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Milk intake and carbon dioxide production of piglets determined with the doubly labelled water technique

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The present study was undertaken to study different methodological aspects of quantifying CO_2 production and milk intake of suckling piglets using the doubly labelled water (DLW) technique. In total, 37 piglets were enriched intraperitoneally with DLW to study equilibration time of ^{18}O (n = 3), to validate the estimation of milk intake and CO_2 production (n = 10) of piglets fed milk replacer and to quantify milk intake and CO_2 production of piglets nursed ordinarily by sows (n = 24). Enrichment of ^{18}O in expired air was analysed without any sample preparation, whereas enrichment of ^{18}O in serum was analysed after a minimum step of sample preparation, which included pipetting of the sample, blowing gaseous CO_2 into the vial for 3 s and equilibrating for 24 h. The ^{18}O enrichment of CO_2 in expired air was constant within 30–40 min of intraperitoneal injection, suggesting that DLW was equilibrated within the body water by that time. For piglets fed milk replacer, the estimation of the daily CO_2 production by the DLW method (64.0 ± 2.7 l CO_2/day) was in agreement with that obtained by respiration trials (64.7 ± 1.8 l CO_2/day). Furthermore, the intake of milk replacer (891 ± 63 g/day) determined by deuterium oxide (D_2O) dilution was similar in magnitude to that found by weighing the milk disappearance (910 ± 58 g/day). The milk intake of piglets fed milk replacer was comparable with that of sucking piglets, but sucking piglets had a remarkably higher CO_2 production than artificially reared piglets, which likely was caused by a higher intake of milk solids and a higher activity level. For sucking piglets, the daily CO_2 production increased curvilinearly with increasing live weight (LW) in kg: piglet CO_2 production (l/day) = $25.75 \times LW - 1.01 \times LW^2$. In conclusion, ^{18}O equilibrates fast within the body water pool when administered intraperitoneally, and the accuracy of assessing milk intake and rate of CO_2 production using the DLW technique is promising. Assessment of excess enrichment of ^{18}O in serum proved to be robust. Finally, the CO_2 production of piglets fed milk replacer differs considerably from that of sucking piglets.

Keywords: doubly labelled water, energy metabolism, heat production, milk intake, sucking piglets

Introduction

Quantitative studies on energy metabolism of sucking piglets are scarce, most likely because of difficulties in deriving reliable estimates of piglet milk intake, digestibility and metabolisability coefficients of ingested sow milk and of heat production (HE) of sucking piglets. In livestock animals, HE is preferably measured by indirect calorimetry because this method allows repeated measurements (Aguilera and Prieto, 1986). However, the technique implies that the sucking piglets and the sow must be kept together in a respiration chamber to allow the piglets to stimulate/maintain milk production of the sow and to avoid stress of the sow and her litter. However, respiration trials are problematic because both the piglets and the sow contribute to the gas exchange (Jakobsen *et al.*, 2005). Different approaches have been proposed to evaluate the HE of lactating animals and sucking offspring in pigs and mink (Verstegen *et al.*, 1985; Noblet and Etienne, 1987; Fink *et al.*, 2003). The use of the doubly labelled water (DLW) technique to derive HE of sucking piglets is an elegant way of overcoming this problem (Jequier and Schutz, 1988), because the DLW technique concomitantly allows estimation of piglet milk intake by the dilution of deuterium oxide (D_2O) and piglet HE (Ritz *et al.*, 1994). Furthermore, if all piglets are enriched with DLW, the total litter HE and the sow milk production can be quantified. Hence, the DLW method applied on piglets is, in combination with respiration experiments and collection trials, a useful technique when studying quantitative energy metabolism of the

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lactating sow. The DLW technique is ideal because it imposes a minimum of stress on the sow and piglets during the recording period (Pettigrew *et al*., 1985). However, assumptions and calculations must be made according to species and live weight (LW) of the animals (Speakman, 1997). Three experiments were carried out to test (1) the equilibration time of ^{18}O in 2-week-old piglets enriched intraperitoneally with DLW, (2) to analyse ^{18}O enrichment in piglet serum with a minimum of sample preparation and subsequent validation of the estimation of CO_2 production (using DLW technique) and milk intake (using the D_2O dilution technique) in piglets fed milk replacer, respectively, and (3) to quantify the CO_2 production and milk intake of piglets nursed by sows. The overall aim of the project was to quantify the energy metabolism of the lactating sow and her piglets when their HE was measured in respiration chambers.

