Supplementary Material

Supplementary Table 1. Excel table with the experimental animals' initial and final body weights.
Supplementary Table 2. Excel table with individual animal data.
Supplementary Table 3. Excel table with experimental and supplier diet δ^2H and $\delta^{18}O$.
Supplementary Table 4. Excel table with quail feather data.
Supplementary Table 5. Excel table with individual measurements used in computing δ^2H_n.

Supplementary Table 6. δ^2H_n and $\delta^{18}O$ of diets from the animal suppliers

	δ^2H_n (‰)	sd	$\delta^{18}O$ (‰)	sd
guinea pig supplier non-lipid diet	-86	2.5	24.6	0.0
guinea pig supplier whole diet	-91	2.4	24.6	0.1
rat supplier non-lipid diet	-66	0.6	25.3	0.1
rat supplier whole diet	-77	2.0	25.4	0.0
quail supplier non-lipid diet	-58	4.4	26.2	0.3
quail supplier whole diet	-64	2.9	26.0	0.1

Supplementary Table 7. Chloroform:methanol (2:1 v/v, CM) vs. petroleum ether (PE) tissue extraction test (n=2 replicates)

	solvent	mean muscle $\delta^{18}O$ (‰)	sd	mean muscle δ^2H (‰)	sd	mean % O	mean % H	$\delta^{18}O$ CM-PE (‰)	δ^2H CM-PE (‰)
rat	chloroform: methanol	13.2	0.3	-97.2	1.1	23.3	5.7	-0.5	2.5
	petroleum ether	13.7	0.3	-99.7	1.6	23.4	5.8		
rat repeat	chloroform: methanol	13.3	0.1	-96.0	1.3	23.1	5.6	-0.5	2.3
	petroleum ether	13.7	0.0	-98.3	0.5	23.4	5.8		
beef	chloroform: methanol	7.1	0.0	-141.9	0.3	23.2	5.6	-0.7	0.4
	petroleum ether	7.7	0.0	-142.3	0.2	24.1	5.7		

Supplementary Table 8. Dentine δ^2H differences by species and t test results

	plant[a]	insect			meat		
	mean difference (‰)	mean difference (‰)	95% conf.	p	mean difference (‰)	95% conf.	p
rat-guinea pig	17.2	13.3	±15.3	0.065	24.2	±12.2	0.0041

