Oxygen and Hydrogen Isotope Composition of Silage Water

ABSTRACT: Silage is an important dietary water source that influences the oxygen and hydrogen isotopic composition of domestic herbivores and their products. Silage sampled fresh from the silo had 18O- and 2H-depleted tissue water when compared with fresh pasture grass sampled around midday during the silage-making seasons. During exposure in the feed bunk, silage water became increasingly enriched in 18O and 2H. When δ18O was plotted against δ2H, the slope of the regression was less during daytime than during nighttime. Exposure to 18O- and 2H-enriched or -depleted water vapor inside sealed glass containers led to strong changes in the isotopic composition of silage water. The results resembled predictions from the Craig–Gordon isotope model of evaporation. The atmospheric conditions during exposure (relative humidity, exposure time, and isotopic composition of the air vapor) in the feed bunk thus strongly affect the isotopic composition of silage water ingested by domestic herbivores.

KEYWORDS: feedwater, labeling, evaporation, turnover, pool kinetic, water isotopes

INTRODUCTION

Dietary water or feed moisture is an important source of water for herbivorous animals. The oxygen (18O) and hydrogen (2H) isotope composition of ingested water is reflected in animal body water, in fecal water, and in tissues such as bones and teeth, hair, and milk. The isotopic composition of dietary water is affected by that of precipitation (meteoric water), which is influenced by geographic features, and also by local environmental conditions and vegetation characteristics. Latitudinal, altitudinal, and seasonal variation in the isotope composition of meteoric water depends on temperature for rain and snow formation. The isotopic composition of water in plant roots, tubers, and stems closely reflects that of the water taken up by roots, but leaf water is isotopically enriched in comparison with source water due to isotope fractionation effects during evaporation. Similarly, free surface water, such as water in puddles on pastures and dew on the leaves, is subject to evaporative enrichment. Enrichment is influenced by relative humidity (RH) and the isotopic composition of water vapor. Local humidity can have an important effect on the oxygen isotope composition of biogenic phosphate. Because of the above relationships there is much interest in food science, ecology, anthropology, and forensic science in using the hydrogen and oxygen stable-isotope composition of animal tissues and animal products as a tracer for the geographic origin of foodstuffs as well as for the detection of adulteration of foodstuffs and the geographic movements of animals (e.g., in birds), the behavioral/nutritional ecology of herbivores, and the production ecology of livestock systems.

Silage is an important feedstuff in cattle production, and silage moisture can contribute a significant fraction of the total water intake of cattle. Therefore, the isotopic composition of silage water affects that of body water and tissues, including milk and meat. To our best knowledge, the isotopic composition of silage water has not been studied to date. Accordingly, there is no empirical knowledge on the relationship between the 18O and 2H of silage water and that of other water sources, such as fresh grass and drinking water, which have been studied comparatively well. Also, there is no empirical knowledge about the main environmental controls of silage-water isotope composition. Thus, for instance, we do not know to what extent observed geographic, seasonal, and production ecology-related patterns in the 18O and 2H composition of milk or beef and lamb meat are related to isotope effects emanating from silage-water consumption.

We hypothesize that silage-water isotope composition is strongly affected by RH and the isotopic composition of atmospheric humidity, as plant tissue becomes disconnected from the (liquid) soil water source when the stand is cut, and subsequent water exchange is dominated by vapor fluxes. RH may vary by 50% during a day, whereas the isotopic composition in air humidity typically varies by 40‰ for hydrogen and 6‰ for oxygen during a year, suggesting that RH and isotopic composition could well be a main control of silage-water isotope composition.

This study presents results derived from data collected on grassland farms in southern Germany and also the results from an experiment conducted under controlled conditions. The work addresses the following specific questions: What is the water isotope composition of silage when taken fresh from the silo? Does its isotope composition differ from that of leaf water sampled in the period of silage production? How does silage-water isotope composition change when silage is exposed to atmospheric conditions in the feed bunk? And, how does silage-water isotope composition respond to altered vapor isotope composition in controlled experiments? The observed changes of silage-water isotope composition were compared with predictions from the Craig–Gordon model. This model has been used extensively to explain the water isotope relationships during evaporation of open water bodies and leaves, and it provides a mechanistic treatment of evaporative fractionation, RH, and vapor isotope composition on water isotope composition at the evaporating surfaces.

