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Dynamic aspects of oxygen consumption and carbon dioxide production in swine

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A model is proposed that allows study of the short-term dynamics of gas exchanges (and heat production) in large open-circuit respiration chambers. The model describes changes in $[O_2]$ and $[CO_2]$ in the respiration chamber by a series of differential equations based on animal metabolism and physical characteristics of gas exchange. The model structure was similar for O_2 and CO_2, although model parameters differed. A constant level of O_2 consumption (and CO_2 production) was assumed for resting animals which was different for fed and fasted animals. The adaptation from a fed to a fasting state was described as a first-order process. Physical activity (standing or sitting) was recorded and was included in the model as a constant. Thermic effect of feed comprised the O_2 consumption and CO_2 production related to several relatively rapidly occurring processes after ingestion of a meal (e.g. ingestion, digestion or absorption). In the model, these processes were pooled into a single phenomenon. Model parameters were obtained statistically by comparing model predictions (based on the numerically integrated differential equations) with the observed $[O_2]$ and $[CO_2]$. The model was evaluated by studying gas exchanges in growing pigs that were fasted for 31 h and re-fed a single meal thereafter. The model fitted the data well over the 47 h measurement range. Traditional methods in which heat production is calculated suffer from noisy data when the interval between observations becomes too short. The proposed method circumvents this by modelling the observed concentration of gases in the respiration chamber rather than the calculated heat production.

Energy metabolism: Calorimetry: Modelling

Animals produce heat from a variety of metabolic processes such as maintenance, thermoregulation, physical activity and production (e.g. deposition of body tissue, milk production). Indirect calorimetry has played an important role in measuring this heat production in both animals and human subjects. In open-circuit respiration chambers, heat production can be calculated based on measurements of O_2 consumption and CO_2 production without hampering the normal behaviour of the animal (Brown *et al.* 1984). Due to the size of the respiration chamber in relation to the subject of study, the technique has been of limited use in studying the short-term dynamics of energy metabolism. An instantaneous change in O_2 consumption (or CO_2 production) by the subject results in a small and prolonged response at the gas analyser. The limited sensitivity of gas analysers in combination with the large volume of the respiration chamber and short interval between measurements leads to noisy data when heat production is calculated (McLean & Watts, 1976; Brown *et al.* 1984; McDonald *et al.* 1988).

The objective of the present study was to propose an alternative method to study the dynamics of gas exchange in open-circuit respiration chambers. In traditional methods,

data for $[O_2]$ and $[CO_2]$ are used in the algebraic calculation of gas exchanges and the resulting heat production. The latter can then be regressed *v.* a series of observations (e.g. time, activity, ingestion of a meal) using an 'animal' model. In the proposed method, it is not the calculated heat production but the observed changes in gas concentrations that are regressed *v.* the observations, using both an 'animal' model as well as a 'respiration chamber' model. This approach circumvents the calculation of heat production based on observations, as predicted values for $[O_2]$ and $[CO_2]$ are compared directly with observed values.

MATERIALS AND METHODS

The approach taken in this study was to identify components of O_2 consumption and CO_2 production by the animal in a model and to estimate, statistically, parameters of this model in order to obtain a suitable fit to observed data for $[O_2]$ and $[CO_2]$ in the respiration chamber. The model is described by a series of differential equations based on animal metabolism and physical characteristics of gas exchange in the respiration chamber. The differential equations are integrated numerically and then serve in a statistical optimization function. If desired, heat production may be calculated as an auxiliary function of estimated parameters.

Equipment

Two open-circuit respiration chambers of approximately 2 and 12 m^3 respectively were used based on a design similar to that of Vermorel *et al.* (1973). The respiration chambers were air-conditioned to maintain constant temperature (26·0 (SD 0·1)°) and humidity (70 % relative humidity) during the experiment. Each chamber contained an individual metabolism cage equipped with two infrared detectors to detect physical activity of the animal. Interruption of an infrared beam for at least 20 s was considered to be a physical activity (i.e. standing or sitting) of the animal. Weight of the feeder was measured continuously. The chambers were lit between 08.00 and 20.00 hours.

Gas was extracted continuously from the respiration chamber by an exhaust fan which was assumed to have a constant extraction rate. Due to the extraction of gas and the limited opening of the gas inlet, the pressure in the respiration chamber was approximately 1 kPa lower than the atmospheric pressure. This underpressure ensured that no gas leakage occurred from the respiration chamber. Temperatures (at the gas meter and in the respiration chamber) and atmospheric pressure were measured continuously. Variations in the atmospheric pressure and temperature were used to calculate the standard temperature and pressure (STP; 0°, 101 kPa) extraction rate. Unless stated otherwise, all volumes were calculated as STP volumes.

The $[O_2]$ was measured with paramagnetic differential O_2 analyser (Oxygor 6N, Maihak AG, Hamburg, Germany) whereas $[CO_2]$ was measured with an absorption infrared analyser (Unor 6N, Maihak AG, Hamburg, Germany). The analysers had a range of measurement of 0·01 (e.g. 0·20–0·21 for $[O_2]$ and 0·00–0·01 for $[CO_2]$) with a sensivity of 0·2 % within the measurement range. The analysers were calibrated daily with a gas of known composition. Gas concentrations were measured between 50 and 60 times/s. To reduce data volume, 10 s averages were stored on a microcomputer awaiting further analysis. Gases other than O_2 and CO_2 (primarily N_2) were calculated as $[N_2] = 1 - [O_2] - [CO_2]$.