[a] performing a t test is not possible because one group has n=1 observation

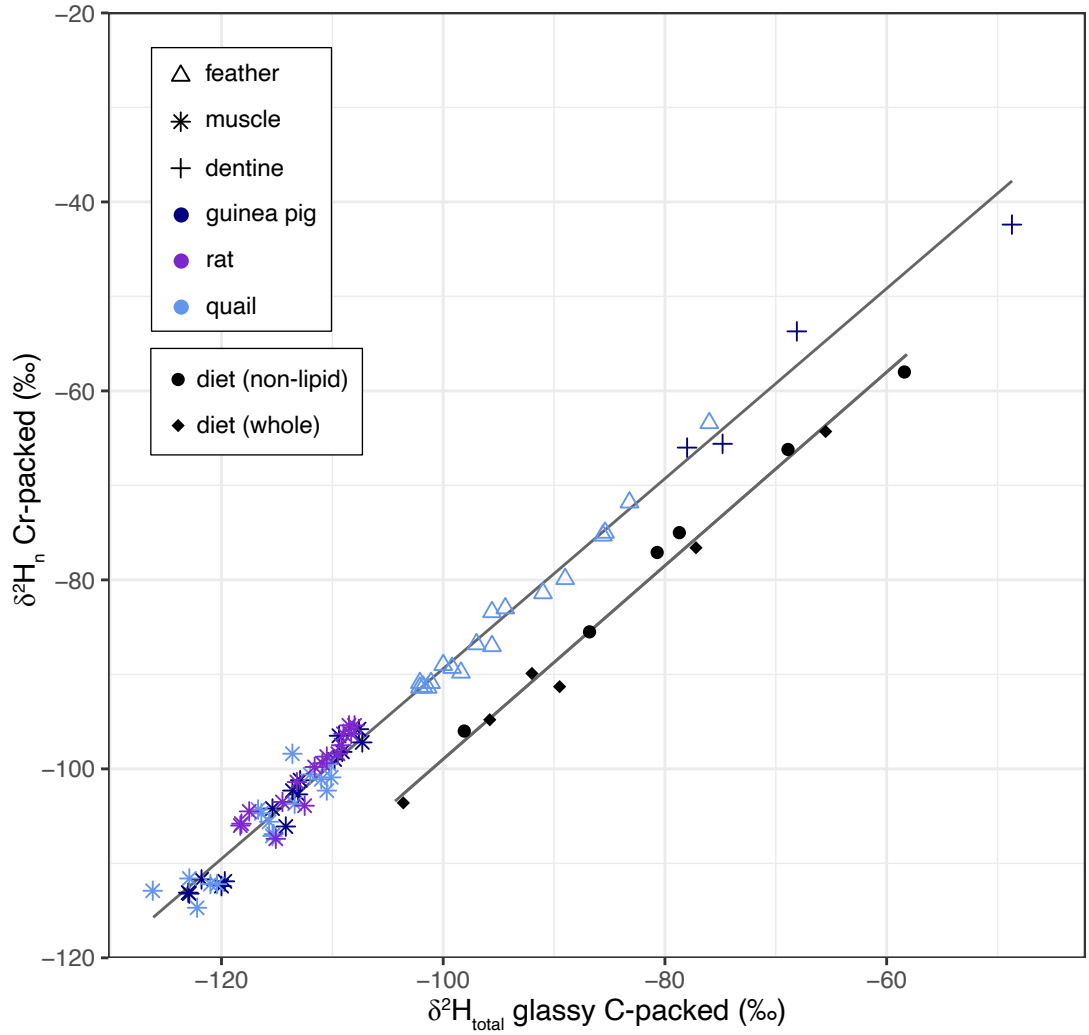
Supplementary Table 9. Paired lipid extract and muscle δ^2H values in a subset of guinea pig samples

sample	lipid extract δ^2H (‰)	muscle δ^2H_n (‰)
1AGC1R2	-156	-112
1AGB6G2	-164	-104
1AGB1G1	-152	-113