Material and methods

Isotopic enrichment and blood sampling

Piglets were fasted for 2 h before an initial blood sample was collected, thereby allowing determination of the background levels of D_2O and $H_2{}^{18}O$. A single intraperitoneal injection of 0.5 ml/kg LW was given to piglets. The DLW was a mix of two-thirds of 97 at% $H_2{}^{18}O$ (Isotech, the Netherlands) and one-third of 99.9% D_2O (Cambridge Isotopic Laboratories Inc., Andover, MA, USA). Based on results obtained from Experiment 1 in the present paper, a second blood sample was drawn 1 h after DLW enrichment to determine the initial body water pool of each piglet. A third blood sample was drawn 3 days later to determine the milk intake and CO_2 production within that period. Blood samples were obtained from the jugular vein using hypodermic needles (1.2 × 40 mm) and 5 ml vacutainers for serum collection (Hemograd Z, Becton Dickinson, Meyland, France). Vacutainers with blood were kept at room temperature until blood was clotted (approximately 2 h), after which they were centrifuged (1558 × **g**, 25 min, 5°C). In order to avoid equilibration of ^{18}O with atmospheric CO_2, serum was sealed in glass ampoules and then stored at −18°C until analysis. Blood and biopsy sampling, housing and rearing were in compliance with Danish laws and regulations for the humane care and use of animals in research (The Danish Ministry of Justice, Animal Testing Act [Consolidation Act No. 726 of 9 September 1993 as amended by Act No. 1081 of 20 December 1995]). Furthermore, the Danish Animal Experimentation Inspectorate approved the study protocols and supervised the experiment.

Experiment 1: breath test

The purpose of the first experiment was to evaluate the time required for the DLW to equilibrate with body water of the piglets, based on ^{18}O enrichment of CO_2 in expired air (referred to as breath test). Three sucking piglets (Yorkshire × Danish Landrace/Yorkshire) were enriched with DLW on day 10 of lactation and their LWs were 5.67, 5.11 and 4.00 kg, respectively. Immediately after DLW enrichment, the piglets were placed individually in small open-air circuit respiration chambers (volume of 30 l) for 3 h. Samples of outgoing air was collected every second minute in 10 ml vacutainers and ^{18}O enrichment of CO_2 in expired air was analysed without any sample preparation. If the piglet urinated, the urine was removed instantaneously to avoid flux of ^{18}O from urinary water to gaseous CO_2 within the chamber. The time required to reach equilibration of DLW was evaluated as the time from piglet enrichment with DLW to the attainment of a constant level of ^{18}O enrichment in expired air.

Experiment 2: validation study

The second experiment was carried out to test the analysis of ^{18}O enrichment with a minimum of sample preparation and to validate the estimation of CO_2 production and milk intake of piglets fed milk replacer using the DLW and the D_2O dilution techniques, respectively. Ten sucking piglets (approximately 1 week old) were removed from the sow and fed with milk replacer until 'weaning' at day 28. The piglets were fed a milk replacer every hour from 0300 to 2300 h with an artificial sow (PigOline, Boss produkter A/S, Ulstrup, Denmark), capable of feeding 1, 2, 3 or 4 piglets individually. The milk replacer used was a commercial mixture (Milky-farm; Nukamel Olen, Belgium), and was mixed twice daily (150 g Milky-farm:1000 g of water). Four groups of piglets (one group with four piglets, three groups with two piglets each) were housed in respiration chambers. To minimise stress, piglets within a group were separated only by wire mesh so that they could hear and see each other and lie down in close proximity. The temperature was kept at 27 ± 0.4°C, and the relative humidity was maintained at 64 ± 1.3%. The CO_2 production was measured daily on a group basis for 22 h in respiration chambers, as described by Jørgensen *et al*. (1996). In addition, the piglets were enriched intraperitoneally with 0.5 g DLW/kg LW at approximately 10 days of age to determine the rate of CO_2 production during a 3-day period. The mean CO_2 productions within a group measured by respiration trials and by the DLW technique were compared to evaluate the accuracy of the DLW method ($n = 4$ groups) when calculated according to a one-pool model (Speakman, 1997). Furthermore, the dilution rate of D_2O was used to derive the water intake of individual piglets. A representative milk sample was collected throughout a 24-h period and chemical analysis of dry matter (DM), protein, fat, lactose and ash allowed to convert water intake of piglets to milk intake. To validate this technique, the daily amount of milk supplied to the group of piglets was recorded, and control weighing of milk delivered to each piglet was performed daily before and after each respiration trial to estimate the individual allotment. The milk disappearance from the artificial sow and the milk intake were compared on an individual basis ($n = 10$ piglets) to evaluate the accuracy of the D_2O dilution technique.

