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Measuring body composition and energy expenditure using (doubly) labelled water

Explanation of the principles and practical application

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13-14.09.2019, ISEP Course on Indirect Calorimetry, Belo Horizonte (BRA)

www.agroscope.ch I good food, healthy environment

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Plan

Use of labelled water techniques in animal sciences

1. Labelled water technique for body composition measurement
   - Principles and applications
3. Doubly labelled water technique for energy expenditure measurement
   - Principles
4. Labelled water administration, sampling and analyses
   - Technical issues

Conclusions

Interest in body composition and energy expenditure measurements in farm animals

Physiology — Animal Health — Nutrition

Understand body reserves management / allocation toward physiological functions

Describe body reserves dynamics and energy expenditure across production cycle

Define animal requirements
⇒ Built-up / renovate feeding systems

Energy expenditure
productive and non-productive parts

Select most feed efficient individuals

Define carcass and products quality

Body composition
chemical: lipid, protein, ash, energy
tissular: adipose, muscles, bones, organs

Products quality — Genetics

Interest in body composition and energy expenditure measurements in farm animals

Gold-standard techniques

- **Body composition**: slaughter and dissection/grinding + analyses => destructive
- **Energy expenditure**: (in)direct calorimetry => confined chamber

Need for *in vivo* techniques allowing

- Repeated measurements on a same individual
- Applicable in « real-life » rearing conditions / Reduced invasivity

reduce physiological and behavioural perturbations

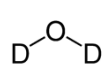
⇒ **Dosing animal with (doubly) labelled water and follow tracers elimination kinetics as a reliable technique?**

Different types of labelled water for diverse applications

Singly labelled water:

Applications

$H_2{}^{18}O$
^{18}O water
BG enrich.: 2000 ppm



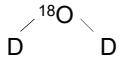
^2H_2O or D_2O
Deuterated water
BG enrich.: 150 ppm

^3H_2O or T_2O
Tritium water
radioactive

Labelled water dilution space
⇒ **Body composition**

+ Labelled water clearance
⇒ **Drinking water**

Doubly labelled water:



Differences in D and ^{18}O elim. rates
⇒ **Energy expenditure**

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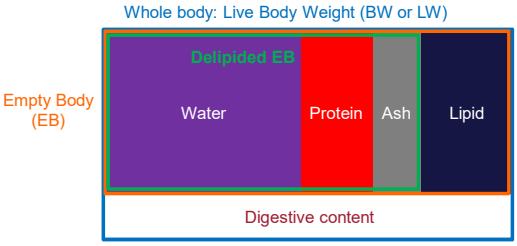
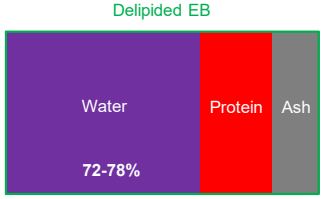
Animal body chemical compartments



Constancy of delipided empty body chemical composition

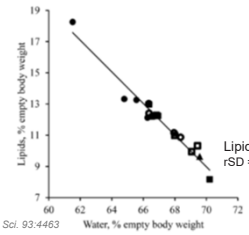


Constant water proportion in delipided EB
within animal model and physiological stage

Implications:
EB water content is correlated:
1. **Positively with EB protein and ash**
2. **Negatively with EB lipid**

Relationship between water and lipids in empty body

Growing goat kids



Lipids (%) = –1.01 × Water (%) + 79.9
rSD = 0.47%, rCV = 3.9%, R^2 = 0.96, n = 17

Lerch et al., 2015, J. Anim. Sci. 93:4463

Quantification of body total and water weights
⇒ Total body composition assessment

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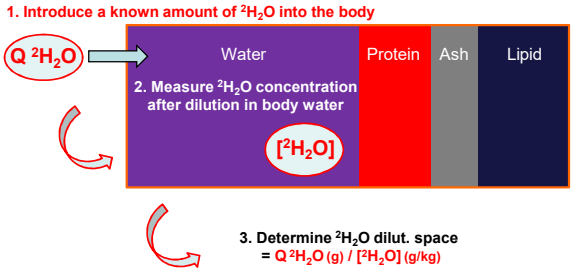
Quantification of total body water through labelled water dilution space measurement

1. Introduce a known amount of ^2H_2O into the body

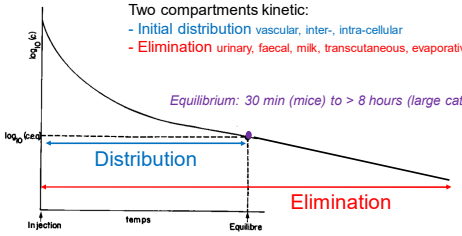


3. Determine ^2H_2O dilut. space
= Q ^2H_2O (g) / [^2H_2O] (g/kg)

Robelin, 1973, Ann. Biol. Anim. Bioch. Biophys. 13:285

Labelled water kinetic following administration

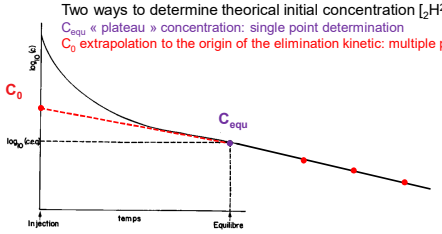
Two compartments kinetic:
- Initial distribution vascular, inter-, intra-cellular
- Elimination urinary, faecal, milk, transcutaneous, evaporative