THEORY

Assuming that silage water is a well-mixed reservoir, the change in water mass per square area (W, mol m⁻²) over an increment of time dt is given by the rates of input (I, mol m⁻² s⁻¹) by rain...
or dew formation, and by the rate of evaporation ($E$, mol m$^{-2}$ s$^{-1}$):

$$\frac{dW}{dt} = I - E$$

(1)

For the conditions in our feed bunk underneath a roof we can neglect $I$. The evaporation flux $E$ is proportional to the vapor concentration difference between the water–air interface (evaporating surface) and the turbulent atmospheric region and, thus, to RH:$$E = C_s(1 - h)/\rho$$

(2)

where $C_s$ denotes the saturation concentration of vapor (which depends on temperature), $h$ is RH in equations, and $\rho$ is a resistance coefficient.

The isotope composition of the evaporation flux is given by the Craig–Gordon model, which accounts for the equilibrium isotope fractionation between liquid and vapor, and the kinetic fractionation resulting from the diffusion across the air boundary layer, and the back flux of atmospheric moisture:

$$R_E = (\alpha R_s - hR) / ((1 - h)\rho/\rho)$$

(3)

$R$ denotes the ratio of the abundances of the heavy and light isotopes ($^{18}H/^{1}H$ or $^{18}O/^{16}O$), and $\alpha$ is the temperature-dependent fractionation factor ($\alpha_{H} = 1.0095$, $\alpha_{H} = 1.0804$ at $T = 24^\circ C$). The subscripts indicate the evaporated water ($E$), silage-water in our case ($s$), atmospheric vapor ($a$), and the heavy isotope ($i$).

Equation 3 can be translated into $\delta$ notation, the conventional notation for the natural abundance of stable isotopes, where $\delta$ is the isotopic composition of water that is in equilibrium with air vapor and also reaches the silage-water pool will gradually exchange and turn over until an equilibrium is reached. We used the reaction progress method to describe the turnover of $O$ and $H$ in silage water. An advantage of the reaction progress approach is that it provides half-life estimates for multiple pool systems. The reaction progress controls the isotopic composition of water condensed in saturated air and thus $\delta_{cond}$. When silage becomes exposed to an infinite vapor source that is not in equilibrium with the water pool in the silage, this silage-water pool will gradually exchange and turn over until an equilibrium is reached. We used the reaction progress method to describe the turnover of $O$ and $H$ in silage water. An advantage of the reaction progress approach is that it provides half-life estimates for multiple pool systems. The reaction progress controls the isotopic composition of water condensed in saturated air and thus $\delta_{cond}$.

(1) $\delta_{eq} = 1 - F$

(10)

where $\delta_{eq}$ is the isotopic composition of water that is in equilibrium with air vapor and $F = 0$ and $F = 1$ represent the beginning of the exchange reaction and its final steady state (equilibrium). The exchange of a single pool follows

$$1 - F = e^{-\lambda t}$$

(11)

where $\lambda$ is the first-order rate constant. Equations 10 and 11 are particularly useful when expressed as

$$\ln(1 - F) = -\lambda t$$

(12)

which follows a straight line in the case of a single pool system, and a graph composed of several straight line segments in systems with several pools.

### MATERIALS AND METHODS

**Silage.** Silage was obtained from the Grünschwaige Experimental Station, Germany: 48°23’ N, 11°50’ E (for details regarding the site, see Schnyder et al.). All grassland was used for beef cattle husbandry with Limousin suckler cows. During the grazing season (approximately from the end of April to the end of October) all cattle were kept on permanent pastures. Meadows and surplus pasture growth were used for silage and hay production. Silage cuts were mainly taken in June and August (yielding 40 and 35% of total on-farm silage production). Silage production generally followed the practices described by Wilkinson. Grass was mowed at around 10:00 a.m., followed by spreading and tedding. In the afternoon of the same day it was raked into rows, and on the following day the wilted grass (approximately 45% dry matter content) was collected using a forage harvester, cut to a length of 4–8 cm, transferred to a drive-in silo without addition of
other fermentable substrates, and consolidated. The silo was sealed not later than 5:00 p.m., and it remained closed until opening for feeding. Silage was the main feed source during the period of sward dormancy when the cattle were housed.