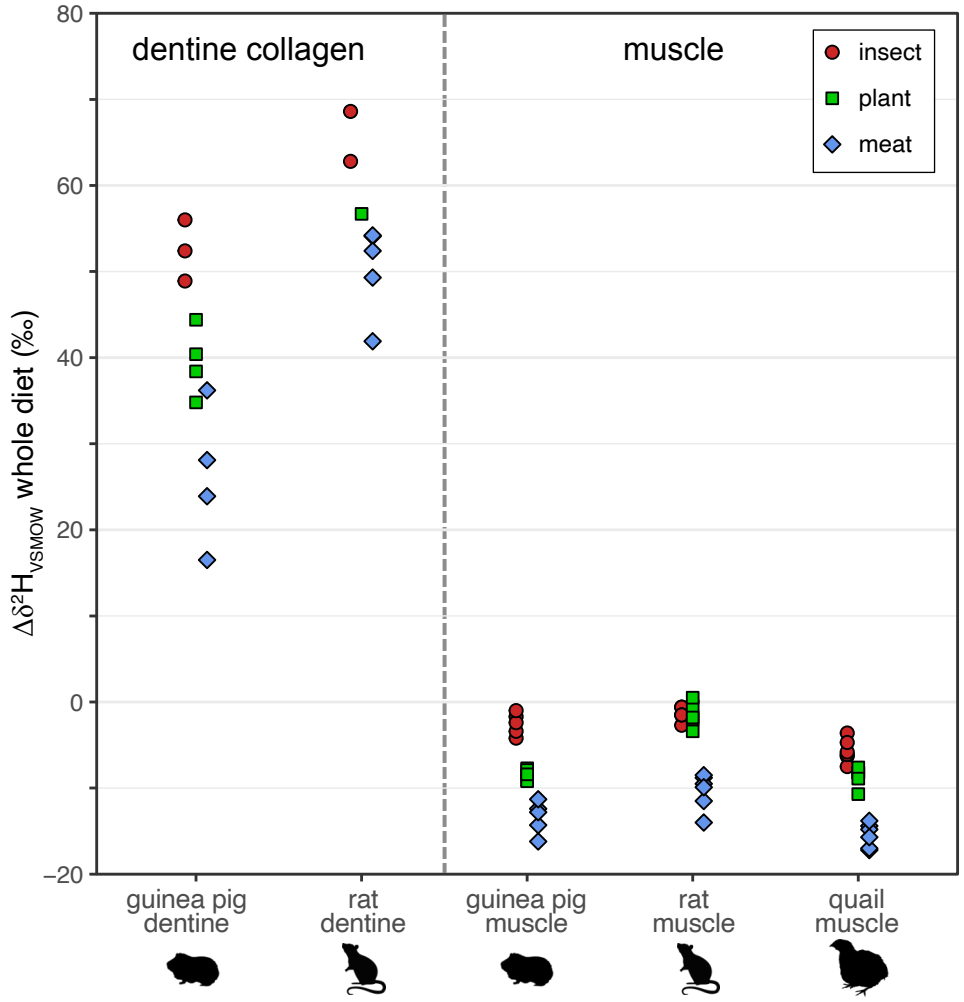
Supplementary Results

Chromium-packed vs. glassy carbon packed reactor

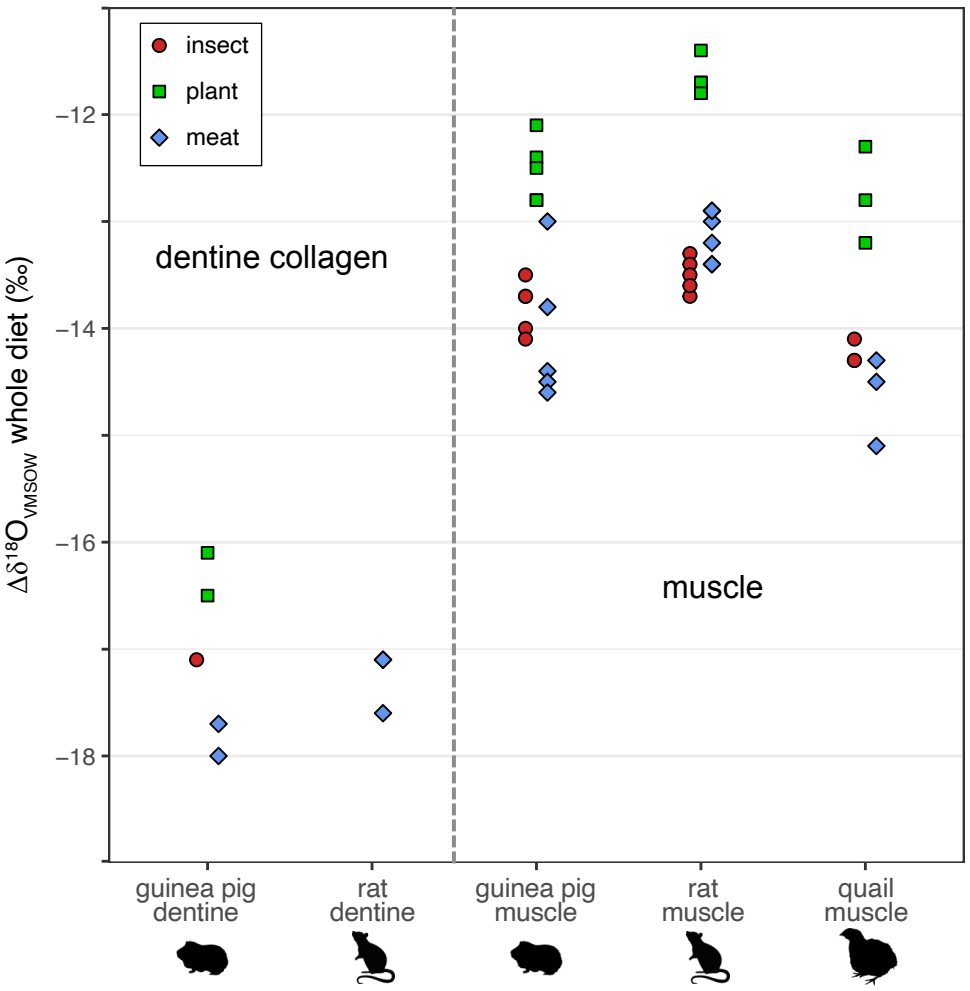
The proteinaceous tissues show a linear relationship between δ^2H_n (with Cr-packed reactor) and δ^2H (with glassy C packed reactor). We include some quail feather samples in this comparison. The model 2 regression line (Legendre, 2014) computed with R software (R Core Team, 2023) is δ^2H_n-Cr = 1.01 δ^2H-glassyC + 11.4 ‰ (Supplementary Figure 1) and the mean offset is δ^2H_n-Cr = δ^2H-glassyC +10.6 ‰ (±1.7 ‰, 1 SD, n=70). This relationship agrees well with previous work in collagen (slope=1.05 and intercept=11.6, mean offset 10.1 ‰, Reynard et al., 2019). The diets also showed a linear relationship between δ^2H_n-Cr and δ^2H-glassyC with a slope near one and a small intercept that overlapped with zero at 95% confidence (Supplementary Figure 1). This near-zero δ^2H isotope difference by method for the diets is expected given the lower nitrogen content of the diets (compared to pure proteins), because the isotopic offset is likely due to HCN formation which increases with increasing N content (Reynard and Tuross, 2016).



