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P. Junghans
M. Derno
M. Gehre*
R. Höfling*
P. Kowski**
G. Strauch**
W. Jentsch
J. Voigt
U. Hennig

Calorimetric validation of ^{13}C bicarbonate and doubly labeled water method for determining the energy expenditure in goats

Kalorimetrische Validierung der ^{13}C-Bicarbonat- und der Wasser-Doppelmarkierungsmethode zur Bestimmung des Energieumsatzes bei Ziegen

Summary The purpose of the present study was to validate the ^{13}C bicarbonate method (^{13}C-M) and the doubly labeled water method (DLWM) for the estimation of the CO_2 production $R(CO_2)$ in goats as a ruminant model. Indirect calorimetry was chosen as the reference method. Studies were carried out in 2 male African dwarf goats at 3 different developing stages (age: 5, 10, and 14 months, body mass: 14.6, 20.3, and 21.7 kg). Animals were fed a balanced feed 14 days before and during the studies. The isotope tracers (4 mg/kg $NaH^{13}CO_3$, 120 mg/kg ^2H_2O, and 75 mg/kg $H_2{}^{18}O$; 99 At.-%) were simultaneously given as a single pulse injection into the jugular vein. Thereafter, the animals were kept for 8 days in two respiration chambers (volume of chamber: 2.85 m^3, air flow rate: 25 l / min) for the estimation of CO_2 production and O_2 consumption. For the determination of $R(CO_2)$ using the ^{13}C-M samples of exhaled breath were drawn from the respiration chambers. The ^{13}C enrichment and CO_2 concentration of breath samples were measured by means of an infrared isotope analyzer. In order to determine $R(CO_2)$ by means of the DLWM, blood serum was used. The ^2H and ^{18}O enrichments were measured by an isotope ratio mass spectrometer. Urine samples were collected over 24 h to quantify renal water losses.

The $R(CO_2)$ was calculated by means of the ^{13}C-M using the area under the ^{13}C enrichment-time curve. The determination of $R(CO_2)$ by means of the DLWM was based on the slopes of the ^2H and ^{18}O disappearance curves and the body water pool obtained from the zero time intercept of the isotope curves.

The values of $R(CO_2)$ resulting from the ^{13}C-M were found to be comparable with those from the calorimetric measurement. Smaller (not statistically significant) values of $R(CO_2)$ - 92% from ^{13}C-M and 87% from DLWM - compared to the indirect calorimetry could indicate the incorporation of ^{13}C and ^2H into metabolites other than CO_2 and H_2O, respectively. The body water contents calculated from the zero time intercepts of the ^2H and ^{18}O disappearence curves amounted to 66% and 63%, respectively. The body water content was found to be not related to the age of animals. The renal water loss was calculated to be 35% of the total water loss (0.76 l/d).

Zusammenfassung Zweck der vorliegenden Untersuchung war die Validierung der ^{13}C-Bicarbonat-Methode (^{13}C-M) und der doppeltmarkierten Wassermethode (DLWM) zur Bestimmung der CO_2-Produktion $R(CO_2)$ bei Ziegen als Wiederkäuermodell. Die indirekte Kalorimetrie war als Referenzmethode ausgewählt worden. Die Untersuchungen wurden an 2 Afrikanischen Zwergziegen bei 3 unterschiedlichen Entwicklungsstadien (Alter: 5, 10 und 14 Monate, Körpermasse: 14,6, 20,3 und 21,7 kg) durchgeführt. Die Tiere wurden 14 Tage vor und während der Untersuchung bilanziert gefüttert. Die Isotopentracer (4 mg/kg $NaH^{13}CO_3$, 120 mg/kg ^2H_2O und 75 mg/kg $H_2{}^{18}O$; 99 At.-%) wurden simultan als einmalige impulsförmige Dosis in die Vena jugularis injiziert. Danach befanden sich die Tiere für 8 Tage in zwei Respirationskammern (Kammervolumen: 2,85 m^3, Luftdurchsatz: 25 l/ min),