Verification of the whole system was performed monthly through gravimetry by infusing either CO_2 or N_2 (to simulate O_2 consumption) in the respiration chamber. After attaining a steady-state for $[O_2]$ and $[CO_2]$ at the gas analysers, the measured 'O_2 consumption' and CO_2 production were compared with the infused quantities. The system was considered operational when the deviation was less than 1 %.

Physical aspects of gas exchange

In the traditional methods, heat production is calculated based on O_2 consumption and CO_2 production (Brouwer, 1965). In the case of O_2 the volume consumed ($\dot{V}_{O_2,consumption}$; litres/h) is calculated as the difference between the inflow ($\dot{V}_{O_2,in}$; litres/h) and outflow ($\dot{V}_{O_2out}$; litres/h) minus the change in volume of gas in the chamber ($\dot{V}_{O_2,chamber}$; litres/h) between two successive measurements (Vermorel *et al.* 1973; Brown *et al.* 1984; Verstegen *et al.* 1987):

$$\dot{V}_{O_2,consumption} = \dot{V}_{O_2,in} - \dot{V}_{O_2,out} - \dot{V}_{O_2,chamber}. \quad (1)$$

The notation $\dot{V}$ indicates the first derivative of V with respect to time (i.e., $\dot{V} = \partial V/\partial t$). The $\dot{V}_{O_2,chamber}$ is calculated as the product of volume of gas in the respiration chamber and the rate of change in $[O_2]$ between successive measurements (i.e. $\dot{V}_{O_2,chamber} = V_{gas,chamber}\partial[O_2]/\partial t$). It is evident that when ∂t approaches zero, one has to measure infinitesimal changes in $\partial[O_2]$ to maintain precision of $\dot{V}_{O_2,chamber}$ and thus $\dot{V}_{O_2,consumption}$.

The proposed model of the respiration chamber was based on a series of differential equations describing the change in volume of O_2, CO_2 and other gases (primarily N_2). Similar to Equation (1), the changes in volume of O_2, CO_2, and N_2 can be described by:

$$\begin{aligned} \dot{V}_{O_2,chamber} &= \dot{V}_{O_2,in} - \dot{V}_{O_2,out} - \dot{V}_{O_2,consumption}, \\ \dot{V}_{CO_2,chamber} &= \dot{V}_{CO_2,in} - \dot{V}_{CO_2,out} + \dot{V}_{CO_2,production}, \\ \dot{V}_{N_2,chamber} &= \dot{V}_{N_2,in} - \dot{V}_{N_2,out}. \end{aligned} \quad (2)$$

The volume of these gases can be obtained through numerical integration of these equations with respect to time. The total volume of gas in the chamber is:

$$V_{gas,chamber} = V_{O_2,chamber} + V_{CO_2,chamber} + V_{N_2,chamber},$$

from which the gas concentrations were calculated (e.g. $[O_2] = V_{O_2,chamber}/V_{gas,chamber}$).

In open-circuit calorimetry, only the outflow of gas is measured. The instantaneous inflow was determined by comparing the STP equivalent of the physical volume of the respiration chamber ($V_{physical,chamber}$) with $V_{gas,chamber}$ so that:

$$\dot{V}_{in} = \left(V_{physical,chamber} \frac{273{\cdot}15 \times P_{chamber}}{T_{chamber}} \right) - V_{gas,chamber},$$

where $T_{chamber}$ is the temperature in the respiration chamber (°K) and the $P_{chamber}$ is the pressure in the respiration chamber (atm). The inflow for each gas is then the product of $\dot{V}_{in}$ and the fraction of these gases in the environment (O_2: 0·2095, CO_2: 0·0003, N_2: 0·7902).

To estimate $V_{physical,chamber}$ precisely, a procedure similar to the monthly system verification was used. N_2 or CO_2 was infused at a constant rate in the respiration chamber, which was fully equipped but did not contain any animals. The differential equations

describing the change in volume of the gases were modified to accommodate the infusion of either CO_2 or N_2.

$$\dot{V}_{O_2,chamber} = \dot{V}_{O_2,in} - \dot{V}_{O_2,out},$$
$$\dot{V}_{CO_2,chamber} = \dot{V}_{CO_2,in} - \dot{V}_{CO_2,out} + \dot{V}_{CO_2,injection},$$
$$\dot{V}_{N_2,chamber} = \dot{V}_{N_2,in} - \dot{V}_{N_2,out} + \dot{V}_{N_2,injection}.$$

The infusion continued until a plateau was approached for $[CO_2]$ or $[O_2]$. After termination of the infusion, $[CO_2]$ and $[O_2]$ continued to be measured until they approached the environmental values of 0·0003 and 0·2095 respectively. The differential equations were solved numerically with the SimuSolv program (Steiner *et al.* 1990). This program was also used to estimate statistically the volume of gas in the respiration chamber and the delay between infusion of gas and first signal appearance at the gas analyser based on the kinetics of the change in $[CO_2]$ and $[O_2]$. These estimates were included in the model and assumed to be constant for the remainder of the experiment. The volume of gas in the respiration chamber was adjusted for the presence of animals assuming a density of 1·0 kg/l for the animals.

Oxygen consumption and carbon dioxide production by pigs

Six pigs (three castrated Large White males, one Large White boar, one Piétrain boar and one castrated Meishan male) were placed in individual metabolism cages. The animals weighed between 28 and 58 kg (Table 1) and were fed *ad libitum* on a diet containing (g/kg): crude protein 220, starch 440, fat 36 and neutral-detergent fibre (NDF) 160. The energy contents of the feed (MJ/kg DM) were 18·1, 15·9 and 15·5 for gross, digestible and metabolizable energy respectively. The animals remained on this diet for at least 10 d (with the last 4 d in the respiration chamber) after which they were subject to a 31 h fast. At the end of this fast, the animals received a single meal of the previously described diet (30 % of their daily consumption). The animals had free access to water throughout the duration of the experiment. The data used in the present study concerned the change in $[O_2]$ and $[CO_2]$ in the respiration chamber from the beginning of the fast to 16 h after ingestion of the test meal (47 h).

