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Lehrstuhl für Grünlandlehre

Water intake and water isotope relations in animal feed

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Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades eines Doktors der Agrarwissenschaften (Dr. agr.) genehmigten Dissertation.

Vorsitzender: Univ.-Prof. Dr. Wilhelm M. Windisch

Prüfer der Dissertation:

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Die Dissertation wurde am 03.12.2013 bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt am 25.02.2014 angenommen.

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Abstract

Introduction: Domestic herbivores show a seasonal variation in oxygen isotopic composition of their body water, which may superpose the geographical isotopic gradient of precipitation. This limits the identification of the geographical origin if animals or animal products are sampled during different times of the year. Therefore, the seasonal variation in oxygen isotopic composition of body water in domestic herbivores should be taken into account for geographical tracing. This requires understanding the mechanisms causing the seasonal isotopic variation. It has been suggested that changes in drinking water intake and oxygen isotopic composition of feed water following a transition from winter to summer diet cause the seasonal variation of oxygen isotopic composition of domestic herbivores. However, these changes and their influence on the oxygen isotopic composition of the body water have not been quantified.

Aims: The subject of the present thesis was to quantify the variation of the oxygen isotopic composition in the total oxygen input of a grazer caused by diet transition from silage to fresh grass. The model organisms were steers. Of particular interest were (i) to predict drinking water intake of steers on pasture, (ii) to understand the mechanisms determining the isotopic composition of silage water prior to ingestion, and (iii) to predict the oxygen isotopic composition of the total oxygen input of steers fed either on fresh grass or silage during the grazing and dormant period, respectively.

Material & Methods: Drinking water intake and daily records of weather conditions were obtained during two grazing seasons with contrasting spring, summer and autumn rainfall patterns.

Silage was sampled from the feed bunk after different times since its distribution to monitor the change in the isotopic composition of the silage water. In model experiments, silage was exposed in sealed exsiccators to heavy and light labelling water for different time intervals to determine the isotopic turnover of silage water. The changes following exposure to different ambient conditions were modelled with an adaptation of the Craig-Gordon model.

The relative mass contribution of different oxygen input components (air oxygen, air vapor, drinking water, free water of feed and chemically bound oxygen in feed) were

predicted based on the quantification methods in Kohn's model (1996). To this end the oxygen isotopic composition in grass, drinking water, air humidity and rain were measured monthly from 2006 to 2012. Finally, the oxygen isotopic composition of the total input was calculated based on a mass balance for steers fed with silage and with fresh grass.

Results & Discussion: The drinking water intake of grazing steers varied largely from 0 to 30 L/d, depending on the environmental conditions. Rainfall and high soil moisture content caused high grass wetness through intercepted rain, formation of dew and guttation, and increased leaf water content. This decreased the drinking water intake. Compared to the water in fresh grass, the water in silage taken from silo was more depleted in heavy oxygen. Silage water may further change its isotopic composition after distribution to the feed bunk as turnover was fast. The changes were highly variable depending on the ambient conditions, which could be well predicted by the Craig-Gordon model.

The changes in drinking water intake and oxygen isotopic composition of feed water caused 6‰ more depletion of heavy oxygen in the total oxygen input of a steer fed on silage during the dormant period than when fed fresh grass during the grazing season. This variation is similar to the variation that can be expected between Sicily and Scotland due to the latitudinal gradient in the isotopic composition of precipitation.

Zusammenfassung

Einleitung: Die isotopische Zusammensetzung des Sauerstoffs ($\delta^{18}O$) variiert im Körperwasser domestizierter Pflanzenfresser saisonal und entlang eines geographischen Niederschlagsgradienten. Werden nun Tiere bzw. Tierprodukte zu verschiedenen Zeitpunkten untersucht, kann der saisonale den geographischen Trend überlagern und damit Rückschlüsse auf die geographische Information verhindern. Daher sollte der saisonale Trend von $\delta^{18}O$ berücksichtigt werden. Dies erfordert ein Verständnis der Einflussgrößen und ihrer Effekte auf den saisonalen Trend. Allgemein bekannte Einflussgrößen sind die Trinkwasseraufnahme und $\delta^{18}O$ im Wasser der Nahrung. Beides ändert sich durch die Nahrungsumstellung von Winter- auf Sommerfutter. Diese Änderungen und ihr zu erwartender Einfluss auf das $\delta^{18}O$ im Körperwasser domestizierten Pflanzenfresser wurden bisher nicht quantifiziert.

Ziele: Die vorliegende Studie untersucht die Variation im $\delta^{18}O$ in allen Sauerstoffquellen, welche durch die Nahrungsumstellung von Silage zu frischem Gras induziert wird, wobei als Modellorganismen Mastochsen dienen. Hauptaugenmerk dieser Studie war, (i) die Trinkwasseraufnahme von Ochsen auf der Weide vorherzusagen, (ii) Einblick in die Mechanismen der Variation von $\delta^{18}O$ im Silagewasser zu bekommen und (iii) $\delta^{18}O$ aller Sauerstoffquellen von Ochsen vorherzusagen, unabhängig ob diese mit frischem Gras oder mit Silage gefüttert werden.

Material & Methoden: Die Trinkwasseraufnahme und Wetterparameter wurden auf Tagesbasis während zweier Weideperioden mit unterschiedlichem Muster der Frühjahrs-, Sommer- und Herbstniederschläge erhoben.

Silagewasser und seine isotopische Veränderung nach der Entnahme aus dem Silo wurde zum einen durch Beprobung aus dem Futtertrog zu unterschiedlichen Zeitpunkten, zum zweiten in Modellversuchen in Exsikkatoren bei Markierung mit schwerem bzw. leichtem Wasser und zum dritten durch Modellierung aufbauend auf dem Craig-Gordon-Modell bestimmt.

Die relativen Massenanteile von allen aufgenommenen Sauerstoffquellen (Luftsauerstoff, Luftfeuchtigkeit, Trinkwasser, freies Wasser der Nahrung und chemisch gebundener Sauerstoff in der Nahrung) wurden entsprechend der Quantifizierungsmethode von Kohn

(1996) bestimmt. Dazu wurde $\delta^{18}O$ von 2002 bis 2012 monatlich in den Komponenten Gras, Trinkwasser, Regen und Luftfeuchtigkeit gemessen. Schließlich wurde $\delta^{18}O$ von allen aufgenommenen Komponenten, sowohl für gras- als auch für silagegefütterte Ochsen mit Hilfe einer Massenbilanz geschätzt.

Ergebnisse und Diskussion:

Die Trinkwasseraufnahme von Weideochsen variierte abhängig von den Umweltbedingungen stark, von 0 bis 30 l/d. Regen und hohe Bodenfeuchte erhöhten die Feuchtigkeit des Grases durch Interzeption, Taubildung und Guttation und erhöhten somit auch den Wassergehalt der Nahrung. Dies verminderte die Aufnahme von Trinkwasser.

Das Silagewasser war weniger angereichert an ^{18}O als das Blattwasser in frischem Gras. Nachdem Silage im Futtertrog verteilt wurde, änderte sich $\delta^{18}O$ im Silagewasser weiter und in unterschiedlicher Richtung, was gut durch das Craig-Gordon-Modell beschrieben werden konnte.

Durch die futterbedingten Umstellungen war $\delta^{18}O$ im aufgenommenen Wasser von silagegefütterten Ochsen im Stall um 6 ‰ abgereichert im Vergleich zum aufgenommenen Wasser von Weideochsen. Dies entspricht etwa der Variation, wie sie auf Grund des breitengradabhängigen Gradienten im Niederschlag zwischen Sizilien und Schottland zu erwarten ist.

1 General Introduction

Oxygen isotopes are applied for tracing the origin or movement of animals and their products because the isotopic gradient of surface water across the globe is incorporated into animal tissue (Bowen *et al.*, 2005). However, several studies showed that domestic herbivores exhibited seasonal variation in oxygen isotopic composition (Renou, *et al.*, 2004; Kornexl *et al.*, 2005). This will overlap with the geographical pattern and thus limits the identification of the geographical origin of an animal or its products (Camin *et al.*, 2007). Therefore, the oxygen isotopic composition of domestic herbivores should consider the season effect for geographical tracing (Boner and Förstel, 2004), which requires the understanding the determinant factors and their effect on the seasonal isotopic variation of domestic herbivores.

You are what you eat, isotopically (DeNiro and Epstein, 1976). Thus, the seasonal variation of oxygen isotopic composition of domestic herbivore is mainly caused by changes in inputs (Podlesak, *et al.*, 2008). Inputs of oxygen considered are air oxygen, air water vapor, chemically bound oxygen in food, free water in food, and drinking water. According to an isotopic mass balance equation, the oxygen isotopic composition of the total inputs ($\delta^{18}\text{O}_{\text{total}}$) is

$$\delta^{18}\text{O}_{\text{total}} = f_{\text{O}} * \delta^{18}\text{O}_{\text{O}} + f_{\text{vapor}} * \delta^{18}\text{O}_{\text{vapor}} + f_{\text{feedO}} * \delta^{18}\text{O}_{\text{feedO}} + f_{\text{drink}} * \delta^{18}\text{O}_{\text{drink}} + f_{\text{dietary water}} * \delta^{18}\text{O}_{\text{dietary water}} \quad (1.1)$$

where f represents the relative mass contributions of the respective input components to the total oxygen input. The oxygen isotopic composition is expressed in the standard ‘delta’ notation on a per mil basis by $\delta = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$, where $R = {}^{18}\text{O}/{}^{16}\text{O}$ and the standard is the standard mean ocean water (SMOW; $R = 0.002005$). The subscripts O, vapor, feedO, drink and dietary water refer to air oxygen, air vapor, chemically bound oxygen in feed, drinking water and free water in diet. Domestic herbivores usually transit the feeding from summer to winter, i.e. from pasture to silage (Elgersma *et al.*, 2004). It was suggested that the seasonal oxygen isotopic variation in herbivores resulted from this diet-switch induced effects: changed relative contribution of drinking water intake to the total oxygen input and the oxygen isotopic composition of feed water (Boner and Förstel,

2004; Camin *et al.*, 2007). However, these changes following diet switch and their effects on the oxygen isotopic composition of domestic herbivores have not been quantified.

Drinking water serves to compensate for any lack of water in the diet to maintain a specific ratio of dry matter to water intake (Castle, 1972; Kume *et al.*, 2010) and to release heat stress of animal (Winchester, 1956). Thus, when the domestic herbivores are fed on silage with constant moisture content, the daily variation of drinking water intake is mainly resulted from the weather condition through affecting the total water requirement (Arias and Mader, 2010). In contrast, except for the effect of weather condition on the physiology of animal, the environmental factors can also affect the dry matter content of grazed forage on pasture and thus influence the drinking water intake. It is a common phenomenon that plants on pastures are wetted by rain, dew and guttation (Burrage, 1971). The internal moisture content of grass decreases under dry soil condition since it cannot supply sufficient water to meet the high-demand of evapotranspiration on hot and dry days (Pirzad, *et al.*, 2011; Chen, *et al.*, 2012). However, the variation of moisture content of forage on pasture has not generally or explicitly been considered as significant parameters causing the variation of drinking water intake. Besides, these sources of dietary water on pasture have not been quantified, which may deteriorate the prediction of the oxygen isotopic composition of total inputs. Thus, DWI of domestic herbivores on pasture needs to be studied further with respect to the variation of the moisture content of grasses.