P. Junghans (✉) · M. Derno · M. Gehre*
R. Höfling* · P. Kowski** · G. Strauch**
W. Jentsch · J. Voigt · U. Hennig
Research Institute for the Biology
of Farm Animals, Department
of Nutritional Physiology „Oskar Kellner"
D-18059 Rostock;
UFZ Centre for Environmental Research
Leipzig-Halle,
*D-04301 Leipzig und
**D-06246 Bad Lauchstädt, Germany

um die CO_2-Produktion und den O_2-Verbrauch zu bestimmen. Für die Bestimmung von $R(CO_2)$ mit Hilfe der ^{13}C-M wurden Proben der Ausatemluft aus den Respirationskammern gezogen. Die Messung der ^{13}C-Anreicherung und der CO_2-Konzentration der Atemproben erfolgte mittels eines Infrarot-Analysators. Für die Bestimmung von $R(CO_2)$ mittels der DLWM wurde Blutserum verwendet. Die ^2H- und ^{18}O-Anreicherungen wurden massenspektrometrisch gemessen. Urinproben wurden zur Quantifizierung des renalen Harnverlusts über 24h gesammelt. $R(CO_2)$ wurde mittels der ^{13}C-M unter Verwendung der Fläche unter ^{13}C-Anreicherung-Zeit-Kurve berechnet. Die Bestimmung von $R(CO_2)$ mittels der DLWM basierte auf den Anstiegen der ^2H- und ^{18}O-Kurven und dem Körperwasser-Pool. Die Werte von $R(CO_2)$ aus der ^{13}C-M war vergleichbar mit denen aus kalorimetrischen Messungen. Die im Vergleich zur indirekten Kalorimetrie kleineren (nicht statistisch signifikant) Werte von $R(CO_2)$ - 92% aus ^{13}C-M und 87% aus DLWM - deuten auf die Inkorporation von ^{13}C und ^2H in andere Metaboliten als CO_2 und H_2O hin. Die Körperwasser-Pools (bezogen auf die Körpermasse), berechnet aus den Ordinatenabschnitten der ^2H- und ^{18}O-Kurven (bei t = 0), betrugen 66% bzw. 63%. Die Körperwasser-Pools zeigten keine Altersabhängigkeit. Der renale Wasserverlust wurde zu 35% des totalen Wasserverlusts (0,76 l / d) berechnet.

Key words Stable isotopes - ^{13}C - ^2H - ^{18}O - doubly labeled water method - carbon dioxide production - energy expenditure - indirect calorimetry - goat - ruminant

Schlüsselwörter stabile Isotope - ^{13}C - ^2H - ^{18}O - Wasser-Doppelmarkierungsmethode - Kohlendioxidproduktion - Energieumsatz - indirekte Kalorimetrie - Ziege - Wiederkäuer

Introduction

Indirect calorimetry using the whole body respiratory technique is widely applied for studying energy metabolism in farm animals. The disadvantage of this technique is that the animals have to be confined to respiratory chambers. The determination of CO_2 production ($R(CO_2)$) and energy expenditure in farm animals under their natural living conditions requires non-restrictive and non-invasive measuring methods. Isotope-aided methods like the ^{13}C bicarbonate (^{13}C-M) (2 - 4, 7, 9, 11) and doubly labeled water ($^2H_2{}^{18}O$) method (DLWM) (1, 5, 6, 10) satisfy this requirement. As the isotope methods are based on the indirect determination of $R(CO_2)$, their calorimetric validation is necessary.

The aim of the present study was to validate and to optimize the ^{13}C-M and DLWM applied simultaneously for the estimation of $R(CO_2)$ and energy expenditure in dwarf goats as a ruminant model. Indirect calorimetry was assumed to be the „golden standard" (4, 11). Dwarf goats were chosen because their medium size permits one to minimize isotope costs, especially costs for ^{18}O labeled water, and to collect sufficient blood with the aid of a catheter at any time (7).