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Experiment 3: sucking piglets

Eight LY-sows (Danish Landrace × Yorkshire) and their second parity litters were used as of parturition to quantify the energy metabolism during lactation (Theil *et al.*, 2004). Lactating sows were fed according to Danish recommendations (Danielsen, 1988) and had *ad libitum* access to a water nipple, whereas piglets had no access to water. Hence, sow milk and metabolically produced water were assumed to be the only sources of water supply to piglets. During two balance periods (days 10–13 and days 17–20), lactating sows were kept in metabolism cages in respiration chambers with their piglets so that milk production was maintained. An adjacent metabolism cage allowed the piglets to escape from the sow when laying down and this cage offered a place for the piglets to urinate and defaecate in one end and to sleep on a rubber mat in the other end. A heating lamp supplied heat to the piglets when they were in pens and in metabolism cages. Milk samples were taken from the sows at the end of each balance period to obtain the chemical composition of sow milk. Milk samples were obtained from three or four random mammary glands by hand-milking the sows after intravenous injection of 10 IU (1 ml) of oxytocin (Løvens Kemiske Fabrik, Ballerup, Denmark) in an ear vein. Milk was filtered through cheesecloth to eliminate dust and scales, and was stored at –18°C until analysis. Piglets were weighed upon entering and leaving the metabolism cages (when blood samples were drawn). Since the costs of DLW (especially ^{18}O water) are significant, only three piglets per litter were enriched with DLW. In a previous study, we showed that determination of milk intake of three sucking piglets per litter was sufficient to estimate the milk intake of the whole litter and thereby the milk production of the sow (Theil *et al.*, 2002). In four of eight litters, piglets that were not enriched with DLW were labelled with D_2O to determine milk intake.

Analyses

The atomic fraction (AF) of deuterium in water hydrogen was measured in serum ultrafiltrate as described by Theil *et al.* (2002). In Experiment 1, the ^{18}O enrichment was analysed as the delta 46/44 m/z value in CO_2 in expired air without any sample preparation (analysed by John Speakman, Aberdeen, Batch #240). In Experiments 2 and 3, the ^{18}O enrichment was measured in serum water, after transferring 50 μl of serum into a 1.5 ml glass vial. The vial was filled with gaseous CO_2 by blowing 99.9% CO_2 into the vial for 3 s (flow rate of 10–15 ml/s) and was immediately closed by a rubber lid. Initial tests revealed that ^{18}O enrichment was constant when amount of serum in vials exceeded 30 μl, indicating that ^{18}O in serum water was in great excess compared with ^{18}O molecules in gaseous CO_2. The ^{18}O in serum was allowed to equilibrate with gaseous CO_2 for 24 h at room temperature. The 46/44 (m/z) ratio was measured against a working standard gas by gas chromatography/isotope ratio MS (Delta S; Finnigan MAT, Bremen, Germany).

Milk samples were analysed for DM, ash, fat, lactose and total nitrogen, and serum was analysed for DM content. DM and ash were determined according to the Association of Official Analytical Chemists (1990). Protein (N × 6.38) was measured by the Kjeldahl method using a Kjell-foss 16200 autoanalyser (Foss Electric, Hillerød, Denmark). Fat was determined after hydrolysis with hydrochloric acid and extraction with diethyl ether (Stoldt, 1952). Lactose was determined using a spectrophotometric method (Beutler, 1984).

Calculations and statistics

Isotope dilution using both D_2O and ^{18}O can be used to derive estimates of the total body water of piglets. The D_2O enrichment was quantified absolutely as an atomic fraction of ^2H relative to ^1H in serum ultrafiltrate, as described by Theil *et al.* (2002). In contrast, the determination of ^{18}O enrichment is more problematic, because an m/z ratio of 46/44 can be obtained not only by a single ^{18}O atom in CO_2 but also by two ^{17}O atoms or by one ^{13}C and one ^{17}O atom in combination with the most abundant isotopes (^{12}C and ^{16}O). Therefore, the DLW injectate was diluted serially with background pig serum to establish a calibration curve. By using the intercept and slope from the calibration curve (Figure 1), the measured m/z 46/44 value of unknown samples was converted to an arbitrary fraction of the injectate, which was equivalent to the ^{18}O enrichment over baseline (EOB) of pig serum. Finally, the DM content of serum was analysed (7.00% – see Results) and a factor of 0.93 was used to convert the ^{18}O EOB in serum into ^{18}O EOB of water:

$$^{18}\text{O EOB of water (with no unit)} = (46/44 \text{ signal} - \text{intercept})/(\text{slope} \times 10^6 \times 0.93) \quad (1)$$

where 46/44 signal is the measured m/z ratio of ^{18}O enrichment in serum of unknown samples, intercept and slope was regression parameters from the calibration curve,