To provide the animals with their ration and to remove faeces, the respiration chambers had to be opened briefly. The quantities of gas entering (O_2) or leaving (CO_2) the chamber were determined from the gas concentration immediately before and after the opening of the respiration chamber and the physical volume of the chamber. The differential equations describing the change in gas concentration were modified accordingly to account for the opening of the chamber.

The model stuctures for O_2 consumption and CO_2 production were essentially similar and only the former will be used for describing the model. Nevertheless, it is important to note that in modelling the dynamics of gas exchange in the respiration chamber, both O_2 consumption and CO_2 production have to be modelled simultaneously. Due to dilution effects, the $[O_2]$ may decrease only because CO_2 is being produced.

Total O_2 consumption (and CO_2 production) by the animal was subdivided into O_2 consumption due to resting metabolism, physical activity, and thermic effect of feeding (TEF). Preliminary results indicated that fed animals have a different resting (i.e. constant) O_2 consumption to that of fasting animals. The adaptation from the fed resting state ($\dot{V}_{O_2,fed}$; litres/h) to the fasting resting state ($\dot{V}_{O_2,fasting}$; litres/h) was described as a first-order decline with mean adaptation time $T_{O_2,adaptation}$ (h). To maintain simplicity of the

model, the animals were assumed to be initially in a fed resting state which was subject to the first-order decline. The animals attained the fasting metabolic state asymptotically. The resting O_2 consumption is then given by:

$$\dot{V}_{O_2,\text{resting}} = \dot{V}_{O_2,\text{fasting}} + (\dot{V}_{O_2,\text{fed}} - \dot{V}_{O_2,\text{fasting}}) \times \exp\left(\frac{-t}{T_{O_2,\text{adaptation}}}\right),$$

where t is time after the beginning of the fasting period (i.e. the beginning of the experiment). No corrections to the resting state were made during or after ingestion of the test meal.

O_2 consumption due to physical activity ($\dot{V}_{O_2,\text{activity}}$; litres/h) was assumed to be constant over the measured period and expressed only when the animal was active (i.e. when at least one of the infrared beams was interrupted for at least 20 s).

TEF was defined as the dynamic component of O_2 consumption related to intake of a meal (i.e. ingestion, digestion, absorption and metabolism). Some of the components of TEF are associated with a temporary increase in O_2 consumption (e.g. ingestion) whereas others result in a more continuous O_2 consumption (e.g. absorption and metabolism). Because these components cannot be identified separately, a model was used in which the O_2 consumption after the ingestion of the test meal was described as a gamma distribution of time. This type of model has been widely used in describing digesta passage in ruminants (Matis *et al.* 1989). In the current context, it represents a distribution of time during which O_2 will be consumed after ingestion of a meal. On ingestion, feed will enter a compartment X (kg; Fig. 1). The inflow to this compartment is determined by the rate of feed ingestion whereas the outflow is determined by a gamma distribution of residence times. Feed intake was assumed to be constant during a meal (i.e. quantity eaten divided by the duration of the meal) and zero between meals. O_2 consumption due to TEF ($\dot{V}_{O_2,\text{TEF}}$; litres/h) was assumed to be proportional to the outflow from X. The gamma-distribution of residence times in compartment X was solved as a series of compartments (in this case: two) with identical fractional outflow rates (k_X; /h). Mathematically:

$$\begin{aligned}
\dot{X}_1 &= \text{rate of intake} - k_X X_1,\\
\dot{X}_2 &= k_X X_1 - k_X X_2,\\
\dot{X} &= \text{rate of intake} - k_X X_2,\\
\dot{V}_{O_2,\text{TEF}} &= O_{2,\text{TEF}} k_X X_2,
\end{aligned}$$

where $O_{2,\text{TEF}}$ is the proportionality factor (litres O_2 consumed/kg feed ingested). The X_1 and X_2 indicate two sequential compartments to solve the system with a gamma distribution of residence times. The mean residence time in X (i.e. the mean time between ingestion of food and its related O_2 consumption) is given by $2/k_X$ and the variance of the distribution by $2/(k_X)^2$. To simplify interpretation, the model was parametrized to include the mean time between ingestion of a meal and its related O_2 consumption ($T_{TEF} = 2/k_X$; h) rather than k_X. O_2 consumption by the animal (litres/h) is then given by:

$$\dot{V}_{O_2,\text{consumption}} = \dot{V}_{O_2,\text{resting}} + \dot{V}_{O_2,\text{activity}} + \dot{V}_{O_2,\text{TEF}}.$$

A data table was constructed containing records for time, $[O_2]$, $[CO_2]$, and the atmospheric pressure. Because the data table in the program to solve the differential equations was limited to 10 000 elements, no more than 2500 records could be used in the data analysis. As the experiment lasted 47 h, mean $[O_2]$, $[CO_2]$, and the atmospheric pressure were calculated for 70 s intervals (based on the 10 s averages stored on the