Regarding to the isotopic composition of feed water, growing leaf water as feed water for domestic herbivores on pasture has been widely studied (Barbour, 2007; Šantrůček *et al.*, 2007). The isotopic composition of leaf water shows diurnal variation, depending on the environmental condition and on the leaf characteristics and thus, results in an isotopic difference of fresh grass water ingested at different times during a day. The enrichment relative to the source water can be described by a model developed initially by Craig & Gordon (1965) and modified by Flanagan *et al.* (1991). However, it is still unknown whether there is an isotopic difference of silage water relative to fresh grass water. Moreover, irrespective of the isotopic variation during production of silage, the last step, distribution to the feed bunk for feeding, could further change the isotopic composition because its removal from silo exposes the silage to atmospheric humidity afterwards. The

response of the isotopic composition of silage water to the ambient environment needs to be analyzed.

The subject of the present study is to broaden our understanding of the isotopic variation of the oxygen intake by herbivores fed on fresh grass during the grazing season and on silage during the dormant period. The following three questions were under focus: a) What is the variation of the daily DWI of domestic herbivores between and within grazing and dormant season? b) Do silage water and growing leaf water differ in their isotopic composition? c) How much varies the isotopic composition of total oxygen input of domestic herbivores in the course of a year? To solve these questions, steers as domestic herbivores kept in the same site (Grünschaige Grassland Research Station) were selected as study subject. The three questions require entirely different methodological approaches. In consequence, the three questions are treated in different, rather independent chapters that have their own introduction, methods and results.

2 Drinking water intake of grazing steers – the role of environmental factors controlling canopy wetness

2.1 Implications

Forage is an important source of water for grazing cattle. A model of drinking water intake (DWI) is presented that accounts specifically for the effects of environmental variables controlling forage moisture content. The model is driven by weather data and the plant-available water (PAW) in the soil to predict DWI of steers at pasture. The approach used to derive the model may be used for further development of mechanistic DWI models. Such tools will be essential for sustainable grazing management under future limited water resources in temperate and arid grazing lands.

2.2 Introduction

Cattle satisfy their water demand by drinking water and from the moisture present in forage. Essentially, drinking serves to compensate for any lack of water in the forage in order that a specific ratio of dry matter to water intake is maintained (Castle, 1972; Kume *et al.*, 2010). This is particularly true for thermoneutral conditions (Khelil-Arfa *et al.*, 2012), which are common for temperate climate conditions. At pasture under temperate conditions, the intake of forage moisture can be very large, due to the low dry matter content of grazed forage (Castle, 1972). Very large variability in daily DWI of cattle has been observed at pasture, and this variation was strongly related to weather factors, such as temperature, humidity, wind speed, sunshine hours, evaporation and rain (Castle, 1972; Castle and Watson, 1973; Ali *et al.*, 1994). While all these factors can affect the dry matter content of the grazed forage (Castle, 1972), they may also influence the surface wetness of the pasture canopy. It is a common phenomenon that plants on pastures are wetted by rain, dew (Janssen and Römer, 1991) and guttation (Meidner, 1977). These sources of dietary water have not generally or explicitly been considered as significant parameters of grazing-related water intake by cattle at pasture.

Monteith (1957) described two vapor sources for dew formation on a canopy: dewfall (a flux of vapor from the atmosphere) and distillation or dewrise (a flux of vapor from the soil). Guttation means the exudation of water from hydathodes, xylem endings (specialized stomata) at the margins or tips of leaves (Meidner, 1977) and occurs when the rate of water supply from the roots is greater than the loss by transpiration (Hughes and Brimblecombe, 1994). Its amount is positively correlated with soil moisture (Hughes and Brimblecombe, 1994), as is dew formation from dewrise. Furthermore, on days with rain, interception directly wets the pasture canopy.

The potential importance of canopy-wetness factors for moisture intake during grazing can be estimated from knowledge of interception, dew or guttation water storage per unit leaf area, leaf dry mass per area, and dry matter intake. The maximum leaf surface water storage (for *Poa pratensis*) ranged from 20 to 90 g/m² (Wohlfahrt *et al.*, 2006). Using these values, a leaf dry mass per unit area of 36 g/m² (Garnier and Laurent, 1994) and a dry matter intake of 7.3 kg/d (estimated for a steer with a body weight of 400 kg and a live weight gain of 0.75 kg/d; Minson and McDonald, 1987), leaf surface water could contribute up to 18 L/d to the water requirement for days on which there is continuous interception water storage.

Measurements of grazing-related moisture intake are extremely time consuming and potentially very imprecise, due to large diurnal and day-by-day variations in grazing activity (Gary *et al.*, 1970; Kilgour, 2012), forage dry matter content (Jordan and Ritchie, 1971) and canopy wetness/surface moisture content (Burkhardt *et al.*, 2009). Also, there is no universal and commonly accepted protocol for dew measurements (Richards, 2004). Moreover, the separate contributions of dewfall, dewrise, guttation and intercepted water to leaf surface wetness and its intake are hardly distinguishable. There is no attempt and reliable procedure to assess these sources of water intake in grazing studies. To avoid the technical issues, an indirect approach was adopted to assess the relationships between DWI and the weather and soil conditions known to affect leaf dry matter content and the formation and persistence of leaf surface moisture, including dew, guttation and intercepted rain. The specific aims of this study were (1) to assess the importance of rainfall (affecting interception storage) on DWI, (2) to verify the hypothesis that soil water conditions (which are known to impact on dry matter content and dew and

guttation incidence) affect DWI, (3) to assess the role of weather factors which affect the occurrence and persistence of leaf wetness and (4) to develop a model predicting the DWI for the herd under contrasting weather and soil water conditions. To this end, we performed a grazing experiment with steers in two years with contrasting spring, summer and autumn rainfall distribution. Daily records of weather conditions and DWI were obtained throughout both grazing seasons.

2.3 Materials and methods

2.3.1 Site description

The study was carried out on a level area of permanent pasture at the Grünschwaige Grassland Research Station, which is located at the north end of the Munich Gravel Plain near Freising, Germany, at 435 m above sea level, latitude 48°23'N, and longitude 11°50'E (Schnyder *et al.*, 2006). The pasture sward was dominated by grasses, of which *Lolium perenne*, *Poa pratensis* and *Agrostis stolonifera* accounted for about 40%, 28% and 9%, respectively, of the biomass. The pasture did not receive any fertilizer for at least 15 years preceding the study, except for the excreta returned by grazing animals. The pasture offered abundant opportunities for shade: rows of >20 m-tall trees lined the western and eastern borders of the paddock, and another row of trees, oriented north-south, was situated in the middle of the paddock. Sward state was held constant by maintaining the compressed canopy height at 5 cm (SD 0.9 cm). This was done by measurements with a rising plate meter (Herbometre®, Agro-Systèmes, La Membrolle-sur-Choisille, France) at about 120 to 150 locations for six times per grazing season and adjustments of the stocking density (animals per paddock area).

The climate is temperate humid with an annual mean air temperature of 9.0 °C (SD 0.8 °C) and an annual precipitation of 775 mm (SD 130 mm) (Schnyder *et al.*, 2006). All meteorological data were obtained from a 3-km distant meteorological station (Munich airport) of the German Weather Authority, Deutscher Wetterdienst (Table 2.1).

Table 2. 1 Mean monthly meteorological conditions at Grünschwaige Grassland Research Station during the grazing periods in 2010 and 2011: daily mean ambient temperature (T_{mean}), relative humidity (RH), potential evapotranspiration (PET), global radiation (GR) and monthly rainfall

Month	T_{mean} , °C	RH, %	PET, mm/d	GR, kWh/m ²	Rain, mm
April	10.3	64.0	2.8	5.0	27
May	13.3	71.8	3.0	4.9	92
June	17.0	75.3	3.2	5.0	141
July	18.5	72.4	3.8	5.6	137
August	18.2	76.5	3.5	4.5	101
September	13.7	81.4	2.1	3.6	61
October	8.1	84.8	1.0	2.2	29

2.3.2 Grazing experiment and drinking water intake measurement

During the whole grazing seasons (April to October) in 2010 and 2011, ten and nine steers (Limousin aged 16 months, SD 4 months; initial body weight 411 kg, SD 91 kg) were kept on the same pasture all day. Each animal had *ad libitum* access to a water bowl (SUEVIA HAIGES GmbH, Kirchheim am Neckar, Germany) and salt block to meet its requirements. DWI was measured for each animal and each drinking bout. The water bowl was placed in a cage that allowed only one animal to drink each time, and the amount drunk was measured simultaneously by two independent systems. One system was a flow meter (B.I.O-TECH e.K, Vilshofen, Germany; resolution of 0.1 kg) that measured the amount of water flowing to the bowl as animals drank water. The other system was a weighing platform (Texas Trading GmbH, Windach, Germany) on which the animals were standing during drinking. It measured the weight of the animal before and after each drinking event, the difference of which provided another measure for DWI. The animal code from electronic ear tags, the drinking duration, the amount of flowing water and animal weight were recorded by a micrologger (PSION Industrial PLC, London, UK). The weighing platform and the flow meter produced similar data (Fig. 2. 1), confirming their general reliability. However, the weighing platform had a lower resolution (1 kg) and fewer valid measurements ($n = 2297$ compared to the flow meter with $n = 4722$) caused by failure to determine the weight when animals moved on the platform. Hence, in the following analysis the flow meter data was used. Daily individual

DWI was calculated as the sum of DWI for each drinking event for an individual steer, while daily mean DWI was obtained by dividing total daily water flow to the bowl by the number of animals in the herd.

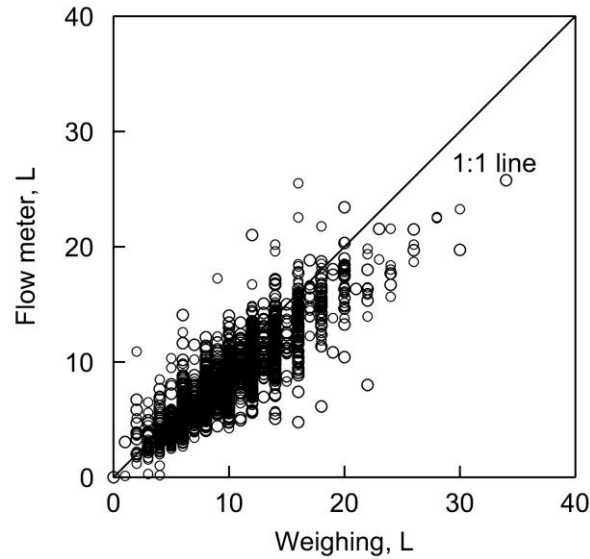


Fig. 2. 1 Comparison of drinking water intake for each drinking event of individual animals from weighing ($n = 2297$) and flow meter data.