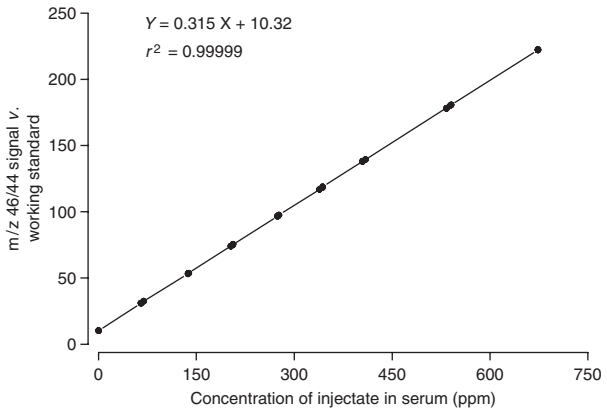

Figure 1 Calibration curve analysed to calculate ^{18}O enrichment over baseline for unknown samples, as described in Equation 1. The most concentrated standard solution in the calibration curve had a concentration similar to the level observed in piglets after DLW was equilibrated, i.e. 1 h after DLW injection.

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the factor 10^6 was used to convert p.p.m. into atomic fraction of the injectate and the factor 0.93 was applied to account for the DM fraction of serum.

The ^{18}O EOB in the first blood sample after enrichment was used to estimate the body water pool in moles ($N_{O\text{-ini}}$) and the body water fraction (BWF_{ini}) at enrichment:

$$N_{O\text{-ini}}\,\text{moles} = m_{inj}/(^{18}\text{O EOB of water} \times M_{H_2O}), \quad (2)$$

$$BWF_{ini}\,(\text{with no unit}) = N_{O\text{-ini}} \times 0.001 \times M_{H_2O}/LW, \quad (3)$$

where m_{inj} is the mass of injectate (inj) in g, ^{18}O EOB is the ^{18}O enrichment over baseline in the blood sample drawn 1 h post injection, 0.001 is used to convert g of body water into kg, M_{H_2O} is the molecular weight of water (18.01499 g/mol), and LW is the live weight of the piglet in kg.

According to our previous study (Theil *et al.*, 2002), the BWF of sucking piglets decreases curvilinearly with increasing LW and may be predicted by

$$BWF_{pred}\,(\text{with no unit}) = 0.825 - 0.130 \times \log E(LW) + 0.026 \times (\log E(LW))^2 \quad (4)$$

where LW of piglets is in kg. Hence, the total body water of piglets at subsequent blood samples (3 days after enrichment) was found by scaling according to Equation 4. The deviation between the initial BWF (BWF_{ini}) and the predicted BWF (BWF_{pred}) was assumed to persist throughout the study, i.e. a lean piglet at enrichment was assumed to remain leaner than the average piglet during the 3-day period. Hence, the BWF (BWF_{end}) and body water pool ($Q_{O\text{-end}}$) after 3 days of suckling were determined as follows:

$$BWF_{end} = 0.825 - 0.130 \times \log E(LW) + 0.026 \times (\log E(LW))^2 + (BWF_{ini} - BWF_{pred}), \quad (5)$$

$$Q_{O\text{-end}}\,\text{moles} = BWF_{end} \times LW \times 1000/M_{H_2O}, \quad (6)$$

where 1000 was used to convert kg of body water into g.

The dilution rates of D_2O (k_D) and ^{18}O (k_O) were found by

$$k_D = (\log E(D_2O\ AF_1) - \log E(D_2O\ AF_2))/\Delta t, \quad (7)$$

$$k_O = (\log E(^{18}O\ EOB_1) - \log E(^{18}O\ EOB_2))/\Delta t, \quad (8)$$

where D_2O AF_1 and D_2O AF_2 are the atomic fractions of deuterium in two consecutive blood samples after enrichment. Respectively, ^{18}O EOB_1 and ^{18}O EOB_2 are the enrichments over baseline on ^{18}O of the same two samples, and Δt is the time difference between samples 1 and 2.