Supplementary Figure 1. Cr-packed δ^2H_n vs. glassy C-packed δ^2H (non-water-equilibrated, total) for feather, muscle and dentine collagen (upper line) and animal diets (lower line). The model 2 regression for tissues is $\delta^2H_n(Cr) = 1.01\ \delta^2H(\text{glassy C}) + 11.2$ ‰, 95% confidence slope ±0.03 and intercept ±3.2, $r^2=0.985$, $p < 0.001$, n=70; and for diets is $\delta^2H_n(Cr) = 1.02\ \delta^2H(\text{glassy C}) + 3.4$ ‰, 95% confidence slope ±0.08 and intercept ±6.5, $r^2=0.988$, $p < 0.001$, n=12. The mean offset for the feather, muscle and dentine collagen is $\delta^2H_n\text{-Cr} = \delta^2H\text{-glassyC} +10.6$ ‰ (±1.7 ‰, 1 SD, n=70), and does not differ between muscle (10.7 ‰ ±1.8 ‰, 1 SD, n=48) or feathers (10.5 ‰ ±1.1 ‰, 1 SD, n=18).



Supplementary Figure 2. Tissue $\Delta\delta^2H$ (from whole diet) in muscle and dentine collagen of animals fed with plant-based (green squares), insect-based (red circles), and meat-based (blue diamonds) diets, in rats, guinea pigs, and quail (the latter muscle only).



Supplementary Figure 3. Tissue $\Delta\delta^{18}O$ (from whole diet) in muscle and dentine collagen of animals fed with plant-based (green squares), insect-based (red circles), and meat-based (blue diamonds) diets, in rats, guinea pigs, and quail (the latter muscle only).

Supplementary References

Legendre P. lmodel2: Model II regression. R Package Version 1.7–3; 2014. https://CRAN.R-project.org/package=lmodel2. (accessed [June 5, 2024])

R Core Team (2023). R: A Language and Environment for Statistical Computing. Version 4.3.1. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/. (accessed [June 16, 2023])

Reynard, L.M., and Tuross, N. (2016). Hydrogen isotopic analysis with a chromium-packed reactor of organic compounds of relevance to ecological, archaeological, and forensic applications. Rapid Commun. Mass Spectrom. 30, 1857–1864.

Reynard, L.M., Ryan, S.E., and Tuross, N. (2019). The interconversion of δ^2H values of collagen between thermal conversion reactor configurations. Rapid Commun. Mass Spectrom. 33, 678–682.