2.3.3 Capacity and actual level of plant available water of the pasture

The average PAW capacity of the soil at the experimental pasture was 135 mm as estimated from rooting depth, soil texture and organic matter content (Schnyder *et al.*, 2006). Lateral surface water flows were not expected due to the flat terrain. Hence, the level of PAW could be estimated for every day of the recording period based on Allen *et al.* (1998), as shown by Schnyder *et al.* (2006) who quantified the effect of PAW on plant community ^{13}C discrimination on all pastures of the Grünschaige Grassland Research Station. PAW (W_i) was derived from the PAW of the previous day ($i - 1$), rainfall ($M_{\text{rainfall},i}$) and actual evapotranspiration (E_i) of the corresponding day (i) as:

$$W_i = W_{i-1} + M_{\text{rainfall},i} - E_i \quad \text{for} \quad W_{i-1} + M_{\text{rainfall},i} - E_i \leq W_{\text{capacity}} \quad (2.1a)$$

$$\text{or else } W_i = W_{\text{capacity}}, \quad (2.1b)$$

E_i was calculated as follows (Allen *et al.*, 1998):

$$E_i = E_{\text{pot},i} \quad \text{for} \quad P_{\text{rel},i} = W_i/W_{\text{capacity}} \geq 0.3 \quad (2.2a)$$

$$\text{or else } E_i = E_i \times W_i / (0.3 \times W_{\text{capacity}}), \quad (2.2b)$$

where E_{pot} is the potential evapotranspiration (mm), which was taken from the meteorological station. Calculation started after snowmelt (middle of March) when the soils were at PAW capacity. Here, a dry soil was defined as a soil at PAW < 30% of the PAW capacity (45 mm in our case) because the plants then already reduce transpiration (Allen, 1998; see equation (2.2)) and a wet soil as a soil at PAW > 95% of the PAW capacity (130 mm in our case).

2.3.4 Dry days and wet days

Rainfall occurred mainly at night (76% of all rainy days had rain between 1900 h and 0700 h of the following day). As wetting by rain may affect the grazing-related water intake on the following day, 'wet days' was defined as the days for which the total rain of the corresponding day and previous day was greater than 2 mm. 'Dry days' were defined as day with less than 0.2 mm of rain. Days not classified into either category were termed 'null days'.

2.4 Results

2.4.1 Drinking water intake and relation to weather variables

Over the two grazing seasons there was a greater number of wet days ($n = 174$) than dry days ($n = 144$), and 52 days were classified as null days. Data from 11 days were not included in the dataset due to failure of the flow meter. Mean DWI varied between 0 and 29.4 L/d. The variation among individuals was even larger (range between 0 and 49.5 L/d) with individual drinking bouts of up to 29.4 L. On average, the SD among individuals within the herd for an individual day was 5.2 L/d (range of SD among days: 0 to 15.2 L/d), indicating considerable variation in the drinking behaviour of individuals. There was, however, no evidence of a systematic deviation between animals. The SD among individuals meant that the daily mean DWI could only be determined with a 95% interval of confidence of 4.1 L/d. In consequence, even a perfect model would not be able to

predict the mean DWI on a particular day better than ± 4.1 L/d within the total range of 0 and 29.4 L/d.

The mean DWI was highest on dry days (13.2 L/d), intermediate on null days (9.5 L/d) and least on wet days (7.1 L/d). It correlated significantly with all weather parameters, except for daily rainfall amount (for all categories of days) and wind speed on dry days (Fig. 2.2). Even within the category of wet days, the amount of rainfall had no influence on DWI. In general, correlations were highest for sunshine hours followed by global radiation, relative humidity, daily maximum ambient temperature and daily mean ambient temperature. The strongest correlation between mean DWI and weather parameters was for wet days, followed by null days and dry days (see R^2 in Fig. II.2). In general, null days were more similar to wet days than to dry days despite the small amount of rainfall that occurred on null days.

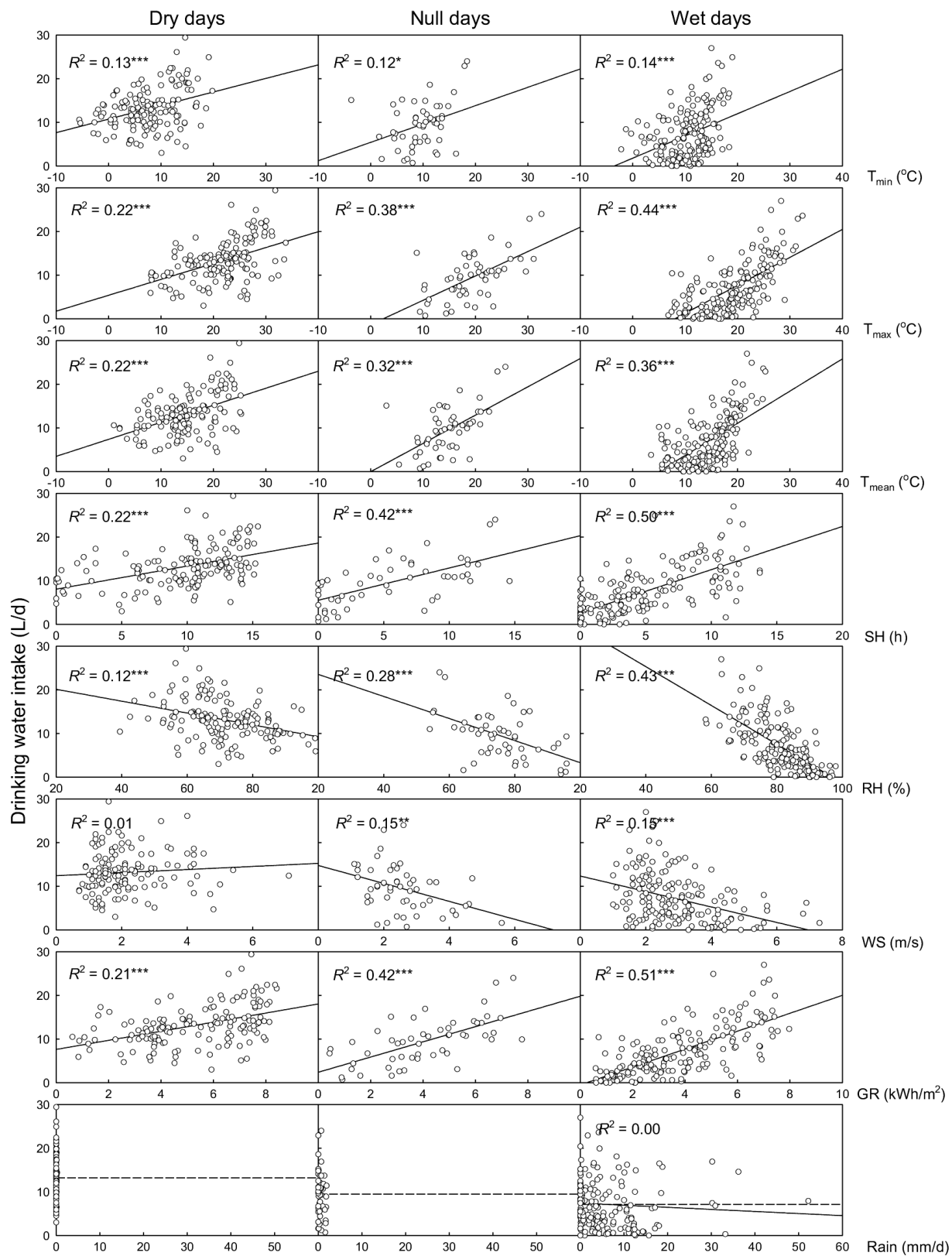


Fig. 2. 2 Relation of daily drinking water intake averaged over the herd and weather variables and respective coefficient of determination, R^2 , of linear regressions (solid lines) during dry days ($n = 144$), wet days ($n = 174$) and null days ($n = 52$). Dashed lines in the bottom panels indicate the averages of drinking water intake for each category.

T_{\min} = daily minimum ambient temperature; T_{\max} = daily maximum ambient temperature; T_{mean} = daily mean ambient temperature; SH = sunshine hours; RH = relative humidity; WS = wind speed; GR = global radiation

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

2.4.2 Drinking water intake under dry soil conditions

Under dry soil conditions (PAW < 30% PAW capacity), DWI on wet days again correlated more closely with weather conditions than on dry days (Table 2.2) and, in general, the correlations were closer than for the whole data set (compare Fig. 2.2 and Table 2.2). The daily mean ambient temperature correlated closely with DWI during dry days and wet days (Table 2.2), especially if a curvilinear relation was used (Fig. 2.3a) to account for the small influence of temperature below 10 °C and the large influence above that temperature.

At the same daily mean ambient temperature, the DWI was always lower on wet days than on dry days if the mean ambient temperature was less than 25 °C (Fig. 2.3a). On hot days (mean ambient temperature > 25 °C), rain had virtually no effect on DWI. The largest difference between dry and wet days of 4.4 L/d occurred at a mean ambient temperature of less than 10 °C. Regressing DWI and mean ambient temperature (T_{mean}) for the combination of dry days and a soil below 30% PAW capacity yielded ($R^2 = 0.7377$ ***, $n = 24$):

$$D_{<30\%,\text{dry}} = 8.8 + 0.0011 \times T_{\text{mean}}^3. \quad (2.3 \text{ a})$$

The same regression for wet days and a soil below 30% PAW capacity yielded ($R^2 = 0.8026$ ***, $n = 17$):

$$D_{<30\%,\text{wet}} = 4.4 + 0.0013 \times T_{\text{mean}}^3. \quad (2.3 \text{ b})$$

Table 2. 2 Coefficient of determination, R^2 , between weather variables and daily mean drinking water intake in dry soil conditions (PAW < 30% PAW capacity) on dry days ($n = 24$) and wet days ($n = 17$)

	Variable	Minimum	Maximum	R^2
Dry days	Minimum ambient temperature, °C	1.2	15.1	0.57***
	Maximum ambient temperature, °C	8.0	31.8	0.52***
	Mean ambient temperature, °C	5.3	24.9	0.63***
	Sunshine hours, h	0.0	15.4	0.36**
	Relative humidity, %	42.5	99.2	0.32**
	Wind speed, m/s	0.8	4.5	0.03
	Global radiation, kWh/m ²	0.9	8.4	0.44***
Wet days	Minimum ambient temperature, °C	5.6	19	0.6***
	Maximum ambient temperature, °C	10.8	32.4	0.64***
	Mean ambient temperature, °C	8.5	25.3	0.78***
	Sunshine hours, h	0.0	13.7	0.37*
	Relative humidity, %	58.3	85.8	0.24*
	Wind speed, m/s	1.0	5.7	0.2
	Global radiation, kWh/m ²	1.2	8.0	0.45**

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

2.4.3 Drinking water intake under wet soil conditions

Under wet soil conditions (PAW > 95% PAW capacity) that can supply sufficient soil vapor, it was assumed and evident (Fig. 2.3b) that rainfall would not increase leaf wetness above that already achieved by dewrise and guttation. Thus, for wet soil conditions, DWI on dry and wet days was described by a unique relation with the most predictive indicator, relative humidity, h ($R^2 = 0.4985$ ***; $n = 41$):

$$D_{>95\%} = 22.1 - 0.22 \times h. \quad (2.4)$$

DWI decreased linearly with increasing relative humidity, and reached zero at a relative humidity near 100 %.