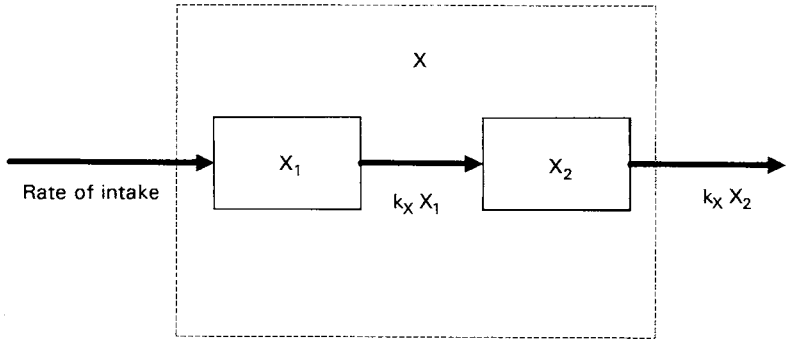

Fig. 1. Compartmental representation describing the dynamics of the thermic effect of feed (TEF). The compartments X_1 and X_2 (with fractional outflow rate k_X, /h) are used to solve the gamma distribution of time between the ingestion of feed and the resulting O_2 consumption and CO_2 production. The O_2 consumption and CO_2 production due to TEF are proportional to the outflow from X (i.e. k_xX_2).

microcomputer). It is evident that for shorter experiments, a smaller time interval can be used.

The system of differential equations (Equations 2) was solved through numerical integration using the Gear's backward difference formulas of the SimuSolv program (Steiner *et al.* 1990). The integration step-size varied but did not exceed the interval between observations (70 s). Intermediate values for atmospheric pressure were obtained through smoothed interpolation of the recorded values. Initial values for the volumes of O_2, CO_2, and N_2 were calculated from the first data record and the STP equivalent of the physical volume of the respiration chamber. Initial values for X_1 and X_2 were based on the meal intake from the previous day assuming a mean residence time in X of 5 h.

Parameters were estimated by maximization of a log likelihood function (Bard's likelihood standard reduced model; Steiner *et al.* 1990) using $[O_2]$ and $[CO_2]$ as dependent variables and time, activity, and feed intake as independent variables and assuming a multivariate normal distribution for the observations. The errors were assumed to be constant and uncorrelated from observation to observation, and from response variable to response variable. Model parameter estimators included $\dot{V}_{O_2,\text{fasting}}$, $\dot{V}_{CO_2,\text{fasting}}$, $\dot{V}_{O_2,\text{fed}}$, $\dot{V}_{CO_2,\text{fed}}$, $T_{O_2,\text{adaptation}}$, $T_{CO_2,\text{adaptation}}$, $\dot{V}_{O_2,\text{activity}}$, $\dot{V}_{CO_2,\text{activity}}$, $O_{2,\text{TEF}}$, $CO_{2,\text{TEF}}$, and T_{TEF}. Likelihood functions describing the change in $[O_2]$ and $[CO_2]$ were analysed simultaneously due to the interrelationship between gases in the respiration chamber mentioned earlier. Heat production (kJ/h) was calculated according to Brouwer (1965) excluding urinary-N and CH_4 production. Standard errors of functions of parameters were calculated from the parameter variance–covariance matrix. Normalized sensitivity coefficients were calculated as $\partial y_j\, \theta_k / \partial \theta_k\, y_j$, where y_j is the response variable (e.g. $[O_2]$) and θ_k is the parameter value (e.g. $\dot{V}_{O_2,\text{fasting}}$) and indicates the percentage change in the response variable due to a percentage change in parameter value.

RESULTS

An example of the evolution of observed values for $[O_2]$ together with the physical activity and the duration of ingestion of the test meal is given in Fig. 2. The $[O_2]$ initially decreases and then increases to attain (approximately) a constant value until the ingestion of the test meal. The initial decrease is due to establishing quasi-equilibrium conditions in the

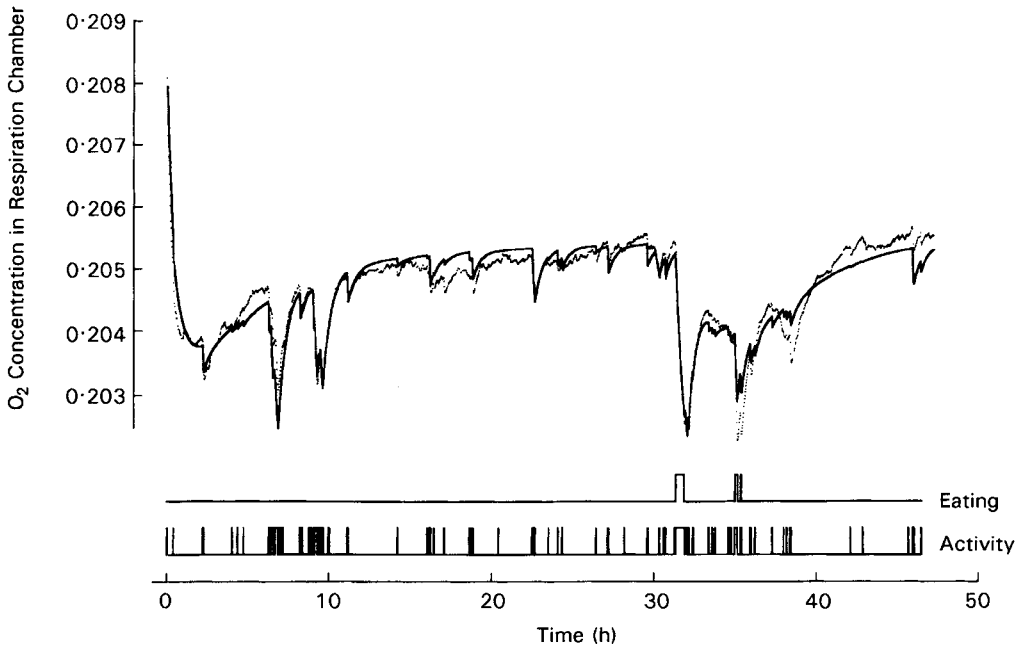

Fig. 2. Dynamics of predicted (———) and observed (·····) O_2 concentration in the respiration chamber containing a 40 kg pig. The animal was subject to a 31 h fast after which it received a test meal (one third of the daily *ad libitum* intake). Physical activity (i.e. standing or sitting) and eating are indicated as true (high value) or false (low value).