Materials and methods

Studies were performed in 2 male African dwarf goats at 3 different developing stages (age: 5, 10, and 14 months, body mass: 14.6, 20.3, and 21.7 kg). Animals were fed a balanced feed 14 days before and during the studies. The isotopically labeled substances (4 mg/kg $NaH^{13}CO_3$, 120 mg/kg ^2H_2O, and 75 mg/kg $H_2{}^{18}O$; 99 At.-%) were simultaneously given as a single pulse injection into the vena jugularis. Thereafter, the animals stayed for 8 days in two respiration chambers (volume of chamber: 2.85 m^3, flow rate: 25 l / min) for the determination of energy expenditure from the CO_2 production and O_2 consumption. For the determination of $R(CO_2)$ using the ^{13}C-M, samples of exhaled breath were drawn from the respiration chambers (12 samples until 12 h after the ^{13}C dose) and transferred to breath bags (Tesseraux, Bürstadt, Germany). The ^{13}C enrichments and CO_2 concentrations of breath samples were measured by means of the IRIS infrared isotope analyzer (WATV GmbH, Worpswede, Germany). In order to determine $R(CO_2)$ by means of the DLWM, blood serum was used (5 samples until 13 days after simultaneous doses of ^2H_2O and $H_2{}^{18}O$). ^2H and ^{18}O enrichments were measured using blood serum by the Delta S isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany). ^2H enrichment was measured in H_2 gas obtained after reduction of serum (4 µl) using chromium at 850 °C. ^{18}O enrichments were determined in ^{18}O labeled CO_2 prepared by 24h equilibration of serum (3.5 ml) with CO_2. Urine samples were collected over 24h to quantify renal water losses.

Calculations

Kinetics of the isotopes ^{13}C in breath CO_2 and ^2H or ^{18}O in blood serum were evaluated by stochastic and compartmental methods using the SAAM31 software package (NIH, Bethesda, MA, USA).

^{13}C Bicarbonate (^{13}C-M) method

The CO_2 production $R(CO_2)$ (units: mol/d) was calculated from the ^{13}C data by means of the Eq. (1) reported by Elia et al. (2, 3):

$$R(CO_2) = (D / A) * F \quad (1)$$

D ^{13}C dose mol
A area under the $^{13}CO_2$ enrichment-time curve (atom % * d)
F fractional recovery of labeled CO_2

For the fractional recovery of labeled CO_2 a value of 0.8 was used (2, 4).

Doubly labeled water method (DLWM)

The calculation of $R(CO_2)$ and $R(H_2O)$ (unit[3]: mol/d) from the ^2H and ^{18}O data was based on Eqs. (2) and (3), respectively (1, 5, 6, 10):

$$R(CO_2) = \{k(^{18}O)*Q(^{18}O) - k(^2H)*Q(^2H) * [(x * (f_2-1) + 1)/(x * (f_1-1) + 1)]\} / (2*f_3) \quad (2)$$

$$R(H_2O) = k(^2H)*Q(^2H) / (x * (f_1-1) + 1) \quad (3)$$

The quantities $k(^2H)$ und $k(^{18}O)$ are rate constants (units: 1/d) which were calculated from the slope of the ^2H and ^{18}O disappearance curves. The body water pools $Q(^2H)$ and $Q(^{18}O)$ (units: mol) were obtained from the zero time intercept of the isotope disappearance curves.

Isotope fractionation of ^2H and ^{18}O leaving the body water as water vapor was taken into account by the factors $f_1 = 0.93$ and $f_2 = 0.99$, respectively. The fractionation of ^{18}O due to the exchange between CO_2 and water was considered by the factor $f_3 = 1{,}039$. The quantity x is the proportion of total water loss that is fractionated and was assumed to be 0.5.

Results

Fig. 1 and 2a show the isotope kinetics after administration of ^{13}C labeled bicarbonate and doubly labeled water, respectively. Fig. 2b presents the relative residuals from the fitted isotope enrichments. $R(CO_2)$ calculated from the ^{13}C-M and DLWM is listed in Table 1 and compared with the indirect calorimetry. Furthermore, water pool (as percent of body mass) and total and renal water loss measured by the DLWM are given. The renal water loss (as percentage of total water loss) amounted to about 35%.

Table 1 CO_2 production, water pool, and water turnover in goats measured by the ^{13}C bicarbonate method (^{13}CM) and the doubly labeled water method (DLWM) compared with the indirect calorimetry.