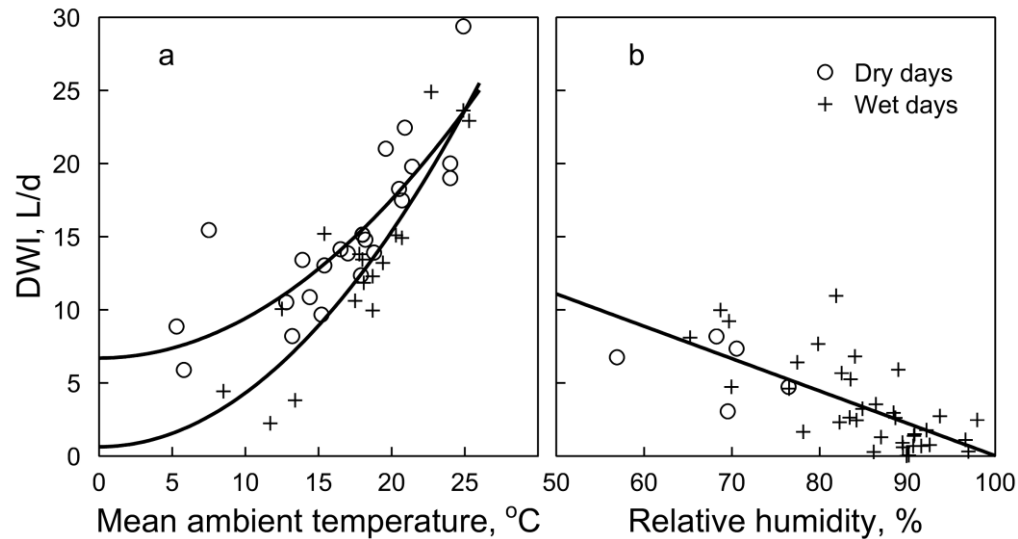


Fig. 2.3 Daily drinking water intake (DWI) (a) for dry soils (PAW < 30% PAW capacity) related to daily mean ambient temperature of dry days ($n = 24$) and wet days ($n = 17$) (b) for wet soils (PAW > 95 % PAW capacity) related to relative humidity of dry days ($n = 5$) and wet days ($n = 36$).

2.4.4 Relation between soil plant available water and daily drinking water intake

PAW covered a large range in both years, from 20 mm to field capacity at 135 mm (Fig. 2.4). However, the two years exhibited almost opposite seasonal patterns of PAW: in 2010 soil was driest in the middle of the grazing season, whereas in 2011 soil PAW peaked in that period of the season. The fluctuations of DWI displayed an inverse pattern relative to PAW, especially in 2010. Even though the negative interaction was not evident for the 2011 grazing-season as a whole, close inspection revealed that for shorter periods the DWI still correlated with PAW. For instance, PAW fluctuated frequently in the beginning of July in 2011, and each of these fluctuations was associated with an opposite variation of DWI.

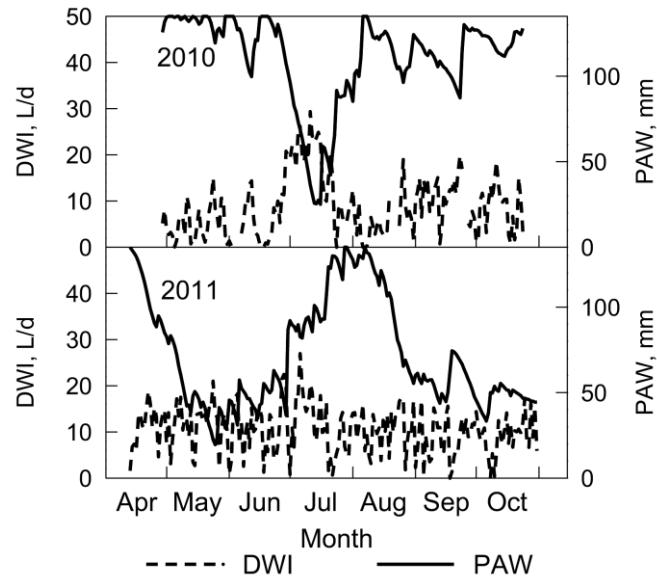


Fig. 2. 4 Soil plant available water (PAW) and daily drinking water intake (DWI) during the grazing seasons of 2010 and 2011. The discontinuities in the record of DWI in 2010 were caused by failure of the flow meter recorder (11 days).

The linear correlations between PAW and the different weather parameters were not significant (rainfall and minimum ambient temperature) or exhibited an $R^2 < 0.1$. The latter included mean and maximum ambient temperature, global radiation, humidity and wind speed (data not shown).

In general, PAW exerted a negative effect on DWI (Fig. 2.5). DWI decreased little until PAW increased up to about 70 mm, and then decreased rapidly. This effect of PAW on DWI was particularly evident when mean ambient temperature exceeded 15 °C. Again, the DWI on wet days was lower than that on dry days under similar weather conditions (mean ambient temperature either above or below 15 °C).

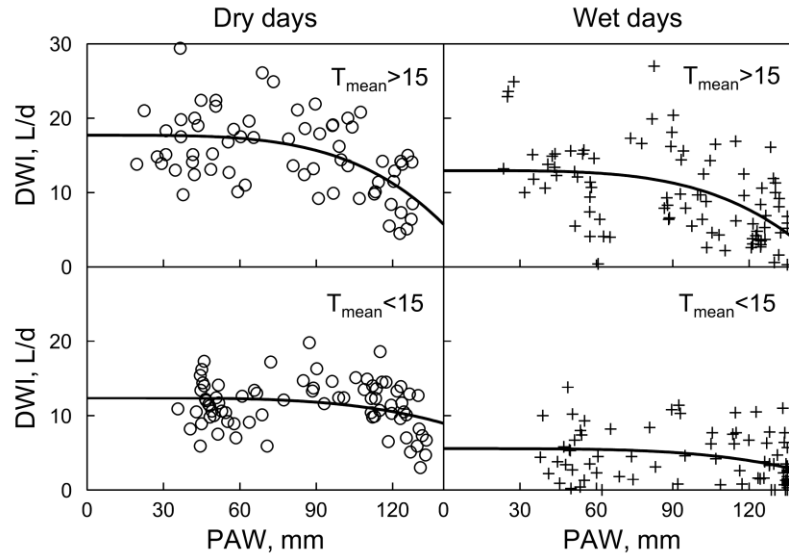


Fig. 2. 5 Relationship between the daily drinking water intake (DWI) and soil plant available water (PAW) for dry days and wet days at high ($> 15\text{ }^{\circ}\text{C}$) and low ($< 15\text{ }^{\circ}\text{C}$) mean ambient temperature (T_{mean}). Lines represent the curvilinear relationship between DWI and PAW as shown in equation (2.5).

2.4.5 Model for predicting drinking water intake

Combining equation (2.3) and (2.4) and optimising the exponent of P_{rel} lead to a model that allowed predicting DWI for any soil moisture and weather condition. The root mean square error (RMSE) for both dry and wet days was least for:

$$D_{\text{dry}} = 0.0011 \times T_{\text{mean}}^3 + 8.8 + (-0.22 \times h + 22.1 - 0.0011 \times T_{\text{mean}}^3 - 8.8) \times (P_{\text{rel}})^4 \quad (2.5a),$$

and

$$D_{\text{wet}} = 0.0013 \times T_{\text{mean}}^3 + 4.4 + (-0.22 \times h + 22.1 - 0.0013 \times T_{\text{mean}}^3 - 4.4) \times (P_{\text{rel}})^4 \quad (2.5b).$$

The measured and predicted DWI clustered along the 1:1 line (Fig. 2.6a) with RMSE 4.2 L/d and 3.6 L/d for dry and wet days, respectively. This uncertainty was similar (or even smaller) than the average confidence interval of the DWI among individuals (4.1 L/d), indicating that practically all variation of the data was explained by the environmental variables included in equations (2.5a) and (2.5b).

Both equations (2.5a) and (2.5b) were also applied for null days. As expected, the model for dry days slightly overestimated DWI and that for wet days slightly underestimated the DWI of null days (Fig. 2.6b), thus yielding slightly higher RMSE values of 4.3 and 4.1 L/d, respectively. Predicting the DWI for null days by using the average of the predictions for dry and wet days decreased the RMSE to 4.0 L/d, which was smaller than the RMSE of dry days even though the data for null days had not been used for the development of the equations.

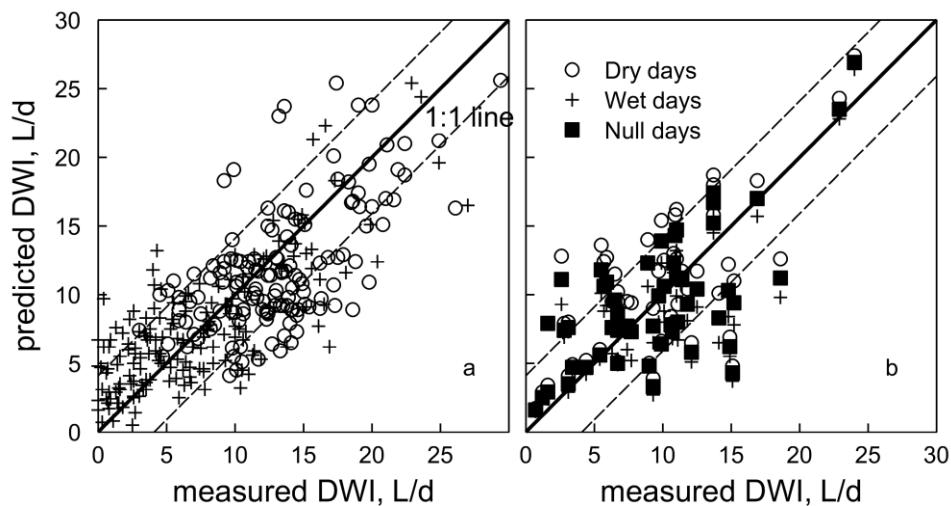


Fig. 2. 6 Comparison of measured and predicted daily drinking water intake (DWI). Dashed lines indicate the mean 95% interval of confidence of the measurements that cannot be explained by a perfect model using perfect soil and weather data. (a) dry days and wet days (b) null days (that are days with > 0.2 but < 2 mm of rain during the previous and actual day) with predictions using equation (2.5a) (applicable for dry days) and (2.5b) (applicable for wet days) and averages of both predictions.

On average, DWI predicted for all days in two grazing seasons with equation (2.5a) (applicable for dry days) and equation (2.5b) (applicable for wet days) differed by 2.2 L/d, significantly less than the measured difference of DWI between dry and wet days (6.1 L/d). The average difference in DWI between dry days and wet days that received rainfall only at night was 2.7 L/d, but the difference became larger when rainfall occurred only in the morning. A further reduction of DWI was brought about by rainfall over a longer period of the day, especially when rainfall occurred throughout the day ('night + morning + afternoon' in Fig. 2.7).