respiration chamber. The door of the chamber had been open until just before the start of the experiment. As a result, the initial $[O_2]$ and $[CO_2]$ were close to environmental values. Due to O_2 consumption by the animal, the $[O_2]$ drops rapidly after the start of the experiment. The kinetics of the initial decline of $[O_2]$ are determined primarily by the volume of the respiration chamber and the gas extraction rate. If the animal maintained a constant level of O_2 consumption, the $[O_2]$ would attain a plateau whose level would be determined by the level of O_2 consumption. In fact, if pressure and temperature in the chamber were constant, the kinetics of the initial decline could have been described by a first-order model. However, the variation in $[O_2]$ indicates that the O_2 consumption is not constant. The increase in $[O_2]$ between $t = 4$ h and $t = 15$ h indicates that the animal is consuming less O_2 than before. In the model, this change is accounted for by a change in resting O_2 consumption from a fed towards a fasting state and the diminishing thermic effects of feed ingested before the start of the experiment. Another marked event is the ingestion of the test meal at $t = 31$ h when the animal ate 0·65 kg in 25 min. The decrease in the $[O_2]$ was much faster than the increase, indicating that ingestion (or digestion and absorption) of feed results in a prolonged increase in O_2 consumption. Other variations in $[O_2]$ were primarily due to physical activity. It contrast to the statistical assumptions that were made, Fig. 2 shows that residuals were correlated over time. As a result, asymptotic standard errors have to be interpreted with caution.

Table 1 lists some of the observed characteristics for the pigs in the respiration chambers, whereas Table 2 lists the parameter estimates that were obtained from maximization of the log likelihood function with precision estimates for the model. The model predictions corresponded reasonably well with the observations for both the small and large respiration chamber. The residual standard error was approximately ten times the sensitivity of the gas analyser. The sensitivity coefficients vary dynamically with time. For

Table 1. *Animal characteristics, physical activity and ingestion of a test meal after 31 h of fasting*

Animal...	1	2	3	4	5	6
Breed	LW	LW	LW	LW	Piétrain	Meishan
Type	castrate	castrate	castrate	boar	boar	castrate
Weight of pig (kg)	28	39	58	42	42	40
Volume of chamber (litres)	2100	2100	11700	11700	2100	2100
No. of physical activities	61	74	60	117	65	86
Total activity (h)	2·3	2·9	3·0	3·9	4·8	5·8
Activities lasting more than 1 min (h)	2·1	2·7	2·8	3·5	4·7	5·5
No. of meals	3	3	3	3	1	1
Test meal (kg)	0·59	0·75	0·94	0·70	0·57	0·64
Duration of eating (h)	0·96	0·71	0·69	0·59	0·72	0·61
Initial X_1* (kg)	0·24	0·31	0·54	0·34	0·15	0·12
Initial X_2* (kg)	0·24	0·18	0·16	0·24	0·15	0·16

LW, Large White.

* Initial values used in the compartmental representation of a gamma distribution of time between the ingestion of feed and the resulting O_2 or CO_2 production.

example, the sensitivity of $[O_2]$ for $\dot{V}_{O_2,fasting}$ is initally important but approaches zero after 20 h. The maximum sensitivity coefficient for $[O_2]$ ranged from −3·0E-2 for $\dot{V}_{O_2,fed}$ to −7·0E-5 for $T_{O_2,adaptation}$. The $[O_2]$ and $[CO_2]$ were least sensitive to $T_{O_2,adaptation}$ and $T_{CO_2,adaptation}$ respectively.

The parameter estimates, together with the model described earlier, allow calculation of auxiliary variables such as the heat production (kJ/h) or RQ. An example of the evolution of the components of O_2 consumption is given in Fig. 3. It clearly illustrates the important contribution of resting fasting O_2 consumption to the total O_2 consumption, and the magnitude of change in O_2 consumption due to physical activity.

DISCUSSION

Calculation method

Rapid changes in O_2 consumption or CO_2 production are difficult to detect when using large respiration chambers due to the large volume of gas in the chamber. Traditionally, heat production is calculated based on physical aspects of the gases (temperature, pressure) and the size of the respiration chamber. The generated data (heat production) are regressed *v.* a series of independent variables (e.g. time, activity, eating) using an 'animal' model. Because heat production is a dynamic phenomenon (its units include 'time') and is subject to rapid changes, calculations based on short time intervals can result in noisy data (Brown *et al.* 1984; McDonald *et al.* 1988). If a statistical model were to be employed to describe the heat production, the difference between observed and predicted values would be a function of the calculation error (noise) and the true error (accuracy of the model). With short ∂t, the calculation error would be much larger than the true error, rendering model evaluation impossible. To reduce the noise, several signal processing techniques have been proposed including calculation of moving averages (Brown *et al.* 1984; Sun *et al.* 1994), fitting cubic splines (Brown *et al.* 1994), Kalman filtering (Even *et al.* 1991) and deconvolution analysis of linear systems (McDonald *et al.* 1988). Nevertheless, the noise observed when ∂t approaches zero is inherent to a system where the calculation of heat production is based on observations.