Subject	Age	BM	BW/BM		$R(H_2O)$		$R(CO_2)$			
	Months	*kg*	%		*l / d*					
			DLWM		DLWM		^{13}C-M	IC	DLWM	IC
			^2H	^{18}O	total	renal	1st experimental day		1st - 8th experimental day	
			(a)	(b)	water loss		(a)	(b)	(a)	(b)
A	5	15.0	67	62	0.75	0.25	255	268	273	289
B	5	14.2	64	60	0.78	0.30	230	248	212	244
C	10	20.0	67	65	0.99	0.35	307	338	297	335
B	10	20.5	66	66	0.77	0.28	296	317	253	327
C	14	21.0	68	64	0.68	0.22	332	358	334	352
B	14	22.5	62	60	0.61	0.20	316	347	262	337

Remarks: BM: body mass, BW: body water pool calculated from the ^2H and ^{18}O isotope dilution (extrapolated to t = 0 days), $R(H_2O)$: water loss;
IC: indirect calorimetry, paired t-test: (a) vs. (b): not significant

Discussion

The ^{13}C kinetics, shown in Fig. 1, result from two influences: (a) animal-related effects and (b) effects related to the respiration chamber („chamber effect"). Concerning the first effect (a) the ^{13}C tracer administered as ^{13}C bicarbonate is mixed with the body bicarbonate pool and washed out subsequently in the breath CO_2. About 10 to 20% of the administered ^{13}C dose is incorporated into intermediary metabolites (3, 4, 11). Referring to the „chamber effect" (b) the delay of ^{13}C elimination observed is mainly caused by mixing of exhaled $^{13}CO_2$ with the steady state CO_2 in the respiration chamber. Because the physical characteristics of the respiration chamber is known (see Materials and methods), its contribution to the ^{13}C kinetics could be separated. However, the stochastic approach chosen to calculate $R(CO_2)$ is based on the area under the enrichment-time curve. Therefore, the shape of the curve does not influence the result and the explicit consideration of the „chamber effect" is not necessary.

The ^2H and ^{18}O kinetics show a monoexponential course (Fig. 2a), which indicate a single body ^2H or ^{18}O pool, respectively. But, small differences between the ^2H and ^{18}O pools calculated from the isotope dilution (extrapolation of the isotope enrichment to the time t = 0 days) must be explained by the exchange the processes of ^2H and ^{18}O. Especially, the exchange of water-bound ^2H with labile ^1H atoms in amino, carboxyl or sulfhydryl groups leads to slightly increased (not statistically significant) ^2H pools compared with the ^{18}O pools as shown

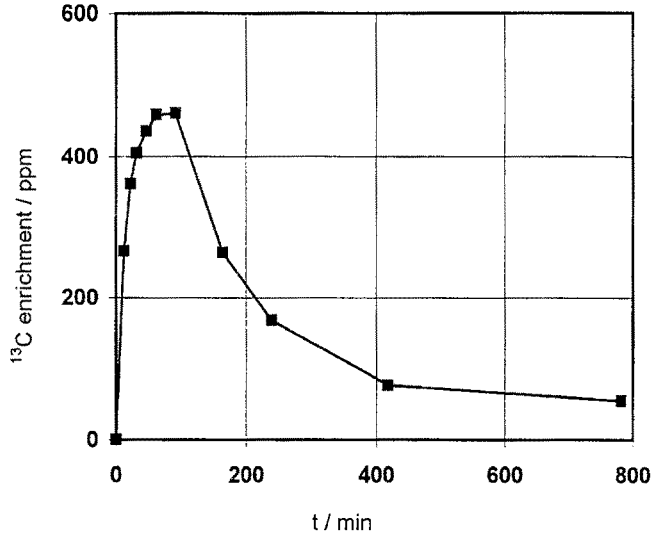

Fig. 1 ^{13}C kinetics of breath CO_2 in a goat studied in a respiratory chamber after a single i. v. dose of $NaH^{13}CO_3$. Breath was sampled from the respiration chamber.

in Table 1. The covariance between ^{2}H and ^{18}O data exhibited in Fig. 2b implies that the deviation from the fitted line affecting both isotopes is mainly caused by physiological variations (e. g., water intake) (5).

The values of $R(CO_2)$ obtained from the ^{13}C-M and DLWM are comparable with the calorimetric ones. Smaller (not statistically significant) values of $R(CO_2)$ yielded from the isotope methods could indicate the incorporation of ^{13}C and ^{2}H into other metabolites as CO_2 and H_2O, respectively. As known in ruminants the isotope ^{2}H is also lost via methane besides the elimination as liquid or gaseous water. Therefore, the total water loss $R(H_2O)$ might have been overestimated and, therefore, $R(CO_2)$ underestimated (5). However, the results in Table 1 show that the underestimation of $R(CO_2)$ was not significant. The calculation of the energy expenditure from $R(CO_2)$ can be performed with the help of formulas reported by, e.g., Haggarty (5). The water content and $R(H_2O)$ (total and renal) presented in Table 1 was found to be not related with the age.