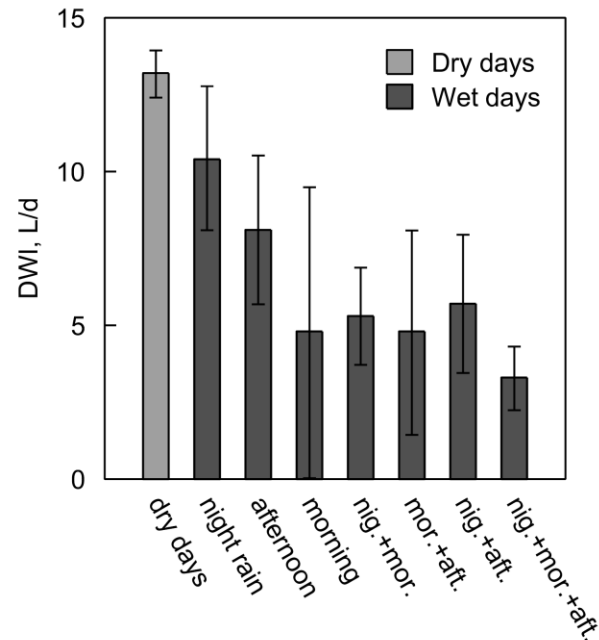


Fig. 2. 7 Daily drinking water intake (DWI) as affected by the diurnal timing and duration of rainfall events: dry days ($n = 144$) are days with no rainfall on the day corresponding to the DWI measurements and the previous day; night rain refers to rainfall only between 1900 h on the previous day and 0700 h on the corresponding day ($n = 47$); morning is only from 0700 h to 1300 h ($n = 20$); afternoon is only from 1300 h to 1900 h of the corresponding day ($n = 11$); nig+mor ($n = 39$) or nig+aft or nig+ mor+aft mean that rainfall occurred at night and additionally in the morning ($n = 24$), or night and afternoon ($n = 31$) or night and morning and afternoon ($n = 52$). Error bars report the 95% confidence interval.

2.5 Discussion

This work reveals close relationships between DWI of steers grazing pasture and the weather and soil conditions that are known to affect plant water status and canopy wetness. This result is consistent with the working hypothesis, that environmental factors enhancing forage-moisture intake during grazing lead to a reduced requirement for DWI, and that such forage moisture includes both plant-tissue internal water and also external water (i.e., dew, guttation and intercepted rain water). A positive correlation between DWI and the dry matter content of grazed forage was reported by Castle (1972); conversely, the same study also found negative relationships between DWI and rainfall

and relative humidity. In the present work, the most striking effects on DWI resulted from (combinations or contrasts of) dry or wet soil conditions, rainfall events (yes or no), and relative humidity of the air. Wet soils (high PAW) facilitate water uptake by plants and increase the relative water content (Volaire and Lelièvre, 2001), and they also promote guttation and dew formation *via* dewrise (Wilson *et al.*, 1999). Rain wets the sward canopy, and high relative humidity enhances dewfall (Xiao *et al.*, 2009) and slows the rate of evaporation of surface moisture from the canopy (Sentelhas *et al.*, 2008). Explicit consideration of environmental factors affecting plant internal water content and canopy wetness led to a new model of the environmental controls on DWI of Limousin steers of specified age and live weight. The model was validated with null-day data (Fig. 2.6b) and also provided an acceptable fit (RMSE = 3.8 and 4.7 L/d for dry days and wet days, respectively) to DWI data obtained in a third year (2012, data not shown).

The model did not account for the possible effects of diet factors (e.g. dry matter intake, protein content or salt consumption) and animal factors (e.g. sex, age, live weight and performance) on DWI. Furthermore, it did not consider direct effects of environmental factors on total and related drinking water demand of cattle, as demonstrated and modelled by others (e.g. Winchester and Morris, 1956; Arias and Mader, 2011; Khelil-Arfa *et al.*, 2012). Any variation of these factors would have affected the RMSE between modelled and measured DWI (which was ≤ 4.2 L/d or $\leq 14\%$ of maximum DWI) or influenced the modelled DWI *via* collinearities with those factors that were included in the model. All steers had *ad libitum* access to a salt block ensuring that physiological requirements were satisfied. Compressed sward height was kept near constant by adjusting the grazing pressure. All animals were of the same breed and sex, had very similar age and live weights and exhibited no statistically significant differences in the relationship between live weight gain and DWI throughout the grazing seasons (data not shown). For these reasons, it suggests that diet (except for dry matter content) and animal factors had a relatively small effect on variation of DWI in this study. Nevertheless, there are several environmental factors, including temperature/exposure to solar radiation, relative humidity and wind, which can exert an influence on the water demands of cattle (Marai and Haebe, 2010; Arias and Mader, 2011). The distinction between such direct effects on drinking-water demand of animals and indirect effects,

which act through variation of forage dry matter content and canopy surface moisture, is not trivial. This is complicated further by collinearities between weather factors (Arias and Mader, 2011). One approach to distinguishing the relative importance of such direct and indirect effects consists in comparisons of the present data with data from controlled environments, or from studies conducted in pens/stables with diets of constant dry matter content and known amounts of drinking and total water consumption. Arias and Mader (2011) performed a comprehensive study of the effects of environmental variables on DWI in cattle finished in unshaded feedlot pens in several contrasting seasons, including summers and winters, in the humid continental climate of Norfolk, Nebraska, USA. Overall (winter plus summer), they found that minimum and mean ambient temperature and the temperature-humidity-index (which were all collinearly related) were the most important predictors of water intake, whereas solar radiation, relative humidity and wind speed had smaller (or non-significant) effects. As minimum temperature increased from 0 to 20 °C, DWI increased approximately 1.7-fold. Winchester and Morris (1956) reported on a controlled environment study with European cattle held at constant temperature and found a 1.5-fold increase in *total* water intake between 5 °C at 25 °C, while dry matter intake varied little in the same temperature range. Brew *et al.* (2011) performed studies in thermoneutral conditions (5 to 20 °C) and found no effect of temperature on total water intake of 7- to 9-month-old growing beef cattle housed in an open-sided barn. It estimates that, for an increase of mean ambient temperature from 5 °C at 25 °C in conditions of dry days with dry soils, total water intake increased by 49%, if it assumed a constant dry matter intake of 7.3 kg/d and a dry matter content of 0.22 kg/kg. This effect is very similar to that reported by Winchester and Morris (1956) and would suggest that the temperature effect on DWI on dry days with dry soils was essentially due to the temperature effect on total water demand of the steers. In comparison, DWI was much lower on wet days with dry soils when temperature was low, indicating a greater grazing-related water intake under these conditions. Indeed cool conditions support a longer persistence of surface moisture on wet days (Dietz *et al.*, 2007).

After accounting for ambient (minimum, mean or maximum) temperature, other climatic factors generally contribute relatively little to the residual between modelled and observed DWI (Cardot *et al.*, 2008). In hot and cold environments, however, the thermal

balance of cattle, and hence water demand, can be affected quite significantly by air humidity, wind speed and solar radiation (Blackshaw and Blackshaw, 1994; Berman, 2005; Mader *et al.*, 2010). Given the ample opportunities for shade and wind shelter and the relative comfort range of daily mean ambient temperature (5 to 25 °C), however, the relevant heat stress and wind chill effects on water demand were probably quite small. On these grounds, it suggests that the observed variations of DWI – observed at a given ambient temperature – were mainly related to counterbalancing variations in grazing-related water intake.

The most convincing argument for strong variations in grazed forage water intake came from the observed relationship between DWI and PAW. Variations in PAW did not correlate, or correlated only marginally, with weather factors, meaning that weather effects on water demand and related effects on DWI must have been small. Increasing PAW decreased DWI up to >10 L/d on both dry and wet days. This effect was greatly enhanced by high atmospheric humidity and wet days. On wet days, the R^2 of each weather parameter with DWI was greater than on dry days, likely due to their effect on the formation or persistence of canopy surface moisture. The fact that rainfall in excess of 2 mm had no effect on DWI is related to the limited water-storage capacity of the sward canopy. Absence of an effect of rainfall >2 mm on DWI is also supported by the raw data presented by Castle (1972). Wet days, high PAW and high atmospheric humidity all promote and sustain high internal and external forage moisture contents (Wilson *et al.*, 1999; Volaire and Lelièvre, 2001), which enhance the intake of forage water.

2.6 Conclusion

The present data and model strongly support the hypothesis that DWI of cattle grazing pasture is balanced by forage moisture intake, including internal tissue water and pasture canopy surface moisture, which includes dew, guttation and intercepted rain water. The originality of the model may be seen in the more mechanistic treatment of the environmental controls on DWI on temperate humid pastures.

3 Variation of isotopic composition of dietary water from pasture to silage

3.1 Introduction

Dietary water is an important water source for animals, and its isotopic composition determines the isotopic signal of animal body water that subsequently is reflected in body tissues like bones and teeth (Daux *et al.*, 2008), hair (Podlesak *et al.*, 2007), or milk (Camin *et al.*, 2008). Studies indicated that local humidity can have an important effect on the oxygen isotopic composition of biogenic phosphate, largely due to the effect of humidity on the isotopic composition of dietary water (Ayliffe and Chivas, 1990; Luz *et al.*, 1990). Concurrently, the application of hydrogen and oxygen stable isotopes as a tracer for geographic movement have motivated interest within the ecological, anthropological, and forensic science communities. This method derives from fact that the geographic isotopic gradient of surface water is propagated to animals. Thus using O and H isotopes for geographical tracing, isotopic variation related to dietary water composition even represents a potentially confounding factor. Therefore, the applications of oxygen and hydrogen stable isotopes of animals (or animal products) require sufficiently detailed information on diet, which is a potential signal of interest for dietary research.

Domestic herbivores normally transit the feeding from summer to winter, i.e. from pasture to silage (Elgersma *et al.*, 2004), resulting in a seasonal isotopic variation and thus making it is impossible to trace geographical origin for the animals from different time if the determinant factors are not quantified. Studies attributed the seasonal isotopic variation in animal to the changed drinking water amount and isotopic composition of dietary water followed diet switch (Boner and Förstel, 2004; Camin *et al.*, 2007), in which, however, until to now there is no data to illustrate the isotopic offset of dietary water between summer and winter.

On smaller time scale resolution, the animal would consume fresh grass water with different isotopic composition daily or even hourly due to the variation of leaf water, which has been widely studied. In contrast, during feedout the silage is exposed to air, providing chance to interact with ambient environment that causes aerobic deterioration

(Chen and Weinberg, 2009) but may also change the isotopic composition of its water. After removal from silo, the strikingly changed determinant environmental factors are relative humidity and isotopic composition of air vapor. Specifically, relative humidity may vary by 50 % during a day (Ephrath *et al.*, 1996) while the isotopic composition in air humidity typically varies by 40 ‰ for hydrogen and 6 ‰ for oxygen during a year (www.waterisotopes.org). Thus, depending on the time of removal from silo and the duration in the feed bunk, the silage is exposed to air differing in humidity and isotopic composition.

In this chapter, the isotopic composition of water of winter diet (grass silage) was compared with summer diet (fresh grass), which is also the origin for the silage. Then, a feed bunk experiment (Exp. A) and an exsiccator experiment (Exp. B) will quantify the effect of environmental conditions on the isotopic composition of silage water after removal from silo. The results from both experiments will be compared to predictions by the Craig-Gordon model.