Finally, the CO_2 production was calculated according to the single-pool model, proposed by Lifson and McClintock (1966):

$$rCO_2 = N_O \times (0.480769 \times k_O - 0.495769 \times k_D) \times 273.15 \times 0.08205, \quad (9)$$

where N_O is the average of the body water pool (in moles) determined initially ($Q_{O\text{-ini}}$) by ^{18}O dilution and predicted at the end of the measuring period ($Q_{O\text{-end}}$), k_O is the fractional turn-over rate of ^{18}O and k_D is the fractional turnover rate of D_2O, the two constants 273.15 and 0.08205 are used to convert the CO_2 production into litres at standard conditions (i.e. corrected to dry air at constant temperature and pressure).

The milk intake of piglets was assessed by determining the fractional water turn-over and the body water pool using the D_2O dilution technique. The water intake was converted to milk intake based on the chemical composition of milk replacer (experiment 2) or sow milk (experiment 3) and by taking into account the metabolically produced water from nutrient oxidation. Milk intake was calculated as described by Coward *et al.* (1982)

$$\text{Milk intake (g per piglet per day)} = \frac{\text{water turn-over} + \text{potential metabolic water stored}}{\text{potential water fraction of milk}}, \quad (10)$$

where water turnover was calculated as the fractional water turnover multiplied by the total body water; potential water fraction of milk was calculated based on the analysed chemical composition of milk and potential metabolic water stored was calculated by assuming that retention of DM in piglets was equal for deposition of both fat and protein. The D_2O dilution technique used to assess piglet milk intake, the chemical analyses of milk components, the underlying assumptions of the technique and calculations are described in detail by Theil *et al.* (2002).

The following normal mixed model (MIXED procedure of SAS (Littell *et al.*, 1996) was used to describe variables related to piglet performance (Table 2):

$$Y_{spij} = \mu + \alpha_i + \beta_j + (\alpha \times \beta)_{ij} + S_s + P(S)_{ps} + \varepsilon_{spij},$$

where Y_{spij} represents the variables related to piglet performance for the Pth piglet ($p = 1, 2, \ldots 13$) of the Sth sow ($s = 1, 2, 3, 4$) at the ith period ($i = 1, 2$) fed with the jth milk source ($j =$ milk replacer, sow milk). Furthermore, μ, α_i and β_j are the grand mean, and the fixed effects related to the factor representing period and milk source, respectively, and $(\alpha \times \beta)_{ij}$ represents the interactions between the two fixed effects. The random components related to sow (S_s) and piglet nested within sow $P(S)_{ps}$, and the residual error component (ε_{spij}) were assumed to be independent and normally distributed and their expectations were assumed to be zero.

Results

Breath test

The enrichment of ^{18}O in expired air increased immediately after DLW was injected intraperitoneally, peaked approximately 15 min later, and then dropped to a stable plateau after 30–40 min. The dynamic changes of ^{18}O enrichment of expired air during the first 3 h after DLW injection are shown for one of three piglets (Figure 2).

Validation study

The DM content of piglet serum was 7.00 ± 0.12%. Dilution of serum from DLW-enriched piglets with serum harvested from a pig not enriched with DLW yielded a perfect linear relationship between the relative (arbitrary) content and the

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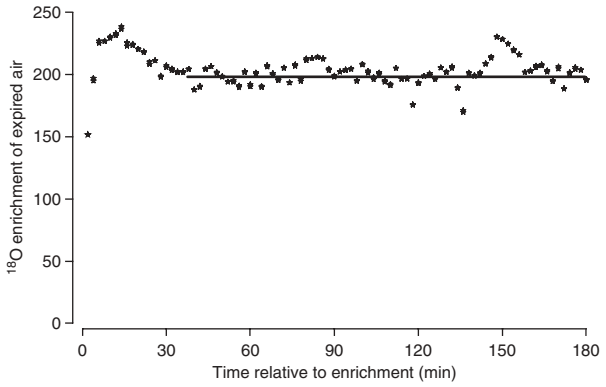

Figure 2 Test of equilibration time of ^{18}O water in piglets. Samples of expired air (breath test) were collected every other minute for 3 h, and ^{18}O enrichment was analysed.