Table 2. *Parameter estimates and derived statistics (energy production) from the animal model with asymptotic standard errors (ASE)*

Animal ...	1		2		3		4		5		6	
	Estimate	ASE	Estimate	ASE	Estimate	ASE	Estimate	ASE	Estimate	ASE	Estimate	ASE
Parameter												
$\dot{V}_{O_2,fasting}$*	14·61	0·04	17·28	0·05	21·29	0·05	19·85	0·03	16·89	0·05	13·09	0·03
$\dot{V}_{CO_2,fasting}$*	11·57	0·04	13·99	0·04	16·49	0·04	16·42	0·02	13·26	0·05	8·94	0·04
$\dot{V}_{O_2,fed}$†	15·81	0·09	19·83	0·07	24·52	0·17	24·18	0·09	19·09	0·09	22·22	0·13
$\dot{V}_{CO_2,fed}$†	13·11	0·08	18·66	0·12	31·88	0·22	28·45	0·14	20·07	0·10	21·88	0·08
$T_{O_2,adaptation}$‡	14·87	0·02	18·05	0·35	3·60	0·05	11·93	0·20	8·89	0·17	4·65	0·03
$T_{CO_2,adaptation}$‡	14·45	0·06	7·79	0·20	3·56	0·04	5·54	0·08	8·56	0·15	10·01	0·11
$\dot{V}_{O_2,activity}$§	13·22	0·06	16·71	0·20	15·13	0·16	9·00	0·13	16·89	0·19	8·79	0·08
$\dot{V}_{CO_2,activity}$§	9·38	0·03	11·30	0·18	13·21	0·14	7·72	0·09	14·24	0·15	7·08	0·07
$O_{2,TEF}$‖	60·65	1·32	52·02	2·65	52·90	1·71	42·50	1·58	41·31	2·41	56·74	1·58
$CO_{2,TEF}$‖	129·12	2·64	98·93	3·37	72·85	1·44	67·48	1·98	47·30	1·87	136·78	3·86
T_{TEF} (h)¶	4·57	0·02	5·17	0·03	3·37	0·02	3·90	0·02	3·58	0·02	7·96	
RSE-O_2**	2·1E-4		2·2E-4		1·6E-4		1·5E-4		2·5E-4		2·1E-4	
RSE-CO_2**	2·7E-4		2·7E-4		2·2E-4		1·1E-4		2·6E-4		2·0E-4	
Energy production††												
fasting (kJ/h)	295	1	350	1	428	1	404	1	340	1	257	1
fed (kJ/h)	322	2	415	1	557	3	534	2	410	2	470	2
activity (kJ/h)	261	1	327	3	311	3	185	2	345	3	178	1
TEF (kJ/kg)‡‡	1·63	0·03	1·34	0·05	1·22	0·03	1·03	0·03	0·91	0·04	1·61	0·03

* Volume of O_2 consumed or CO_2 produced in a fasting and resting state (litres/h).
† Volume of O_2 consumed or CO_2 produced in a fed and resting state (litres/h).
‡ Mean time to adapt from a fed–resting state to a fasting–resting state assuming an exponential distribution of adaptation time (h).
§ Volume of O_2 consumed or CO_2 produced during standing or sitting (litres/h).
‖ Volume of O_2 consumed or CO_2 produced due to the thermic effect of feed (litres/kg).
¶ Mean time between the ingestion of a meal and the resulting O_2 consumption or CO_2 production assuming a gamma-distribution of time (h).
** Residual standard error.
†† Calculated energy production according to Brouwer (1965).
‡‡ Thermic effect of feed.

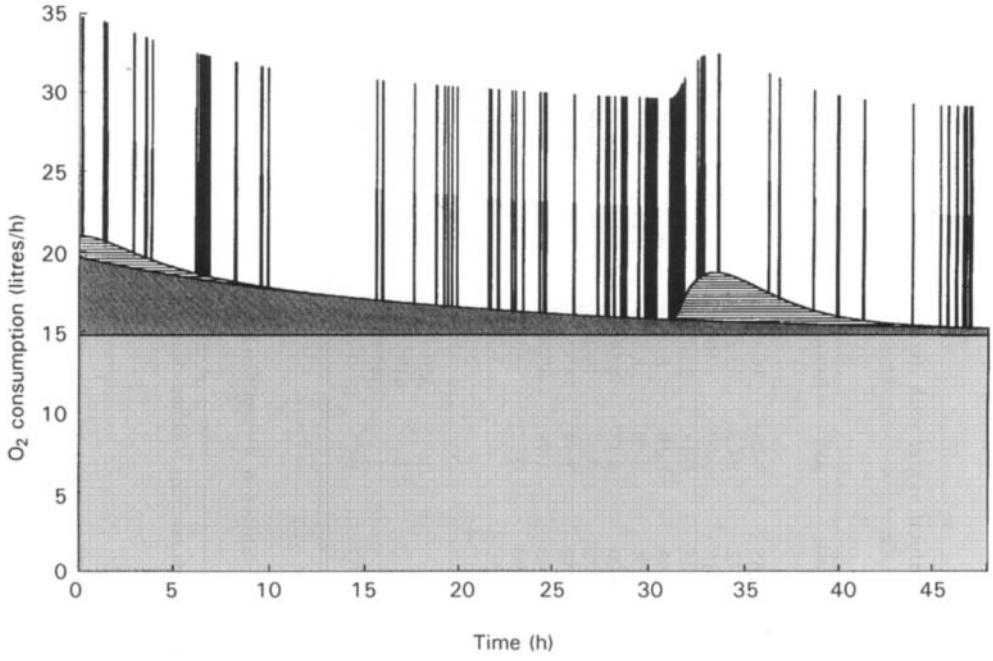

Fig. 3. Components of O_2 consumption in a 40 kg growing pig. The animal was subject to a 31 h fast after which it received a test meal (one third of the daily *ad libitum* intake). The components included fasting–resting O_2 consumption (▨), the adaptation from a fed–resting to a fasting–resting state (▧), the thermic effect of feed (▤) and O_2 consumption due to physical activity (■).

