surrounding water, it cannot be measured in the living animal. However, if lactic acid accumulation also reaches a stable concentration during anoxia, there is no net lactic acid production. So if both ethanol and lactic acid accumulation reach a stable concentration, the fish is in thermodynamic steady-state with respect to ethanol and lactic acid production. In that case the formulae can be used with $L = 0$ and E = excreted ethanol.

The present formulae can therefore be used if the animal reaches thermodynamic steady-state during anaerobic metabolism. Biochemical determinations of metabolite concentrations of whole animals will be necessary to establish whether

steady-state is reached for the endproducts of anaerobic metabolism. According to Van den Thillart *et al.* (1976) the lactate level of whole goldfish reaches a stable concentration after approximately 6 h of anoxia. If for one of the endproducts the animal does not reach steady-state, this unknown production can be determined by simultaneous direct and indirect calorimetry: H measured by direct calorimetry equals H measured by indirect calorimetry. The amounts of oxidized carbohydrate and fat can then be calculated with the respective indirect calorimetric formulae. The normoxic and anoxic heat production of goldfish measured by direct calorimetry was recently described by Van Waversveld *et al.* (1987).

As suggested by Gnaiger (1983*a*), simultaneous direct and indirect calorimetry can assist in exploring mechanisms of metabolic energy expenditure. Our new approach of indirect calorimetry adds an important new perspective to the suggestion of Gnaiger (1983*a*). Finally it should be stated that the formulae have to be adjusted for every new situation. This can easily be done with the help of Tables 1 and 2 by adjusting the constants $a-k_I$ inclusive, according to the new situation. The formulae given for goldfish are to be seen only as examples. In this example the formulae are derived for goldfish, partly based on the constants given by Brafield (1985). Whenever other constants are required, the formulae have to be adjusted. For example Brafield (1985) did not account for CO_2 excretion into the buffer system of the aquatic environment, which is thermodynamically different from CO_2 excretion into air. Because of this difference, the heat production is raised by 0–8 kJ mol^{-1} (Gnaiger, 1983*c*). If desired, this can be treated according to the neutralization reaction of lactic acid as described in the example.

Indirect calorimetric formulae for the calculation of heat production and carbohydrate and fat oxidation of all species under all possible experimental conditions can easily be derived with the three general formulae of Table 2.

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