Table 1 *Chemical composition (mean ± s.e.) of milk replacer (n = 2) and sow milk (n = 16) from experimental sows in weeks 2 and 3 of lactation*

	Milk replacer (Exp. 2)	Sow milk (Exp. 3)†
Dry matter (DM) (g/100 g milk)	$12.7^{b} \pm 0.01$	$17.6^{a} \pm 0.3$
Protein (6.38 × N) (g/100 g DM)	$23.5^{b} \pm 0.3$	$29.7^{a} \pm 0.7$
Fat (g/100 g DM)	$25.7^{b} \pm 2.8$	$37.4^{a} \pm 1.1$
Lactose (g/100 g DM)	32.7 ± 2.3	30.9 ± 0.9
Non-lactose carbohydrate (g/100 g DM)	10.7 ± 0.4	–
Ash (g/100 g DM)	$10.4^{a} \pm 0.2$	$4.7^{b} \pm 0.1$
Gross energy (MJ/kg DM)	$21.69^{b} \pm 0.19$	$25.52^{a} \pm 0.19$
Potential water fraction‡	$0.948^{a} \pm 0.001$	$0.942^{b} \pm 0.001$

[a,b]In a row indicate significant differences between milk replacer and sow milk based on Wald's test.
†No difference was found in chemical composition of sow milk between weeks 2 and 3 of lactation.
‡Potential water fraction is the water content of milk (milk replacer or sow milk) corrected for metabolic water that would be produced if all the digested dry matter was oxidised. The digestibilities of protein, fat, non-lactose and lactose were assumed to be 0.88, 0.98, 1.00 and 1.00, respectively. The analysed dry matter content was corrected to 100% before calculating the potential water fraction, as reported by Theil *et al.* (2002).

analysed ^{18}O enrichment over baseline (Figure 1). The ^{18}O excess enrichment of piglets in background samples (i.e. pre-injection) was 1.2 ± 0.9 p.p.m. (range −2.3 to 6.8 p.p.m.), whereas the excess enrichment after steady state was attained at 679 ± 21 p.p.m. (range 555–773 p.p.m.).

The milk replacer contained 12.7% DM and on a DM basis, the protein, fat, lactose, non-lactose carbohydrates and ash contents amounted to 22.3%, 25.0%, 31.9%, 10.4% and 10.3%, respectively (Table 1). The energy content was 21.69 MJ/kg DM and the potential water fraction of milk was 0.948.

The 10 piglets reared artificially with milk replacer ingested on average 910 ± 58 g/day as evaluated by the rate of milk disappearance. Based on the D_2O dilution technique, the milk intake was estimated to be 891 ± 63 g/day (Table 2). The fitted linear regression between estimated milk intake (*Y*-axis) and weighed milk disappearance (*X*-axis) was $Y = 0.98 \times X$ if using a no intercept model ($P = 0.25$ for no intercept; Figure 3). On an individual basis, the difference between weighed milk disappearance and milk intake estimated by D_2O dilution was in the range of −24 to +90 g/day. Artificially reared piglets were housed in four groups in respiration chambers. The daily rate of CO_2 production, determined by gas exchange, per piglet in the groups averaged 64.7 ± 1.8 l/day per piglet. In accordance, the daily rate of CO_2 production, as assessed by the DLW method, amounted to 64.0 ± 2.7 l/day per piglet. The fitted regression using a no intercept model ($P = 0.14$ for no intercept) between CO_2 production assessed by the DLW method (*Y*-axis) and by indirect calorimetry (*X*-axis) was close to a perfect fit ($Y = 0.99 \times X$).

Sucking piglets

Sow milk contained 17.6% DM and on a DM basis, the protein, fat, lactose and ash contents were 28.9%, 36.4%, 30.1% and 4.6%, respectively (Table 2). The gross energy content of milk was 25.52 MJ/kg DM, and the potential water fraction was 0.942.

Table 2 *Data on artificially reared piglets and sucking piglets on days 10–13 and days 17–20* post partum

	Artificially reared piglets (Exp. 2)		Sucking piglets (Exp. 3)	
Days *post partum*	10–13	17–20	10–13	17–20
No. of piglets	10	10	47	47
Mean live weight (g)	$3.02^{c} \pm 0.36$	$3.96^{b} \pm 0.36$	$3.32^{bc} \pm 0.13$	$4.82^{a} \pm 0.13$
Live-weight gain (g/day)	$138^{b} \pm 26$	$154^{b} \pm 26$	$171^{b} \pm 9$	$219^{a} \pm 9$
Body water (%)	$77.6^{a} \pm 1.0$	$75.8^{b} \pm 1.0$	$73.6^{c} \pm 0.4$	$71.5^{d} \pm 0.4$
Milk intake (g/day)†	$891^{bc} \pm 101$	$1182^{a} \pm 101$	$864^{c} \pm 46$	$1055^{ab} \pm 46$
Energy intake (MJ/day)	$2.46^{c} \pm 0.43$	$3.26^{b} \pm 0.43$	$3.88^{b} \pm 0.20$	$4.74^{a} \pm 0.20$
CO_2 production (l/day)‡	$64.0^{b} \pm 2.7$	–	$93.7^{a} \pm 5.1$	–

[a,b,c,d]Means with superscripts in a row indicates a significant effect of period (comparison within an experiment) or a significant effect of milk source (comparison between experiments).
†Determined by the D_2O dilution technique.
‡Determined by the DLW method ($n = 24$ piglets in experiment 3 for this parameter).