In large respiration chambers, gas concentrations fluctuate less than the calculated gas exchanges or heat production. In the proposed method, a statistical model was employed to predict directly the change in $[O_2]$ and $[CO_2]$ in the respiration chamber. The model was based on a series of differential equations which were integrated numerically. With decreasing ∂t, the solution of numerical integration approaches the true solution of a system of differential equations. As a result, precision of the prediction is increased and the difference between observed and predicted values is determined primarily by the appropriateness of the proposed model. The method has the advantage that short-term dynamics of energy metabolism can be studied with few numerical constraints. Nevertheless, the combination of short ∂t (e.g. < 1 s) between measurements and long duration of the experiment (several days) results in large data matrices that have to be analysed statistically. Statistical analysis of an experiment on a SUN SPARCStation 10 typically required less than 20 min of the central processing unit (CPU). With the rapid development of computer hardware and software, it is unlikely that data matrix size and computer CPU requirement will impose major barriers for the type of analysis proposed here.

Resting metabolism

Data on feed intake of the day preceding the start of the experiment indicated that three animals (2, 3 and 4) had eaten shortly before the withdrawal of food, which resulted in higher initial values for X_1. Although this was accounted for in the calculation of TEF, no adjustments were made for the resting metabolic state. In other words, the last meal for the

other animals occurred earlier, and at the start of the experiment these animals were 'fasting voluntarily'. Therefore, $\dot{V}_{O_2,fed}$ and $\dot{V}_{CO_2,fed}$ have to be interpreted with caution as it is difficult to define precisely a point in time at which animals will start adapting their resting metabolism.

The mean adaptation time from a fed resting state to a fasting resting state was 10·3 h for O_2 and 8·3 h for CO_2 but this was highly variable between animals. The $[O_2]$ and $[CO_2]$ are relatively insensitive to the adaptation time even though the asymptotic standard errors for $T_{O_2,adaptation}$ and $T_{CO_2,adaptation}$ are small. The adaptation time will play a role during the first few hours of the experiment. During these hours, the most important source of variation in $[O_2]$ and $[CO_2]$ is the closing of the respiration chamber which is controlled by constants such as the volume of the respiration chamber and air extraction rate. As a result, the adaptation from the fed to a fasting state only accounts for a small proportion of the observed variation in gas concentration. The limited sensitivity of the gas concentrations for the adaptation time indicates that the model and parameters that are used to describe the adaptation from a fed to a fasting state have to be interpreted with caution. Nevertheless, suppression of the adaptation part of the model (i.e. assuming that $\dot{V}_{O_2,fasting} = \dot{V}_{O_2,fed}$ and $\dot{V}_{CO_2,fasting} = \dot{V}_{CO_2,fed}$, suppression of $T_{O_2,adaptation}$ and $T_{CO_2,adaptation}$ and re-adjusting the remaining parameters) did not result in an accurate fit of the data. In order to study precisely the adaptation from a fed to a fasting state, it is important to minimize the variation in gas concentrations due to external sources. Opening of the respiration chamber should therefore be limited as much as possible.

For some of the animals, the observed $[O_2]$ was consistently slightly lower than predicted between 12 and 24 h after the start of the experiment. The observed $[O_2]$ remained stable or even decreased whereas the model predicted an increase in $[O_2]$ in the respiration chamber due to the reducing O_2 consumption when adapting from the fed to the fasting–resting state. Although total heat production is generally reduced after prolonged fasting, there are indications that heat production in human subjects increases during the first 2 d of starvation (Elia, 1992; Webber & Macdonald, 1994; Macdonald & Webber, 1995) compared with an overnight fast. This has been attributed to increased gluconeogenesis, ketogenesis and differences in energetic efficiencies between glucose and fat utilization for ATP synthesis.

Both O_2 consumption and CO_2 production decreased during fasting, although the latter decreased to a greater extent (Table 2). As a result, the resting RQ will be lower in the fasting state than in the fed state. The fact that $RQ_{fasting}$ is less than 1 indicates that lipids and/or proteins are oxidized to produce energy. Differences in resting metabolism between animals are partially due to differences in (metabolic) body weight. The fasting energy production (at zero activity) averaged 0·51 (SD 0·07) MJ/d per $kg^{0.75}$ which was intermediate between values found by McDonald *et al.* (1988; 0·45 MJ/d per $kg^{0.75}$) and Bernier & Noblet (1996; 0·55 MJ/d per $kg^{0.75}$) for pigs of similar weight.

Activity

During the 47 h period, between sixty and 117 periods of activity lasting more then 20 s were recorded; the longest period was associated with the ingestion of the test meal (Table 1). The O_2 consumption and CO_2 production due to physical activity were of similar magnitude to those of the fasting state. In other words, a fasting animal doubles its heat production when it is sitting or standing. The energy produced during activity averaged 16·8 (SD 5·0) kJ/$kg^{0.75}$ body weight (BW) per h (6·7 (SD 2·2) kJ/kg BW per h), a value close to those compiled by Noblet *et al.* (1993) for pigs, ranging in weight between 27 and

208 kg. McDonald *et al.* (1988) reported that in fasting pigs, physical activity increased heat production by 95 % for events lasting longer than 4 min and 119 % for shorter events (standing activities lasted 7 min 5 s on average whereas sitting never lasted longer than 4 min). In contrast, the average duration of sitting or standing activities in the present experiment was 3 min and a total of only 3·8 h activity was observed during the 47 h experiment. These results indicate that in growing pigs the limited contribution of activity to the total energy production is due more to the duration than to the energetic cost of activity.