Advantages of the isotope methods are, first, animals can be studied under freeliving conditions, and second the incorporation of ^{13}C and ^{2}H into other metabolites as CO_2 and H_2O, respectivley, gives information on further intermediary metabolic processes (e.g., gluconeogenesis, methanogenesis). Third, ^{13}C-M can be applied for short-term measurements (6 - 12 h) of $R(CO_2)$. DLWM is suitable for long-term measurements (5 - 20 d) of $R(CO_2)$. Fourth, additionally DLWM enables the determination of body water pool and total (renal + nonrenal) water loss. The disadvantage of both isotope methods is that the respiratory quotient (RQ) can not be determined by them. As the value of RQ lies almost around 0.8, the energy expenditure can be often estimated with a good accuracy from $R(CO_2)$. Another drawback of the ^{13}C-M is that ^{13}C bicarbonate can not be administered orally because of the acidity in the stomach. For this purpose the doubly labeled (^{13}C,^{15}N) amino acid approach was proposed (8).

Conclusions

^{13}C-M and DLWM can be considered as alternative methods for the indirect calorimetry, especially if investigations aim to measure energy expenditure in animals under free-living conditions. The isotope methods are applicable to ruminants like goats. Falsifying influences on the determination of $R(CO_2)$ caused by methanogenesis and inhomogeneity of the body water pool proved not to be significant. The isotope methods have an additional advantage of providing information on body composition when compared to indirect calorimetry.

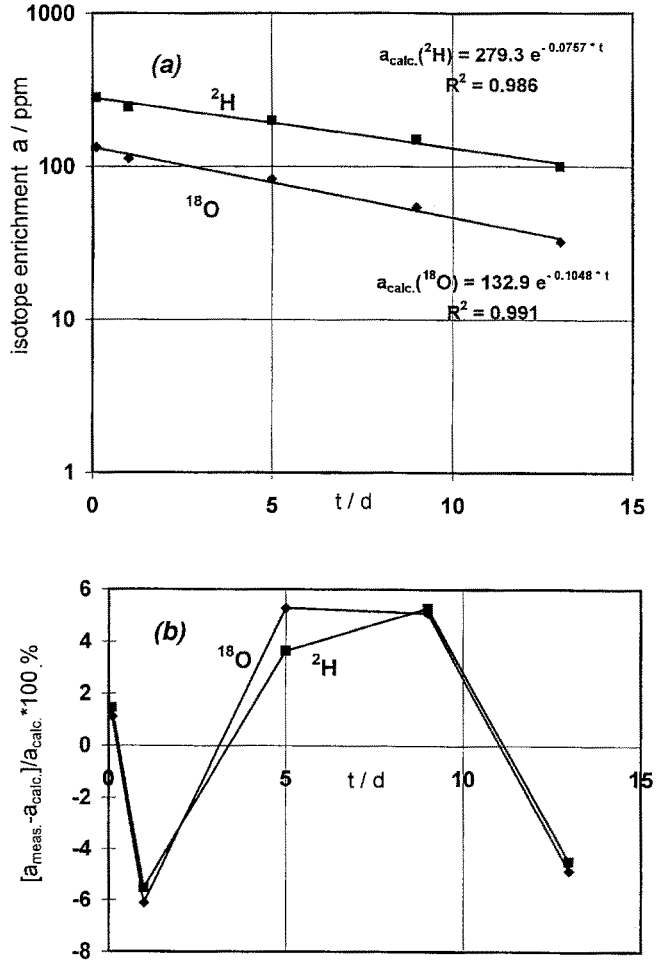

Fig. 2 The top graph (a) show the ^{2}H (■)and ^{18}O (◆)kinetics of blood serum in a goat after a single dose of $^2H_2{}^{18}O$. The isotope enrichments were fitted by a single-exponential approach (—). The bottom graph (b) show the relative residuals from the fitted isotope enrichments.

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