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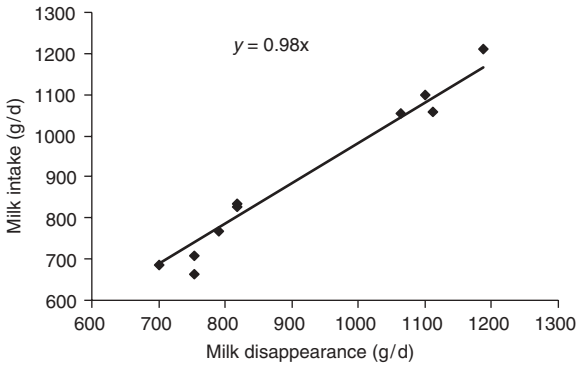

Figure 3 Validation of piglet milk intake determined by the D_2O dilution technique and by weighing the amount of milk allotted to individual piglets (milk disappearance) on days 10–13 *post partum*. Piglets were fed milk replacer as of day 8 *post partum*.

LW ($P < 0.001$), LW gain ($P < 0.001$), milk intake ($P < 0.01$) and energy intake of sucking piglets increased ($P < 0.01$) from days 10 to 13 to days 17 to 20, whereas body water percentage decreased with increasing age ($P < 0.01$; Table 2). The average CO_2 production of the three median piglets according to LW within litters ($n = 24$) was 93.7 l/day. For sucking piglets, the following regression between live weight (LW, kg) and daily CO_2 production was found:

$$\text{Piglet } CO_2 \text{ production (l/day)} = 25.75 \times LW - 1.01 \times (LW)^2.$$

Discussion

The fast stabilisation of ^{18}O enrichment in expired air showed that ^{18}O was rapidly transported from the intraperitoneal cavity and distributed throughout the entire body of the piglet via the blood. After 15 min, the enrichment peaked and then dropped quickly to a steady-state level. This pattern was probably caused by an initial equilibration of ^{18}O with blood and intercellular water, whereas it obviously takes longer (approximately 40 min) for the ^{18}O to equilibrate with intracellular water. The result is comparable with the 20 and 40 min required for D_2O to equilibrate after intravenous and intramuscular injections, respectively (Rudolph *et al.*, 1988).

The serial dilutions of DLW-enriched serum yielded a perfect linear response in ^{18}O enrichment over baseline. To convert the ^{18}O abundance in serum into ^{18}O abundance in water, the water content of piglet serum (93.0%) was measured. When the ^{18}O abundance was expressed as enrichment over baseline (i.e. excess enrichment) and corrected for DM content, values were essentially 0 p.p.m. in blood samples drawn prior to enrichment. Hence, the presented approach proved to be a robust and precise technique and required only a minimum of sample preparation. Another advantage with the presented method is that it avoids correction for ^{17}O and ^{13}C abundances in piglet serum. The analysis of ^{18}O enrichment in serial dilutions using serum was similar to the approach used to measure D_2O enrichment of piglet serum (Pluske *et al.*, 1997).

The milk replacer used in the validation study differed chemically from the reported composition of sow milk (Darragh and Moughan, 1998; Theil *et al.*, 2004): When the milk replacer was mixed according to the manufacturer's recommendations, it contained only 12.7% DM, whereas sow milk contains 18–19%. Furthermore, the milk replacer had lower contents of both protein and fat and higher ash content than sow milk (Table 1). In contrast to sow milk, the milk replacer contained carbohydrates other than lactose which constituted approximately 10% of the DM fraction. As a consequence of the altered chemical composition, the energy content of the milk replacer was lower than that reported for sow milk (21.69 *v.* 25.52 MJ/kg DM, respectively), and the piglets fed milk replacer likely had a lower fat deposition as indicated by the higher BWF on days 17–20. The lower fat retention may explain for the lower growth performance after weaning observed for piglets fed milk replacer before weaning (Wolter *et al.*, 2002).