Fresh pasture grass was sampled at approximately biweekly intervals during the growing seasons (from April to October) of 2006 to 2012. All grass samples were collected around noon, immediately put into extraction vials, and sealed in the field.

Silage samples were taken randomly from silos on the experimental farm in March/April of 2011, 2012, and 2013. In addition, 28 samples of silage were obtained from silos of regional farms within a radius of about 20 km around the experimental farm. The samples comprised silage from 2012 and 2013, from different months (May–October), from the first to the fourth cut, and from grass-clover mixtures and seminatural grasslands to capture the range that can be expected.

Experiment A: Isotopic Composition of Silage Water in the Feed Bunk. This experiment assessed the variation of the isotopic composition of silage water after distribution in a feed bunk during a day. During the period of sward dormancy when cattle were housed, the daily requirement of silage was distributed on the cleaned feed bunk in the open-front stall once every morning. Silage was sampled on three consecutive days (April 25, 26, and 27, 2012) immediately after distribution in the feed bunk and at 0.5, 1, 2, 4, and 24 h thereafter. On a given day, the last sample (of the 24 h period) was collected on the next day just before the leftovers were removed and new silage was provided. A handful of silage was sampled from the upper layer of silage in the feed bunk where the cattle tended to consume. Each sample was conserved in a self-sealing bag and kept at −4 °C until water extraction started.

Weather data, including RH and air temperature, which exhibited clear diurnal cycles (Figure 1), were obtained from a meteorological station in Freising (distance = 7 km; http://www.lfd.bayern.de/agm/daten.php?statnr=8).

Figure 1. Weather conditions (RH, solid line; air temperature, dashed line) for four consecutive days during the feed bunk experiment. The arrows indicate the removal of leftovers from the previous day and the distribution of fresh silage in the feed bunk when 0-h and 24-h samples were taken. Markers show sampling after 0, 0.5, 1, 2, 4, and 24 h since distribution in the morning (8:00 a.m.) on April 24 (□), 25 (△), 26 (○), and 27 (×) of 2012, respectively.

Additionally, air vapor was sampled by cryogenic distillation in April (from 2006 to 2011) to assess its variation in δ18O and δD during this time of the year. Rain was sampled and measured every month from 2006 to 2011 for construction of the local meteoric water line (LMWL).

Experiment B: Vapor Labeling of Silage Water. The second experiment analyzed how the isotopic composition of air vapor influences the isotopic composition of silage water. Silage, which had been produced the year before, was collected from the silo of the experimental farm using a silage corer on March 31, 2011, and then kept in a self-seal bag at 4 °C until placement in a closed container (an exsiccator vessel with drying agent removed). To allow quick sampling from the container during the experiment, about 2 g of silage was allocated to each of six or eight aluminum trays per container as individual samples (see below). The container was then closed for about 12 h to effect a homogenization of moisture content and isotopic composition in all of the samples.

The silage samples were placed on the perforated shelf of the (exsiccator) container. Under the shelf, 40 mL of labeling water was placed in a plate of 10 cm diameter. To promote air mixing, a fan was installed in the headspace of the container. The labeling water and silage samples were quickly placed in the container and the lid was closed. Thus, the initial conditions in the container were the same as in the room with temperature, RH, δ18O, and δD around 24 °C, 40%, −18‰, and −130‰, respectively.

The experiment was divided into two groups, with different sampling intervals. Each group had two containers with differing isotope composition of the labeling water. The labeling water was either enriched in 18O and H2 or depleted (heavy or light water) relative to silage water. In the first group with short time intervals between openings, six samples were exposed to the labeling atmosphere and two were removed randomly after 1, 2, and 4 h from each container. In the second group with long intervals between openings, each container contained eight samples and two samples were collected after 8, 24, 48, and 96 h. In each case, one of the duplicate samples was used to measure the isotopic composition of water and the other was used to measure relative moisture content (g/g, water mass in fresh mass of silage). The labeling water in each container was sampled at the beginning and end of the labeling experiment.