3.2 Theory

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

$$dM_{\text{area}}/dt = I - E \quad (3.1)$$

For the conditions in the feed bunk I can be neglected. The evaporation flux E is proportional to vapor concentration difference at the water-air interface with that in the turbulent atmospheric region, and thus to relative humidity h (Gonfiantini, 1983):

$$E = C_s (1 - h)/\rho \quad (3.2)$$

where C_s denotes the saturation concentration of vapor depending on temperature and ρ is a resistance coefficient.

The isotopic composition of the evaporation flux is given by the Craig-Gordon model (1965), which accounts for the equilibrium isotope fractionation between liquid and vapor, the kinetic fractionation resulting from the diffusion across the air boundary layer and the back flux of the atmospheric moisture (Gat, 1996):

$$R_E = (\alpha^* R_s - h R_{\text{vapor}}) / ((1 - h) \rho_i / \rho) \quad (3.3)$$

R denotes the atom ratio between the heavy and the light isotopes ($^2\text{H}/^1\text{H}$ or $^{18}\text{O}/^{16}\text{O}$), α^* is the temperature-dependent fractionation factor. The subscripts indicate the evaporated water (E), the water in the silage (s), the vapor in the atmosphere (vapor) and the heavy isotope (i).

Eqn 3.3 can be translated into the δ notation ($\delta = R/R_{\text{std}} - 1$), which expresses the atom ratio relative to that in a standard, then leading to:

$$\delta_E \approx (\delta_s - h \delta_{\text{vapor}} - \varepsilon^* - \Delta\varepsilon) / (1 - h) \quad (3.4)$$

where ε^* and $\Delta\varepsilon$ represent the equilibrium enrichment and kinetic enrichment that are associated with the phase change and the diffusion. The change in δ_s is then given by (Helliker and Griffith 2007):

$$d(M_{\text{area}} R_s) / dt = -E R_E \quad (3.5)$$

For the silage, the volume and isotopic ratio in the remaining water are hence only controlled by the evaporation rate E and the isotopic composition of the evaporation flux R_E , which both depend on relative humidity h (Eqn 3.4 and 3.5). The remaining fraction of the initial amount of water M_{s0} is defined as $f_{\text{remaining}} = M_{\text{area}} / M_{s0}$. Eqn (3.5) can be rearranged to describe the change in isotopic composition of the remaining silage water, $d\delta_s$, with $f_{\text{remaining}}$ (Welhan and Fritz, 1977; Gibson *et al.*, 1999),

$$d\delta_s / d \ln f_{\text{remaining}} = \delta_E - \delta_s \quad (3.6)$$

As $f_{\text{remaining}}$ approaches zero, δ_s approaches a steady state isotopic composition, δ_{steady} , (Welhan and Fritz, 1977) under local atmospheric conditions due to the back diffusion from atmosphere. The change of δ_s , $d\delta_s$, thus approaches zero, which, by integrating with respect to $f_{\text{remaining}}$, yields (Gibson *et al.* 1999):

$$(\delta_s - \delta_{\text{steady}}) / (\delta_{s0} - \delta_{\text{steady}}) = f_{\text{remaining}}^m \quad (3.7)$$

where δ_{s0} is the initial isotopic composition of silage water (at $f_{\text{remaining}} = 1$), δ_s is the isotopic composition of silage water at any instant in $f_{\text{remaining}} < 1$, $m = (h - \varepsilon_k - \varepsilon^*) / (1 - h + \varepsilon_k)$ as defined by Welhan and Fritz (1977), Gonfiantini (1983) and Gibson *et al.* (1999).

In the case of evaporation in completely dry air ($h = 0$), the Rayleigh fractionation results and Eqn (3.7) reduces to (Gat 1996):

$$(\delta_s + 1) / (\delta_{s0} + 1) = f_{\text{remaining}}^{\alpha^* - 1} \quad (3.8)$$

In the case with air of 100 % humidity, only the exchange of vapor and liquid occurs but without evaporation. δ_s would approach to the equilibrium with air vapor because the silage water is a limited volume while air vapor can be regarded infinite.

In the humidity above 100 %, condensation may occur. Thus, the measured isotopic value of extracted water from silage (δ_s') is a mixing function from condensation of air vapor ($\delta_{\text{cond.}}$) and original silage water (δ_s) in exchange with air vapor.

$$\delta_s = \frac{(M_s + M_{\text{cond.}}) * \delta_s' - M_{\text{cond.}} * \delta_{\text{cond.}}}{M_s} \quad (3.9)$$

where M_s and $M_{\text{cond.}}$ represent mass of silage water and increased mass resulted from condensation. The equilibrium fractionation controls the isotopic composition of water condensed in over-saturation (Wen *et al.*, 2011). Thus, δ_{cond} is assumed to be isotopically equal with corresponding labeling water.

The reaction progress method was used to describe the turnover of O and H in the silage water (Cerling *et al.*, 2007). Benefits of using the reaction progress variable include calculating the half-life for multiple pool systems (Cerling *et al.*, 2007). Besides, results from experiments with isotopically different labeling water can be combined together. The fractional approach to equilibrium leads to a reaction progress variable (Criss, 1999; Cerling et al, 2007),

$$\frac{\delta_s - \delta_{\text{eq}}}{\delta_{s_0} - \delta_{\text{eq}}} = 1 - F \quad (3.10)$$

where δ_{eq} is the isotopic equilibrium with air vapor, $F = 0$ and $F = 1$ represents the beginning of the exchange reaction and the final steady state (equilibrium). The exchange of a single pool follows

$$1 - F = e^{-\lambda t}, \quad (3.11)$$

where λ is a first-order rate constant. Equation 3.10 and 3.11 are particularly useful when cast as:

$$\ln(1 - F) = -\lambda t \quad (3.12)$$

which has the property of being a straight line in case of a single pool while it yields a graph composed of several straight segments for low number multi-pools systems.

3.3 Materials and methods

3.3.1 Silage material

Silage was obtained from the Grünschwaige Experimental Station, Germany; 48°23' N, 11°50'E (for details regarding the site, see Schnyder *et al.*, 2003). All the grassland was used for animal husbandry. During the grazing season (approximately from end of April to end of October) all cattle were kept on part of the grassland. The other part was used for silage production at maturity of growth in June and regrowth and August. Both months contributed about 40% and 35% of the total silage produced on the farm. The silage production generally followed Wilkinson (2005). After mowing, the grass was wilted on the field for two days and then filled into drive-in silos without adding any fermentable substrate. The silage was kept in the silo until opening for feeding, which was the feed source during the dormant period when the cattle were housed.

Growing grass was sampled monthly at daytime during growing seasons from 2006 to 2011 to compare the summer feed with the silage but also to characterize the precursor of the silage. The silage was taken randomly from silo in April from 2011 to 2013.

3.3.2 Experiment A: Isotopic composition variation of silage water in the feed bunk

The purpose of the first experiment was to quantify the variation of isotopic composition of silage water after distribution in feed bunk during a day. During the dormant period, the daily requirement of silage was distributed on the cleaned feed bunk in the open-front stall once in the morning. Silage was sampled on three consecutive days (25, 26 and 27/04/2012) shortly after distribution in the feed bunk (~ 5 min) and 0.5 h, 1 h, 2 h, 4 h and 24 h later. The last sampling (24 h) happened on the next day before cleaning the leftovers and provision of new silage. All samplings were accomplished in the morning (Fig. 3.1). Thus, there was a 24-h sample taken on the day 25/04 resulting from the feeding for day 24/04, while no 24-h sample from silage distributed on 27/04 was taken. A handful was sampled from the upper layer of silage in the feed bunk where the cattle tended to consume. The sample was conserved in the self-seal bags kept at -4 °C. Hourly RH and air temperature, which exhibited clear diurnal cycles (Fig. 3.1), were obtained from a meteorological station in Freising (distance 7 km; <http://www.lfl.bayern.de/agm/daten.php?statnr=8>). Additionally, the air vapor was

sampled by cryogenic distillation in April from 2006 to 2011 to assess its variation in $\delta^{18}\text{O}$ and $\delta^2\text{H}$ during this time of the year. Rain was sampled and measured every month from 2006 to 2011 for the construction of the local meteoric water line (LMWL).

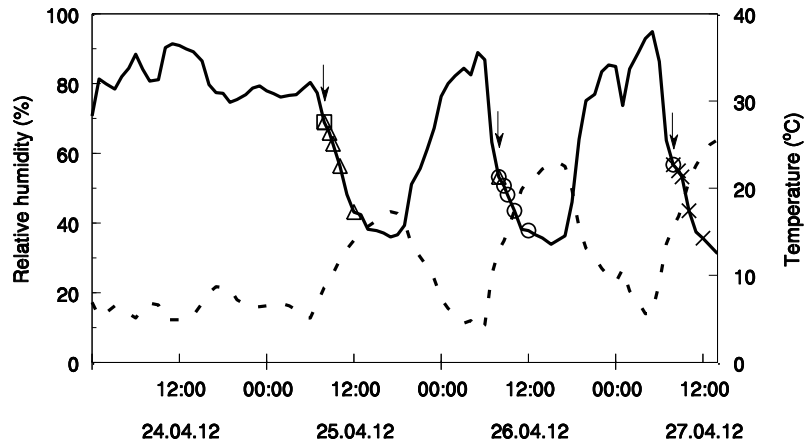


Fig. 3. 1 Weather conditions (relative humidity, solid line; air temperature, dashed line) for four consecutive days during the feed-bunk experiment. The arrows indicate the removal of leftovers from the previous day and the distribution of fresh silage in the feed bunk when 0-h sample and the 24-h sample were taken. Markers show sampling after 0, 0.5, 1, 2, 4 and 24 h since the distribution in the morning (08:00) on 24 (\square), 25 (Δ), 26 (\circ) and 27/04/2012 (\times), respectively.

3.3.3 Experiment B: Exsiccator manipulations to study the effect of δ_{vapor} on δ_s

The second experiment analyzed how the isotopic composition of air vapor influenced the variation of isotopic composition of silage water to characterize the response of silage to a new environment.

Silage was collected directly from silo with a silo corer on 31st March, 2011 and then kept in a self-seal bag at 4°C until the start of the experiment. To allow quick sampling from exsiccator during the experiment, about 2 g of silage were allocated into individual aluminum trays as individual sample. Then, all the samples were kept in an empty air-tight exsiccator for about 12 hours to reach homogeneous moisture content and isotopic composition prior to the experiment.

The silage samples were placed on the perforated shelf of the exsiccator. Under the shelf, instead of a desiccant, 40 ml of labeling water contained in a plate of 10 cm diameter was placed. In order to promote air mixing, a fan was installed in the headspace

of the exsiccator. The labeling water and silage samples were quickly put into the exsiccator and the lid was closed. Thus, the initial condition in the exsiccator (e.g. temperature, RH and isotopic composition of air) was the same as in the room.

The experiment was divided into two groups, each of which had two exsiccators differing in the isotopic composition of labeling water inside, either more enriched or more depleted (heavy or light water) relative to silage water. In the first group with short intervals between openings and short labeling time, six samples were exposed to the labeling atmosphere and randomly two were removed quickly after 1, 2, and 4 hours from each exsiccator. One was used to measure the isotopic composition of water and the other was used to measure the moisture content. In the second group with relatively long intervals between openings and long labelling time, each exsiccator contained eight silage samples. After 8, 24, 48 and 96 hours again two at a time were sampled. The labeling water in each exsiccator for each group was sampled before and after the whole exposure procedure.