In the current experiment, a constant O_2 consumption and CO_2 production was assumed during physical activity. Specific actions such as standing up and lying down could have been included in the model but were not considered here. There are indications (McDonald *et al.* 1988) that the energy expenditure during standing is far from constant due to movement, raising and lowering of the head and investigative behaviour. Also during lying down, stretching and positional changes may result in a different heat production. To account for the type of the activity, observation systems other than the ones used in this experiment have to be used.

The marginal RQ for activity ($\dot{V}_{CO_2,activity}/\dot{V}_{O_2,activity}$) averaged 0·79 which is similar to the RQ during fasting (average 0·78). This indicates that fatty acids (the contribution of protein oxidation is generally considered insignificant during mild exercise; Roberts *et al.* 1996) were the primary energy source for activity. Another explanation for the low marginal RQ for activity may be the bicarbonate transport in the blood. The CO_2 resulting from substrate oxidation may have remained dissolved in the blood and been released from the lungs later than detection of the (relatively) short-duration activity.

Thermic effect of feed

Although pigs in the present study consumed the test meal in two or three separate meals, more than 87 % was consumed during the first meal. In the current experiment, 6·9 % of the gross energy was used for TEF (8·3 % ME), a value similar to that found in adult human subjects (Piers *et al.* 1992).

The mean duration of TEF was 4·8 h. However, the distinction between TEF and fasting (or fed) resting metabolism is arbitrary. The function chosen to describe the dynamics of TEF aggregates events ranging from instantaneous changes in energy expenditure (e.g. ingestion) to more gradual ones (e.g. substrate assimilation). The latter may also be partially accounted for in the resting metabolism. As a result of this aggregation, no mechanistic interpretation should be attributed to this function other than an empirical description of time between the intake of feed and the resulting heat production. We also tried to describe the dynamics of TEF by a first-order exponential function, but this function over-estimated TEF during the first hours after ingestion of a meal (the maximum energy expenditure for a first-order model
is predicted at the ingestion of the meal). Reed & Hill (1996) used the model $y = A + Bt\exp(-t/C)$ to describe the dynamics of TEF. The model used here, which analytically can be described as $y = O_{2,TEF}X_{1,initial}(1/T_{TEF})^2 t\exp(-t/T_{TEF})^2$, is a special case of that used by Reed & Hill (1996) (i.e., $A = 0$, $B = O_{2,TEF}X_{1,initial}(1/T_{TEF})^2$, and $C = T_{TEF}$). It has the advantage that it can be written as a compartmental system, which simplifies solution if is to be used to describe ingestion of several meals during a day.

The marginal RQ for TEF averaged 1·76 causing the RQ to rise during digestion, absorption and assimilation of a meal. Although not often observed in human subjects, RQ values greater than unity are commonly observed in growing animals on low-fat, high-

carbohydrate diets (Jakobsen & Thorbek, 1991). Energy from carbohydrate supplied in excess of oxidation and glycogen storage repletion has to be stored as fat. Lipogenesis is composed of the conversion of glucose to fatty acid (RQ ∞) and glucose oxidation to regenerate NADPH (RQ 1). Depending on the stoichiometry of the latter, the RQ for *de novo* fatty acid synthesis from glucose has been reported to range from 1·9 to 9·6 (Elia & Livesey, 1988).

Strengths, weaknesses and the future of modelling

The model proposed here was based on three main components (resting, activity, and thermic effect of feeding). Although these components explained a large part of the variation, some systematic deviations between observations and predictions were apparent (Fig. 2). Some of these deviations may be attributed to the adaptation from a fed–resting state to a fasting–resting state. This adaptation is a complex one, and further refinement of the model would require additional observations (e.g. blood glucose and free fatty acid levels) in order to explain this adaptation physiologically. Other deviations between observed and predicted values may be attributed to the all-or-none notion of physical activity that was adopted for the model (i.e. standing or lying).

An important thing to consider is that the proposed model is a data analysis tool and not a simulation model. The goal is not to obtain the best fit of the data *per se*, but to obtain the best fit of a mechanistic model given a limited number of independent variables. The deviations between the observed and predicted values may be used to guide further research in this area, but do not exclude analysis of the current model parameters (although caution is required).

In the traditional analysis method, one could calculate energy production without a model (other than the assumptions made in the calculations) and visually evaluate the results. In the method proposed here, one is forced specifically to define a model in order to interpret the changing $[O_2]$ and $[CO_2]$ in the respiration chamber. Nevertheless, it is questionable whether a similar detailed analysis could have been performed with the classical methods. The problem of 'noisy data' would make analysis of physical activity more difficult and would probably mask some of the systematic deviations between the observed and predicted values seen here.

In conclusion, a (compartmental and statistical) modelling tool is presented to analyse dynamically and compartmentalize heat production in swine. Parameter estimates obtained from the model were coherent with current biochemical and physiological knowledge. Although the model that is used to explain the observed variation in gas concentrations has to be adapted to specific experimental conditions, the proposed methodology has general applicability. For instance, in animal production it may be used to further refine energy systems or energy requirements.

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