In a preliminary experiment, air-tightness of the containers was verified by filling the containers with N2 and measuring CO2 and humidity changes using a LiCor LI-6262 infrared gas analyzer. The evolution of humidity inside containers was examined after placing a dish of water in the container. Humidity increased rapidly from an initial 48 to 100% with a half-life of 1.8 min (r2 = 0.96 with n = 233). The measurements were not repeated during the experiments with silage, because it would have required additional fittings, hoses, pumps, and instruments, increasing the probability of leaks and failure.

Extraction and Measurement of Silage Water. Silage water was extracted by cryogenic vacuum distillation. For the regional samples and for experiment A, δD and δ18O were measured using a L2120-i Analyzer (Picarro, Santa Clara, CA, USA). Mean analytical uncertainties quantified as SD of about four replicate measurements for each sample were ±0.1‰ for δ18O and ±0.5‰ for δD. To examine whether the measurements using the L2120 are biased by organic volatiles in the water extracts, we compared the measurements of the L2120 with measurements of an IsoPrime isotope ratio mass spectrometer that was interfaced to a low-flow equilibration unit (both GVI, Manchester, UK). This comparison was made for a large number of samples (n = 177 for O and n = 58 for H) of differing origins. We found no indication that water samples extracted from plants or soil were subject to a larger error than pure water (rain, tap water, groundwater, air humidity). The mean bias (difference) between both instruments for SS plant samples (O, 1.1‰; H, 1.6%) and S1 soil samples (O, 0.0‰; H, −2.4‰) did not differ from the mean bias of 70 pure water samples (O, 0.4‰; H, −1.4‰). Also, the scatter between both measurement methods as quantified by the root mean squared error did not differ between plant samples (O, 1.4‰; H, 5.8%), soil samples (O, 0.2‰; H, 2.5%), and pure water samples (O, 0.6‰; H, 3.5%)

For experiment B, which was more sensitive to such bias by organic volatiles, H2 and 18O values were determined by IRMS with a Thermo Finnigan DELTAplus XL with TC/EA converter (Finngan MAT, Bremen, Germany). Analytical uncertainties were ±0.5‰ for δ18O and ±2‰ for δD in this case.

All three measurement systems were calibrated using secondary isotope standards (i.e., V-SMOW, SLAP, and GISP) and checked at regular intervals using previously calibrated (again against secondary isotope standards) laboratory water standards for possible instrument drift. These laboratory standards were produced from local deionized tap water by evaporation/condensation, so that their isotope range spanned the range of samples.

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RESULTS

Comparison of Fresh Grass and Silage. The water isotope composition of fresh grass sampled in June and August, the two main periods of silage making, differed slightly (about 1.2‰ in $\delta^{18}$O), reflecting the differences in weather conditions between the two periods. In contrast, for silage there was a pronounced depletion by about 14‰ in $\delta^{18}$O and 60‰ in $\delta^2$H relative to fresh grass (Table 1). In the feed bunk, the silage became less depleted but, on average, the effect was small (about 1‰ in $\delta^{18}$O).

Table 1. Oxygen and Hydrogen Isotopic Composition ($\delta^{18}$O and $\delta^2$H) of Water in Silage of Regional Farms and on the Experimental Farm and in Fresh Grass

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>$\delta^{18}$O (%)</th>
<th>$\delta^2$H (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silage from silo</td>
<td>n</td>
<td>mean</td>
</tr>
<tr>
<td>Regional farms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silage from silo</td>
<td>28</td>
<td>−12.9</td>
</tr>
<tr>
<td>Silage from feed bunk</td>
<td>36</td>
<td>−8.1</td>
</tr>
<tr>
<td>Fresh grass in June</td>
<td>23</td>
<td>2.8</td>
</tr>
<tr>
<td>Fresh grass in August</td>
<td>32</td>
<td>4.0</td>
</tr>
</tbody>
</table>

**Silage was directly taken from either the silo or the feed bunk. Fresh grass was collected at around noon in the months when grass was cut for silage production (June and August).**