Based on Equation 10, it is evident that characteristics related both to piglets (water turn-over and potential metabolic water stored) and to the sow (potential water fraction of milk) are important for assessing the milk intake of piglets based on the D_2O dilution technique. However, in spite of the different chemical composition (milk replacer *v.* sow milk), the potential water fraction of the milk replacer was comparable with that of sow milk and indicates that variations in chemical composition of milk should have only negligible impact on the potential water fraction of milk. In agreement, we have previously found that the potential water fraction of milk does not change during lactation and does not vary among experiments in spite of differences in chemical composition (Theil *et al.*, 2002 and 2004). For future experiments, we, therefore, suggest that a constant of 0.942 be used as the potential water fraction of sow milk. However, if D_2O dilution is used to quantify colostrum intake, the potential water fraction is somewhat lower (approximately 0.88), because the digestibilities of fat and proteins in colostra are lower than in milk (Devillers *et al.*, 2004).

There was agreement between values for milk intake measured by the D_2O dilution technique and by weighing milk ingested while artificially fed, the deviation between the two methods amounted to approximately 2%. The discrepancy between the two methods may be caused by an underestimation of the milk intake by the D_2O dilution technique, or a slight overestimation of the actual milk intake of piglets evaluated by milk disappearance. In favour of the latter explanation, minor amounts of milk was retained in the tubes leading milk replacer into the drinking cup inside the cage and some piglets spilt minor amounts. The present study supports the conclusion of Prawirodigdo *et al.* (1990) that the D_2O dilution technique predicts the amounts of milk disappearing from artificial nipples with an accuracy of 97.9–100.5%. The D_2O dilution estimates are evidently superior to the weigh–suckle–weigh estimates of sow milk production (Pettigrew *et al.*, 1985; Theil *et al.*,

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2002), due to metabolic and salivary losses and due to disruptions of sow–piglet interactions.

Estimation of CO_2 production rate by means of the DLW method depends on whether the water dilution spaces are based only on ^{18}O (one-pool model) or on both D_2O and ^{18}O (two-pool model). According to Speakman (personal communication), a one-pool model should always be used for animals weighing up to 2 kg, and a two-pool model should always be used for animals weighing >10 kg. A two-pool model is most appropriate for large animals in order to account for ^{18}O incorporation into fat tissue. Accordingly, the present paper suggests that the one-pool model described by Lifson and McClintock (1966) does estimate the CO_2 production accurately on a group basis (two piglets in three groups, four in the fourth group), since a fitted regression between CO_2 production estimated by the DLW method and that measured by indirect calorimetry yielded a nearly perfect fit (slope = 0.99 with a no-intercept model). The low BW of sucking piglets and their low fat content in early lactation may explain why the one-pool model was the most appropriate. In contrast to the accuracy, the precision was rather low, indeed the deviation between the two methods was in the range of −8.7 to 6.9 l/day. The DLW method estimated the CO_2 production for the four groups within 89.8–114.4% of that measured by indirect calorimetry. A higher initial ^{18}O enrichment would likely improve the precision of measurement. However, determination of the body water pool at the end of the measuring period will also improve the precision, because the body water pool changes rapidly in pigs (Haggarty *et al.*, 1994) and the water pool greatly affects the derived estimate of CO_2 production (Speakman, 1997).

The CO_2 production of sucking piglets (93.7 l/day) on days 10–13 was considerably higher than that of piglets fed milk replacer (64.0 l/day) most likely due to different activity and feeding levels (piglets fed milk replacer consumed less energy, due to low fat and solid content of the milk replacer). The activity level of sucking piglets was higher compared to that of artificially reared piglets, because sucking piglets spent time and energy fighting for their gland position and stimulating the sow's udder. Another reason for the higher CO_2 production observed in sucking piglets is the higher intake of milk energy, which is known to increase O_2 consumption (Noblet and Etienne 1987) and, therefore, also increases CO_2 production. Noblet and Etienne (1987) measured the gas exchange of sucking piglets between sucking bouts ('resp–suckle–resp' approach) and reported a daily CO_2 production of 63.4 l per piglet, which is similar to that found in the present study for artificially reared piglets, but not for the sucking piglets. Initially, we planned to adopt their 'resp–suckle–resp' approach but realised that piglets slept most of the time between the sucking bouts, which, therefore causes an underestimation of the litter HE.

In summary, the DLW method is a useful technique for quantitative studies of nutrient metabolism in sucking piglets and their dam. If CO_2 production and milk intake of sucking piglets are assessed by the DLW method, and if these data are combined with measurements of the gas exchange (indirect calorimetry) and with collection of faeces and urine of the lactating sow and her litter, it is possible to quantify the energy and the protein balances of both the lactating sow and her litter.

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