3.3.4 Extraction and measurement of silage water

Silage water was extracted using cryogenic vacuum distillation. For experiment A, $\delta^2\text{H}$ and $\delta^{18}\text{O}$ were measured by L2120 – *i* Analyzer (Picarro, California, USA). Analytical uncertainties were ± 0.07 ‰ for $\delta^{18}\text{O}$, and ± 0.5 ‰ for $\delta^2\text{H}$. For experiment B, ^2H and ^{18}O values were determined by IRMS with a Thermo Finnigan DELTA^{plus} XL with TC/EA converter. Analytical uncertainties were ± 0.5 ‰ for $\delta^{18}\text{O}$, and ± 2 ‰ for $\delta^2\text{H}$.

3.4 Results

3.4.1 Isotopic composition of fresh grass water and silage water

In April and October, when feeding of silage but also feeding of fresh grass likely may occur, silage was depleted by 14 ‰ in $\delta^{18}\text{O}$ and 60 ‰ in $\delta^2\text{H}$ as compared to the fresh grass (Table 3.1) causing a large contrast in dietary water. Furthermore, the silage had smaller moisture content (about 50%) than the fresh grass (about 75 %). In June and August, when the majority of the silage was produced, the fresh grass was only slightly more enriched than in April or October reflecting the higher evaporative conditions in

mid summer. The variation of silage water expressed as standard deviation SD was similar to that of fresh grass.

Table 3. 1 Oxygen and hydrogen isotopic composition ($\delta^{18}\text{O}$ and $\delta^2\text{H}$) of dietary water for cattle kept at Grünschwaige Grassland Research Station during grazing and dormant periods. Silage is without exposure in the feed bunk. Fresh grass was obtained at the time when the animal switched diet (April and October) and when the grass was cut for silage production (June and August).

Water source	$\delta^{18}\text{O}$ (‰)			$\delta^2\text{H}$ (‰)	
	n	Mean	SD	Mean	SD
Silage	24	-9.1	2.8	-80	23
Fresh grass in April and October	12	4.9	2.5	-19	11
Fresh grass in June and August	50	3.7	2.3	-15	11

3.4.2 Experiment A: Feed bunk experiment

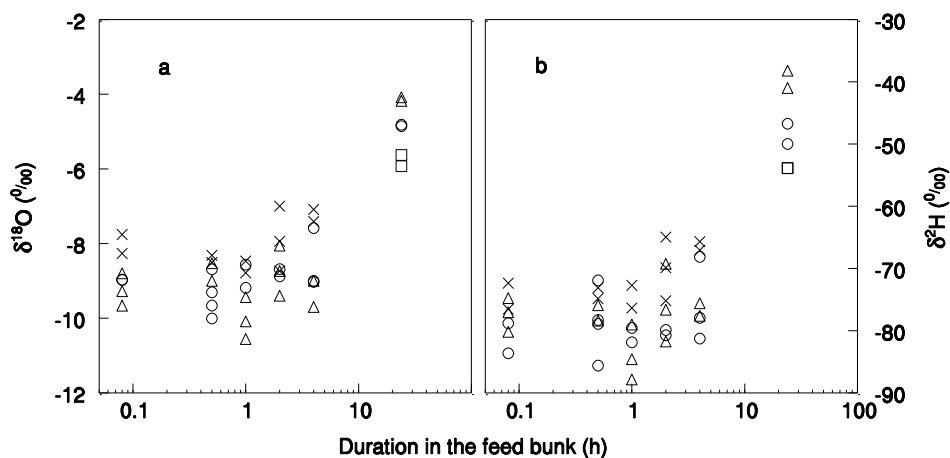


Fig. 3. 2 Variation in oxygen (a) and hydrogen (b) isotopic composition of silage water immediately after distribution in the feed bunk at 8 am (~5 min after distribution, $n = 7$) and 0.5 h ($n = 8$), 1 h ($n = 7$), 2 h ($n = 8$), 4 h ($n = 7$) and 24 h ($n = 6$) later on day 24/04 (\square), 25/04 (Δ), 26/04 (\circ) and 27/04 (\times). Note: x axes are log scaled.

The silage water became increasingly enriched in ^{18}O and ^2H in the feed bunk (Fig. 3.2). After 24 h $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of silage water increased by 4 ‰ and 30 ‰, but still was considerably more depleted than the fresh grass (Table 3.1).

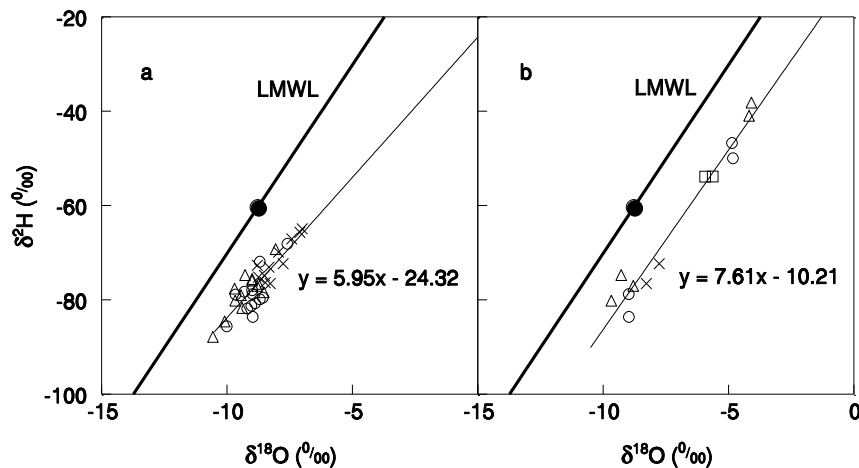


Fig. 3. 3 Relationship between $\delta^{18}\text{O}$ and $\delta^2\text{H}$ (evaporation line) of silage water. (a) Sampling immediately after distribution at about 8 a.m. in the feed bunk and 0.5, 1, 2 and 4 h later ($n = 37$); (b) immediately after distribution and 24 h later ($n = 13$). Explanation of symbols is given in Fig. 3.2. The solid circle (●) shows the isotopic composition of water derived from equilibrium fractionation (at 22 °C) with measured vapor composition. The bold line is the local meteoric water line (LMWL) derived from monthly rain water samples between 2006 and 2011 ($n = 73$, $R^2 = 0.99$).

The initial silage water as taken from the silo in April located at the right side of LMWL, while the water derived from equilibrium fractionation with air vapor fell very close to the LMWL and exhibited almost equal $\delta^{18}\text{O}$ but higher $\delta^2\text{H}$ than the initial silage water. The silage water samples were divided into two groups according to the RH that silage experienced in the feed bunk. During the morning hours the RH was relatively low and decreasing (from about 60% to 40%; Fig. 3.1) inducing evaporation. The samples during this time fell on an evaporation line with a slope of 5.95 (Fig. 3.3 a). During night RH increased to about 80% and remained there for several hours (Fig. 3.1). In consequence, the slope between the initial samples and the samples taken after night was

steeper (7.61; Fig. 3.3b). It should become vertical at 100% RH when only equilibrium fractionation occurs.

3.4.3 Experiment B: Exsiccator experiment

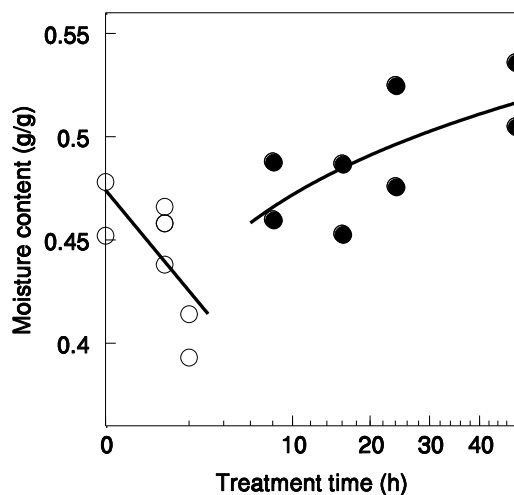


Fig. 3. 4 Moisture contents of the silage after depending on the time since labeling started. Open circles indicate the exsiccator with short intervals between openings. Solid circles indicate the exsiccator with long intervals between openings. The time axis is square root scaled.

The moisture content of the silage in the exsiccators with short intervals between openings decreased due to the repeated opening that allowed air of low RH to enter the exsiccator (Fig. 3.4). In contrast, in the second group with long intervals between openings where the first sampling was carried out after eight hours, the silage moisture even reached higher moisture contents than the initial silage.

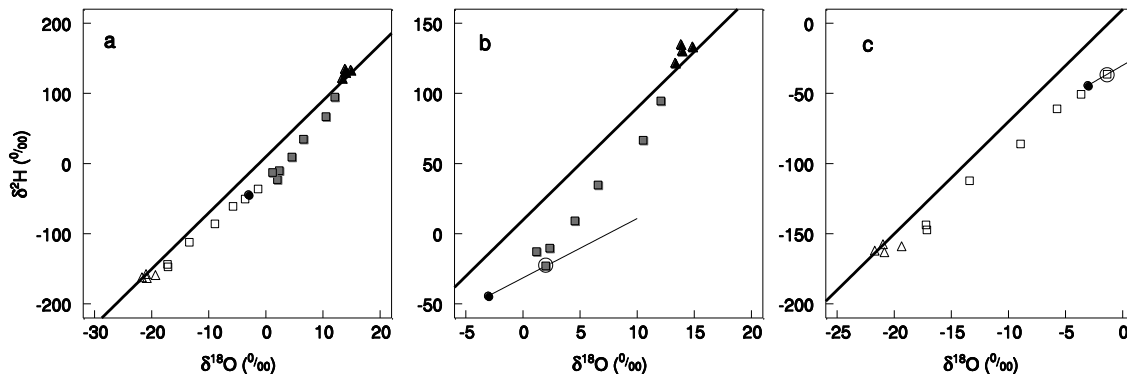


Fig. 3. 5 $\delta^2\text{H}$ versus $\delta^{18}\text{O}$ plot of the silage water exposed to heavy water and light water (a) and silage water exposed to heavy (b) or light (c) labeling water. The black circle (\bullet) represents the initial silage water without exposure. Triangles indicate the heavy (\blacktriangle) and the light (\triangle) labeling water. Gray and white squares represent the silage exposed to heavy and light labeling water, respectively. The circle highlights the first sample, which was taken after one hour, and the thin line connects this sample with the initial silage water. The thick line is the local meteoric water line (LMWL).

The water from silage sampled directly from silo did not fall on the LMWL (Fig. 3.5a) as it was already the case in experiment A (Fig. 3.3). During exposure to labeling water, the silage water approached the corresponding labeling water (Fig. 3.5a). However, close inspection revealed that especially the sample taken after 1 h deviated from the line connecting the initial silage water and corresponding labeling water whereas the others were almost on one connecting line (Fig. 3.5b, c).