The silage from the regional farms, on average, was even more depleted than the silage of the experimental farm. The standard deviation was large, and the range included the silage from the silo in April lay at the right side of the LMWL. On average, air vapor in April had a $\delta^{18}$O of −18‰ (SD 2‰) and a $\delta^2$H of −132‰ (SD 16‰), respectively, whereas the water estimated from air vapor and equilibrium fractionation was very close to the LMWL and exhibited almost identical $\delta^{18}$O but higher $\delta^2$H than the water of silage taken fresh from the silo. The silage-water samples from the feed bunk were divided into two groups according to the RH of the air (Figure 3). During the morning (8:00 a.m. to 12:00 p.m.) the RH was relatively low and decreasing (from about 60 to 40%; Figure 1), thereby accelerating evaporation. Samples taken in this time fitted an evaporation line with a slope of 5.95 (standard error of the slope, 0.45, $r^2 = 0.76$; Figure 3a). During the night-time the RH increased to about 80% and remained at this level for several hours (Figure 1). The slope of the $\delta^2$H versus $\delta^{18}$O regression for samples taken at daily intervals, between 8:00 a.m. of 1 day and 8:00 a.m. of the next day, was significantly steeper (7.61 with a standard error of 0.55, $r^2 = 0.96$; Figure 3b).

**Experiment B: Vapor Labeling Experiment.** The moisture content of the silage decreased in the sealed glass containers with short opening intervals of 1, 2, and 4 h ($y = -0.03 + 0.47x$, $r^2 = 0.46$), probably due to exchange of vapor-saturated air with vapor-depleted air when the container was opened frequently (Figure 4). In contrast, when the container was opened infrequently, silage moisture content increased with time due to condensation ($y = 0.41x^{0.08}$, $r^2 = 0.45$).

The water from silage sampled before exposure to the labeling water did not fall on the LMWL (Figures 5 and 6), as had been observed in experiment A (Figure 3). With the exception of the samples taken after 1 h, the successive samples showed a gradual exchange of the initial silage water with vapor derived from the labeling water (Figure 5). Thus, silage water became gradually enriched when exposed to heavy labeling water and depleted when exposed to light labeling water.

The gradual exchange of silage water with labeling water via vapor fluxes was especially evident when plotted as a function of labeling duration (Figure 6). A different isotopic trend of silage water was apparent, however, in the container with short intervals between openings compared with the container with long intervals between openings (compare panels a and b of Figure 6 and panels c and d of Figure 6). During the first hour,
the silage water became enriched in $^{18}$O by on average 3‰ and in $^2$H by on average 17‰ (Figure 6a,c). However, the initial enrichment was less (only about one-third) when the labeling water was isotopically depleted (Figure 6a,c). With longer exposure time, the silage water approached the corresponding labeling water (Figure 6a,c) and almost attained the value of labeling water after 48 h in the case of light labeling water. Conversely, a notable change still occurred with the heavy labeling water due to the larger isotopic spacing between silage water and heavy labeling water.

For the containers with long opening intervals and long exposure times, it was assumed that the disturbance due to the opening was very small and, hence, did not affect the estimation of reaction progress (Figure 7). The effect of condensation, however, had to be considered, as this was evident from the increasing moisture content (Figure 4). After exclusion of this effect (eq 9), the transformed data fell on one straight line during the first 48 h (Figure 7). The slope of the regression line was $-0.035$ (standard error 0.002, $r^2 = 0.957$), which corresponds to a half-life of approximately 20 h. The measurements after 96 h of exposure time fell clearly outside the range that could be expected from the prediction interval of the regression. Following the reaction progress approach, the intercept of the regression for the slow pool indicated that approximately 10% of the total silage water had a half-life longer than 20 h.

**Model Simulations.** Under zero humidity, the δ values follow a Rayleigh line when plotted against the remaining fraction of silage water (Figure 8). With RH increasing up to 50%, the convexity of the line decreased as compared to the Rayleigh line until a straight line was reached at RH = 50%, with a positive slope in the case of labeling water being heavier than the silage water and a negative slope in the opposite case (Figure 8a,b). With RH > 50%, the silage water approached a constant value during drying. This constant value was lower and was reached earlier at higher RH. At 100% humidity the water in the silage approached the isotopic composition of the labeling water.