Robelin, 1973, Ann. Biol. Anim. Bioch. Biophys. 13:285

Labelled water kinetic following administration

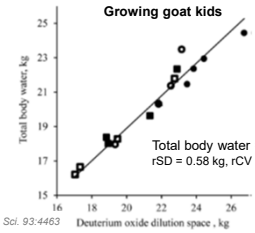
Two ways to determine theorical initial concentration [$_2H^2O$]:
C_{equ} « plateau » concentration: single point determination
C_0 extrapolation to the origin of the elimination kinetic: multiple points determination



C_{equ} technique only reliable when none input of water occurs during the equilibration phase food and water deprivation

C_0 is the most accurate technique when a repeatable scheme of sampling is performed same times over the nychtemeral cycle

Relationship between ^2H_2O dilution space (extrapolation technique) and total body water



Lerch et al., 2015, J. Anim. Sci. 93:4463

Overestimation of total body water due to deuterium exchange with non water hydrogen
Dilution space also include digestive content water

⇒ **Need to calibrate predictive equations relying ^2H_2O space with body composition**

Predictive equations of empty body composition from ^2H_2O dilution space

Growing goat kids (n=17) *Lerch et al., 2015, J. Anim. Sci. 93:4463*

EB lipids (kg) = 0.523 × body weight – 0.588 × ^2H_2O space rSD = 0.34 kg, **rCV = 13.1%, R^2 = 0.83**

EB protein (kg) = 0.066 × BW + 0.084 × ^2H_2O space rSD = 0.18 kg, **rCV = 4.9%, R^2 = 0.89**

EB energy (Mcal) = 5.27 × BW – 5.06 × ^2H_2O space rSD = 3.54 Mcal, **rCV = 7.9%, R^2 = 0.88**

High accuracy largely confirms in other animal models

EB lipids prediction from BW and $^2H^2O$ space

Animal	R^2	rCV (%)
Lactating Ewe	0.87 – 0.97	8 – 14
Lamb	0.98	8.5
Dairy goat	0.85 – 0.95	14 – 16
Dairy cow	0.87	16
Piglet	0.91 – 0.93	12 – 17
Lactating sow	0.86	7.5

16 references

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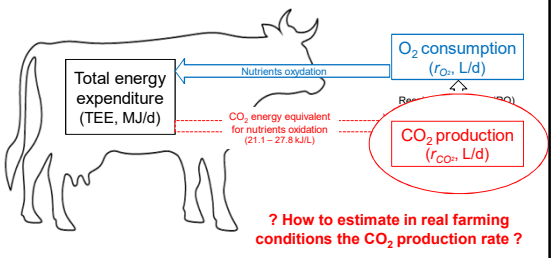
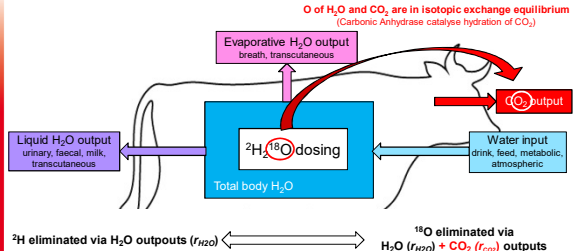
Estimate of energy expenditure from CO_2 production rate



? How to estimate in real farming conditions the CO_2 production rate ?

Lifson and McClintock, 1966, J. Theoret. Biol. 12:46

H_2O and CO_2 balances in animal body

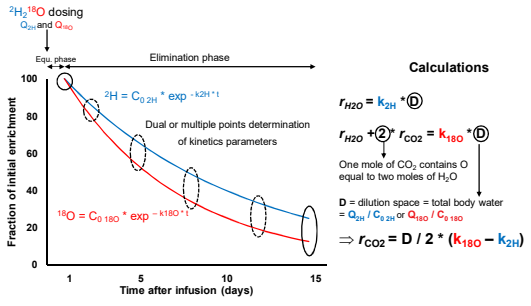


^2H eliminated via H_2O outpouts (r_{H2O}) ⟺ ^{18}O eliminated via H_2O (r_{H2O}) + CO_2 (r_{CO2}) outputs

Difference in ^{18}O and ^2H elimination rates ⇒ Estimate of r_{CO2}

Lifson and McClintock, 1966, J. Theoret. Biol. 12:46

Comparison of ^2H and ^{18}O elimination kinetics



Calculations

$r_{H2O} = k_{2H} * (D)$

$r_{H2O} + (2) * r_{CO2} = k_{18O} * (D)$

One mole of CO_2 contains O equal to two moles of H_2O

D = dilution space = total body water = $Q_{2H} / C_{0\,2H}$ or $Q_{18O} / C_{0\,18O}$

