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O182 Deuterium oxide elimination kinetics for estimation of body water mass and flux: Developments for a thrifty, rapid and non-invasive method in dairy goats

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Introduction
The *in vivo* deuterated water (D_2O) elimination kinetics is a precise method to estimate body water mass, turnover and intake, and body chemical composition (Al-Ramamneh et al., 2010). Nonetheless, the reference methodology in ruminants requires intravenous (iv) injection of D_2O followed by serial blood sampling, water cryoextraction and deuterium enrichment analysis by isotope-ratio mass spectrometry (IRMS). The aim was to investigate non-invasive sampling (milk vs. blood), fast water extraction (centrifugation vs. cryoextraction) and cheaper analysis method [isotope-ratio infrared spectroscopy (IRIS) vs. IRMS] to determine D_2O elimination kinetics in dairy goats.

Material and Methods
Eighteen Alpine goats (3.0 ± 0.6 years old; 226 ± 10 days in milk) weighing 47–71 kg and milked once a day (08h00) were used (Lerch et al., 2021). Jugular blood (16h00) and milk (08h00) were collected for five days following iv injection of D_2O (0.2 g/kg BW), plus one sample

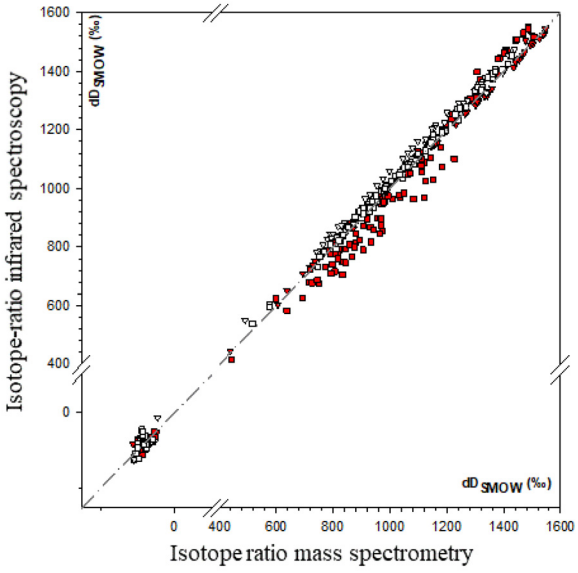

Figure 1. Comparison of isotope analysis methods on deuterium enrichment measurements. ▼ : cryoextracted blood serum, ■ : centrifuged blood serum, ▽ : cryoextracted skimmed milk and □ : centrifuged skimmed milk.

Table 1
Comparison between method combination (serum/milk × cryoextraction/centrifugation × IRMS/IRIS) for the study of deuterated water elimination kinetics and relative total body water (TBW) mass and daily rate of body water inflow/outflow (rH_2O) estimates in dairy goats (n = 18).

	Body water traits		Regression vs. reference method[1]			
			TBW		rH_2O	
	TBW (kg)	rH_2O (kg/day)	RMSE	R^2	RMSE	R^2
Blood serum						
Cryoextraction						
IRMS[3]	39.1 (3.1)[2]	5.1 (1.9)				
IRIS[4]	39.3 (3.2)	4.9 (1.9)	0.4	0.99	0.06	1.00
Centrifugation						
IRMS	39.8 (3.4)	5.1 (1.9)	0.6	0.97	0.08	1.00
IRIS	38.8 (3.5)	5.8 (1.9)	1.2	0.87	0.29	0.98
Skimmed milk						
Cryoextraction						
IRMS	39.1 (3.5)	5.1 (1.5)	1.7	0.77	0.39	0.96
IRIS	38.3 (3.4)	4.9 (1.3)	1.8	0.75	0.42	0.95
Centrifugation						
IRMS	39.5 (3.7)	4.9 (1.5)	1.8	0.73	0.37	0.96
IRIS	39.0 (4.0)	4.8 (1.4)	1.9	0.70	0.41	0.96

[1] Serum/cryoextraction/IRMS considered the reference method.
[2] Mean (SD).
[3] IRMS, isotope-ratio mass spectrometry.
[4] IRIS, isotope-ratio infrared spectroscopy.

before injection. One mL of blood serum or skimmed milk was cryodistillated to extract water (West et al., 2006), using quartz wool to trap volatile organic compounds (VOCs). Three mL of blood serum or skimmed milk were mixed with 150 mg charcoal followed by centrifugation (4000 g, 15 min, 4 °C). The recovered supernatant was centrifuged (2000 g, 30 min, 4 °C) in 10 kDa deproteinisation-tubes (Vivaspin6). Extracted water was analysed by IRMS (EuroVector-Isoprime, UK) and IRIS (Picarro L2140 i, USA) connected to a micro combustion module to eliminate VOCs. For each method combination (serum/milk × cryoextraction/centrifugation × IRMS/IRIS), parameters of D_2O elimination kinetics were computed by nonlinear regression (Proc NLIN, SAS 9.4): $C_t = C_0 \times \exp^{-k \times t}$. Total body water (TBW; D_2O dilution space/1.08) and daily rate of body water inflow/outflow (rH_2O; TBW × k) were computed and compared by linear regression (Proc GLM) against the reference (serum/cryoextraction/IRMS).

Results and Discussion

D_2O elimination kinetics presented a $R^2 = 0.99$ for all method combinations, except for serum × centrifugation × IRIS ($R^2 = 0.96$, Figure 1). The VOCs present in serum may have interfered with IRIS analysis, which was not observed with centrifuged skimmed milk. Ruminants absorb VOCs from ruminal fermentation, and these metabolites are at greater concentrations in plasma than in milk (Billa et al., 2020). When compared to the reference method, estimated TBW and rH_2O were related ($0.70 \geq R^2 \geq 0.99$) across all method combinations. The R^2 for serum was lower when estimated from centrifugation × IRIS compared to centrifugation × IRMS (Table 1). Milk had lower R^2 (0.74 for TBW and 0.96 for rH_2O) than blood serum, whatever the extraction and analysis method. Differences between milk and serum may be explained by divergent water turnover between mammary gland (once a day milking and low milk yield) and blood sampled at discrete time points.

Conclusion and Implications

No deviation was observed between IRIS and IRMS analyses, except for centrifuged serum which maybe due to VOCs residues. Cryoextraction and centrifugation yielded similar D_2O enrichment results, except for serum analysed by IRIS. Centrifugation is rapid and does not require custom-made cryoextraction lines. The TBW and rH_2O estimates from milk D_2O kinetics were of similar magnitude, but only moderately correlated with the reference blood serum method. The use of milk is non-invasive, less time consuming and limits animal handling which facilitate its implementation in experiments compared to blood sampling.

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