The influence of RH on the pattern of slopes for the relationship between δ$^2$H versus δ$^{18}$O (evaporation line) depended on the relation of the initial composition of silage
When the water in equilibrium with air vapor was isotopically heavier than the silage water, the slopes increased with RH (Figure 8c). In contrast, the \( \delta^2\text{H} \) versus \( \delta^{18}\text{O} \) line of remaining water rotated clockwise as RH increased if the silage became exposed to vapor, resulting in equilibrium water that was more depleted than the silage water (Figure 8d). For the isotopic composition chosen for Figure 8c, the line became almost vertical at about 60% RH, indicating that under this condition only \( \delta^2\text{H} \) would change while \( \delta^{18}\text{O} \) would remain constant.

**DISCUSSION**

This work presents the first analysis of silage-water isotope composition. It demonstrates a large contrast to the isotopic composition of fresh grass and a dominant role of RH and atmospheric water-vapor isotope composition on the isotopic composition of silage water. The changes in the isotope composition of silage water behaved similarly with the expectations from the simulation with the Craig–Gordon evaporative model, a classical mechanistic framework which has been applied extensively in empirical studies of the water isotope composition of open bodies of water and leaf water.\(^{11}\)

Silage water, extracted from silage removed fresh from the silo, was strongly depleted in \( ^{18}\text{O} \) and \( ^2\text{H} \) relative to water extracted from fresh grass that was collected at about the same time of day as grass mowing for silage production took place, even though the moisture content of fresh grass (approximately 80%) was much higher than that of silage (about 50%). Thus, silage water must have become depleted in \( ^{18}\text{O} \) and \( ^2\text{H} \) at one or more stages of the production process between mowing and storage. Such processes may have involved vapor exchange with soil and atmosphere and condensation–evaporation cycles involving dew rise (vapor flux from the soil) and dew fall (atmosphere) during diurnal periods between mowing and carting of silage to the silo. Both atmospheric vapor and soil water are depleted in \( ^{18}\text{O} \) and \( ^2\text{H} \) relative to leaf water collected at noon.\(^{42}\) Also, leaf water follows strong diurnal cycles,\(^{42}\) with predawn values of \( \delta^{18}\text{O} \) approximately 10‰ depleted relative to leaf water collected at noon (Inga Schleip, Ulrike Gamnitzer, Hans Schnyder, unpublished data). Certainly, additional empirical studies of diurnal vapor exchange fluxes, and related condensation–evaporation phenomena on silage water \( \delta^{18}\text{O} \) and \( \delta^2\text{H} \) between mowing and closing of the silo are needed.
After removal from the silo, the isotopic composition changed depending on the moisture conditions of the surrounding air, including its isotopic composition and the duration of exposure. In a vapor-labeling experiment, initial drying led to a transient enrichment but, with continuing exposure to the high humidity environment, exchange reactions became predominant (Figure 6). The Craig−Gordon model accounts for the effects of RH and isotopic composition of the vapor (Figure 8), and it was able to explain the results of all experiments. For instance, the silage in the feed bunk followed a steeper trend during the night, when RH was high, than during the daytime when humidity was low, in agreement with the prediction of the Craig−Gordon model (Figure 8). Also, in experiment B, the data points at 1 h cannot be explained as a pure exchange with vapor, but considering drying, they conformed to expectations from the Craig−Gordon model (Figure 8).

Depending on the amount and the isotopic composition of the air vapor and the degree of drying of the silage after removal from the silo, a large variation of silage water is possible. This variation may span >200‰ for $\delta^2$H, as suggested by the simulations with the Craig−Gordon model (Figure 8). Under more realistic scenarios, where the atmospheric vapor did not differ strongly from the equilibrium vapor of silage water and drying on the feed bunk was limited, the observed variation of silage-water isotope composition in the feed bunk was much smaller (approximately 40‰ for $\delta^2$H; Figure 2).