⇒ $r_{CO2} = D / 2 * (k_{18O} - k_{2H})$

Lifson and McClintock, 1966, J. Theoret. Biol. 12:46

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Overview of the use of (doubly) labelled water techniques in animal sciences

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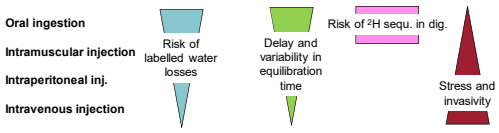
How to administrate and where to follow the kinetic of labelled water ?

Route of administration of the dose



Compartments in which the elimination kinetic is follow

Blood => central compartment, homogeneous, could induce stress esp. for multiple sampling

Urine => elimination compartment, largely validate, needs the second collection urine

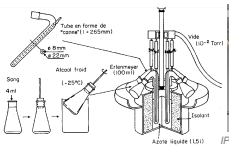
Saliva => reliable compared to blood (human), needs validation in farm animals!

(Milk) => Non-invasive alternative for lactating mammals

Speakman, 2001, Bod. Comp. Anal.:56

Water extraction from samples

Reference method: water distillation / sublimation



IPHC, CNRS Strasbourg (FR)
Cryo-distillation line, 2019

From a « warm » to a « cold » point under vacuum

- Highly accurate and reproducible
- Specific « home made » equip.
- Time consuming 10-20 samp. / day

Robelin, 1973, Biol. Anim. Bioch. Biophys. 13:285

Alternative method: centrifuge tubes

Use of centrifuge tubes for deproteneization of blood plasma

- High throughput and convenient
- Uncompleted water extraction + isotope exchange => potential bias

Chery et al., 2015, Rap. Comm. Mass Spectr. 29:562

Quantification of ^2H and ^{18}O enrichments

Infrared spectroscopy	Isotope-ratio mass spectrometry (IRMS)	Laser spectroscopy
		
1950	**2000**	**2010**
Calibration vs gradient enriched standards curve	Online reduction in TC-EA for $^2H^1H$ / $^1H^2H$ or $C^{18}O/C^{16}O$ gases ratio determination in IRMS	Injection of the sample as a gas in a tube scan by a laser
Moderate accuracy and poor sensitivity => Need high enrichment level: high dosing	High accuracy and sensitivity => Fit with lower enrichment level (/5 vs IR spectroscopy)	Fast and cheap On-field applications Accuracy in the frame of DLW study? Risks of artefacts?

Approximate prices for ^2H_2O and $^2H_2{}^{18}O$ technics (in €)

	^2H_2O - body composition		$^2H_2{}^{18}O$ - energy expenditure	
Model size	Equ. point n=2	Mult. points n=6	Dual points n=3	Mult. points n=6
Poultry ≈ 1.5 kg	40 (0.3%)*	120 (0.1%)	110 (8%)	220 (4%)
Sheep-goat ≈ 65 kg	45 (10%)	125 (4%)	500 (80%)	600 (65%)
Pig ≈ 120 kg	50 (17%)	130 (7%)	800 (85%)	900 (77%)
Cattle ≈ 600 kg	80 (50%)	160 (87%)	3'700 (97%)	3'900 (95%)

*% of total cost due to the dose is reported in brackets
Considering: - a dosing at 0.1 g ^2H_2O/kg BW and 0.05 g $H_2{}^{18}O$/kg BW
- a price of 0.7 €/g 2H2O and 120 €/g H218O
- an analytical cost of 20€ / point for $^2H/^1H$ and 35 € for both $^2H/^1H$ and $^{18}O/^{16}O$ ratios determ.

Conclusions: Deuterated water dilution space for *in vivo* body composition measurement

More than 85 years of use, still in use with several analytical improvement

- Need rigorous experimental procedures *(injection, sampling)*, and specific analytical equipment and skills
- Non negligible cost

⇒ When calibrated predictive equations are used: **accuracy only equalled/overpassed by few imaging techniques**
CT-scan, MRI, DXA, *need complete anaesthesia + not suitable for large cattle*

Direct comparison of several techniques *(^2H_2O dil. space, US, CT, DXA, 3D imaging, BCS, adipocyte size, impedancemetry…)* currently ongoing in goats *Pires, De La Torre, Lerch et al.*

Conclusions: Doubly labelled water technique for *in vivo* energy expenditure measurement

More than 60 years of use, especially in human

Alternative to indirect calorimetry, suitable for on-field study

Accuracy relative depending on corrections for isotope exchange/incorporation
especially for ^{2}H during fat accretion in fast growing animal

Extremely expensive: until now only few applications in farm animal
< 10 published articles, reduced sized models in most of the cases

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Thank you for your attention

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