Vapor labeling and pool modeling indicated that the bulk of silage water comprised a pool that could be exchanged rapidly with the surrounding vapor. With a half-life of 20 h, 75% of the water inside this pool was exchanged within about 1.7 days. The fast change of the isotopic composition after exposure of the silage to an environment with altered vapor isotope composition is not unexpected given that the tissue/cell structures of the leaf are weakened or disrupted due to the mechanical and chemical modifications of the production process.36 The vapor-labeling study also suggested that a minor fraction of the silage water (corresponding to approximately 10% of total silage water or 5% of the initial amount of tissue water in the cut grass if it is assumed that moisture decreases from an initial 80 to 40% during silage production) was included in a less rapidly exchanging water pool. We do not know the actual physical identity of this pool. Numerically it is similar in size to the water content of air-dry grass,43 which is probably associated with the bound-water fraction of cell walls.

The increase in silage moisture in the sealed containers is likely to have been due to condensation induced by the more negative osmotic and matrix potential of the silage water, relative to that of the deionized labeling water of zero potential36,37 but not to dew formation, which was excluded in the isothermal containers. Dew could possibly provide air humidity much more rapidly to the leaves. This may explain the much shorter (apparent) half-life (1−3 h) of leaf water in intact leaves under pronounced dew formation.44 Dew formation is

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**Figure 8.** Effect of relative humidity ($h$) and isotopic composition of air vapor on the change of the hydrogen isotopic composition of silage water ($\delta^2$H) (a, b) and on the slope of the $\delta^2$H−$\delta^{18}$O relationship (c, d) during drying. The air vapor was assumed to be in equilibrium with either heavy water (heavy vapor, $\delta^{18}$O = 4.2‰, $\delta^2$H = 42.9‰; a, c) or light water (light vapor, $\delta^{18}$O = −31.1‰, $\delta^2$H = −226.9‰; b, d) of experiment B. All calculations assume $T = 24$ °C, initial $\delta^{18}$O = −3.0‰, initial $\delta^2$H = −47.1‰, and kinetic fractionation $\alpha_k^{18}$O = 1.0189 and $\alpha_k^2$H = 1.017 (see refs 31 and 32). Dashed lines in panels a and b represent the labeling water, which was also the equilibrated state for silage water at $h = 100%$. Solid lines in panels c and d represent the local meteoric water line. The solid circle represents the same initial silage water in all panels.
likely to occur during the wilting phase of silage production, especially at night-time, but may also occur in open feed lots.

In each case, the silage water was found to be considerably more depleted in $^{18}O$ and $^2H$ than the water in fresh leaves (Table 1; Figure 2). The use of water isotopes as an indicator of the geographical origin, which has been well demonstrated in wildlife,\(^5\) may be useful in domestic animals only if the type of feed is known (i.e., fresh grass vs silage). In domestic animals, water isotopes are better suited to indicate the type of feeding:

A small seasonal variation can be expected when silage is fed throughout the year, whereas water isotopes in feed will show pronounced variation with season in the case of summer-grazed animals. In turn, the isotopic composition of the body water of an animal also changes when the diet is switched, for example, as occurs between the grazing period and the stall-feeding period in late autumn. Thus, barn-fed and pasture-fed lambs from the United Kingdom differed more than pasture-fed lambs from the United Kingdom and from Sicily in $^2H$,\(^6\) and the isotopic composition of milk water changed significantly between winter and summer.\(^7\) Large seasonal changes in $^{18}O$ and $^2H$ related to a diet switch were also found in cow hair\(^8\) and beef.\(^9\) A change in feed moisture content may also influence body-water isotopic composition via a changed requirement and intake of drinking water.\(^10\) However, both factors, the changed isotopic composition and an altered drinking water requirement, would probably change the isotopic composition of body water in the same direction as silage water, as drinking water is isotopically depleted relative to leaf water. Also, silage-water isotopic composition plotted close to the LMWL. Drinking water should also plot on this line if it is derived from local meteoric water. In accordance with this, we also expect that the lower water requirements of animals during winter than during summer, due to lower temperature,\(^11\) would not modify the picture.

In summary, our study demonstrated that silage water differs markedly from leaf water, and it can change during the feeding process. Thus, body water in domestic animals will differ depending on whether the feed is silage or fresh grass. This feeding pattern will overlay the geographic pattern. Given that this is the first study on silage water, more information would be useful about the influence of the production process on isotopic composition of silage water.

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