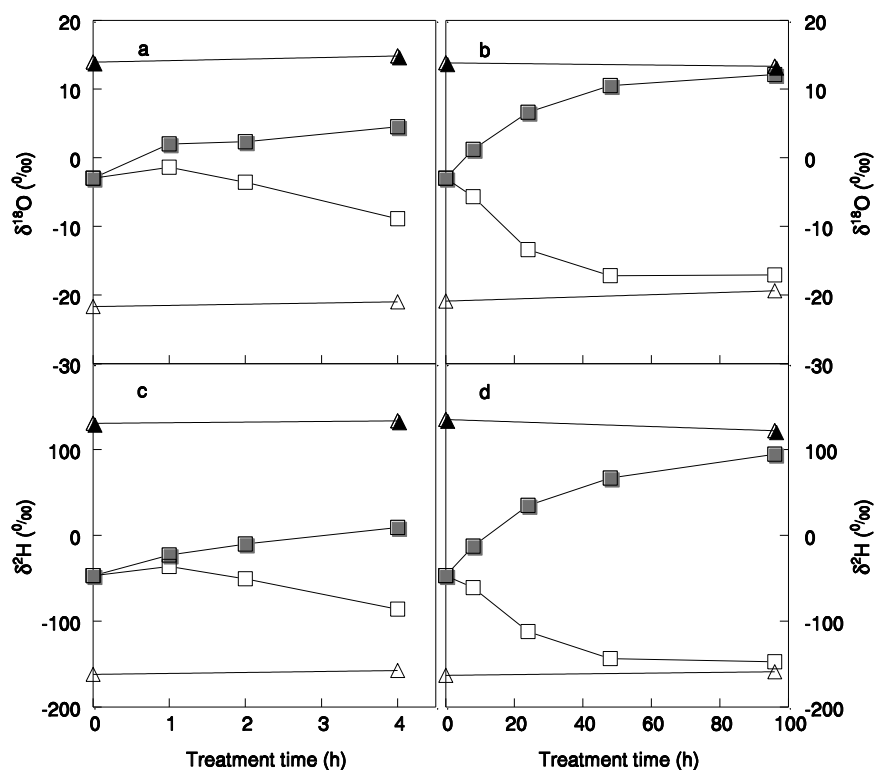


Fig. 3. 6 Time course of oxygen and hydrogen isotopic composition ($\delta^{18}\text{O}$ and $\delta^2\text{H}$) of silage water (squares) and labeling water (triangles) for up to 4 hours (a, c) and for up to 96 hours (b, d); closed and open symbols indicate heavy and light labeling water, respectively.

The silage water showed a different isotopic trend in the exsiccator with short opening intervals compared with that in the exsiccator with long opening intervals (c.f. Fig 3.6a with b, c with d). During the first hour, the silage water became more enriched in both ^{18}O and ^2H (Fig. 3.6a, c), no matter whether it had been exposed to heavy or light labeling water. However, the silage exposed to light labeling water became less enriched than that exposed to heavy water (Fig. 3.6a, c). With longer exposure time, the silage water approached the corresponding labeling water (Fig. 3.6a, c). In the second group where the first sampling from the exsiccators was carried out after eight hours, the silage water followed an exponential pattern approaching the corresponding labeling water (Fig. 3.6b, c). The isotopic composition of silage water over light labeling water had almost reached the composition of the labeling water after 48 h and hence the further change until 96 h exposure time was small, while the change then still was considerable with the heavy labeling water due to the larger spacing between silage and labeling water.

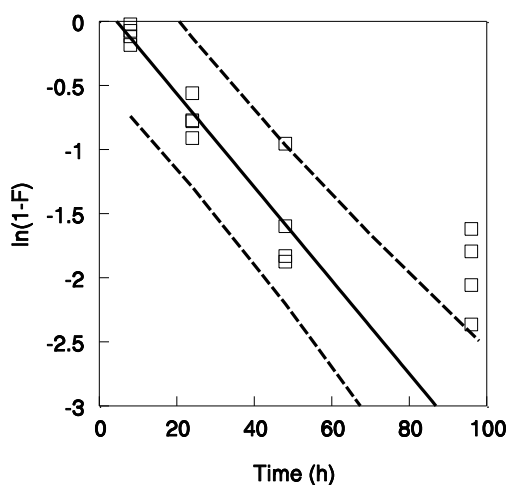


Fig. 3. 7 Reaction progress variable ($\ln(1-F)$) calculated from data obtained from exsiccators with long opening intervals and long labeling time after excluding the effect of condensation. Dashed

lines represent the 95 % prediction intervals for the forecasted reaction progress variable for the measurements from after 8, 24 and 48 hour of labeling.

For the exsiccators with long opening intervals and long exposure time it was assumed that the disturbance due to the opening was sufficiently small to calculate the reaction progress (Fig. 3.7). However, the effect of condensation due to the increasing moisture content (Fig. 3.4) had to be considered. Even after excluding this effect (Eqn 3.10) the transformed data only fall on one straight line during the first 48 hr (Fig. 3.7). The slope of the regression line was -0.04, which corresponds to a half-life of approximately 17 h. The measurements after 96 h exposure time fell clearly outside the range that could be expected from confidence interval of samples that resulted from this regression. Roughly 10% of the total silage water thus had a longer half-life larger than 17 h.

3.4.4 Model simulations

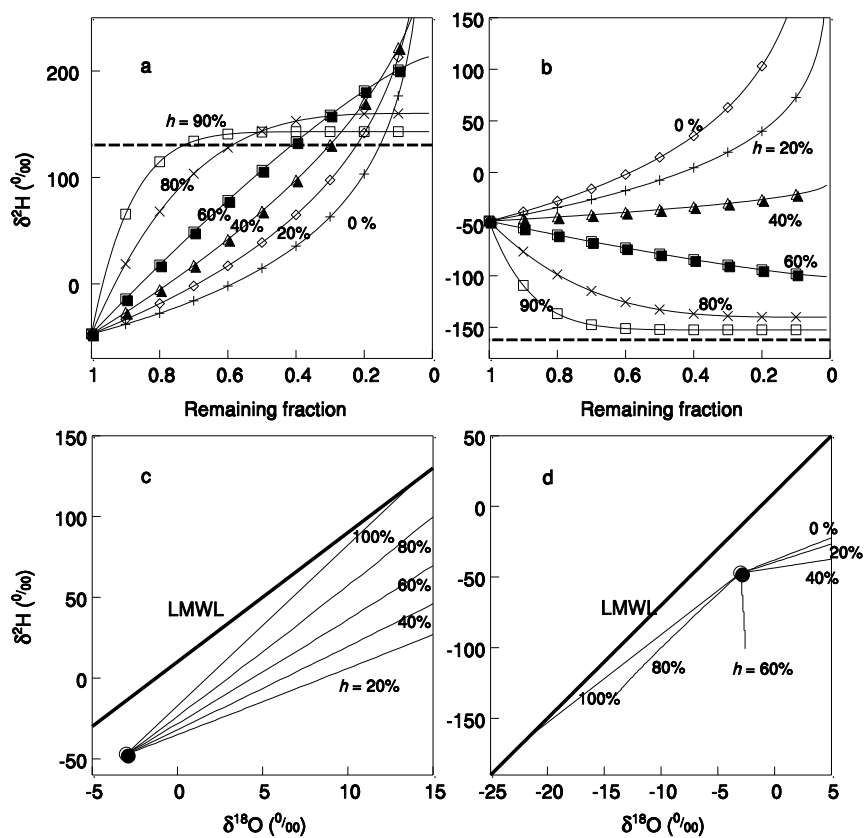


Fig. 3. 8 Effect of relative humidity (h) and the isotopic composition of air vapor on the hydrogen isotopic composition ($\delta^2\text{H}$) of silage water (a, b) and on the slope of the $\delta^2\text{H}$ - $\delta^{18}\text{O}$ relationship (c, d) during drying. The air vapor was assumed to be in equilibrium with either heavy water ($\delta^{18}\text{O}_{\text{vapor}} = 4.2\text{‰}$, $\delta^2\text{H}_{\text{vapor}} = 42.9\text{‰}$; panels a, c) or light water ($\delta^{18}\text{O}_{\text{vapor}} = -31.1\text{‰}$, $\delta^2\text{H}_{\text{vapor}} = -226.9\text{‰}$; panels b, d). All calculations assume $T = 24\text{ °C}$, initial $\delta^{18}\text{O}_s = -3.0\text{‰}$, initial $\delta^2\text{H}_s = -47.1\text{‰}$, and kinetic fractionation $\alpha_{\text{kO}} = 1.0189$ and $\alpha_{\text{kH}} = 1.017$ (Kays and Crawford, 1980; Merlivat, 1978). Dashed lines represent the labelling water which was also the equilibrated state for silage water under relative humidity of 100%.

Under zero humidity, the δ values follow a Rayleigh line when plotted against the remaining fraction (Fig. 3.8). For larger RH but below 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 case of air vapor being heavier than the silage water and a negative slope in the opposite case (Fig. 3.8a, b). With RH further increasing above 50%, the silage water approached a constant value with increasing water loss. This constant value was higher and reached earlier the higher RH became. At 100% humidity the water in the silage approached an isotopic composition given by the isotopic composition of the air vapor and the equilibrium fractionation

The influence of RH on the slope pattern of slope for the relationship between $\delta^2\text{H}$ versus $\delta^{18}\text{O}$ (evaporation line) depended on the relation of the initial composition of silage water and the isotopic composition of the vapor (Fig. 3.8c, d). When the water in equilibrium with air vapor was isotopically heavier than the silage water, the slopes increased as RH increased (Fig. 3.8c). 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 (Fig. 3.8d). For the isotopic composition chosen for Fig. 3.8c, the line became almost vertical at about 60% RH, indicating that under this condition only $\delta^2\text{H}$ will change while $\delta^{18}\text{O}$ remains constant.

3.5 Discussion

The isotopic composition of silage water differed pronouncedly from that of fresh grass. 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.

The difference in isotopic composition of both feed types will cause a large change in the body water when the diet of an animal is switched, e.g. with beginning of the stall period in late autumn. This explains the large variation in milk water that does only weakly correlate with the geographical origin of the milk (Chesson *et al.* 2010), while it changed pronouncedly between winter and summer seasons (Kornexl *et al.* 1997). Similar large seasonal changes were also found in cow hair (Auerswald *et al.* 2011) and beef (Boner and Förstel, 2004). The change in moisture content will also have an influence on the dietary water because more drinking water is needed by the animal if the drier silage is fed. Both influences, the different isotopic composition and a higher drinking water requirement, will change the body water in the same direction as silage water and drinking water are more depleted in heavy isotopes than leaf water. Silage water always was close to the local meteoric water line, on which also the drinking water should plot if it is derived from local groundwater. The influence of the change in drinking water demand when switching from the moist grass to the dryer silage or even hay, which previously was assumed to be the main cause of the observed changes e.g. in beef (Boner and Förstel, 2004), thus only partly explains the difference. The reason is that the moisture content from fresh grass to silage only changed by one third, which has to be replaced by drinking water, while two thirds are still provided by the roughage. Furthermore, water requirements during winter are usually smaller than during summer due to the lower temperatures (Winchester and Morris, 1956). Sun *et al.* (2014) found that drinking water requirements increase with the third power of ambient temperature, which lets us expect that – depending on the stall temperature – drinking water intake during winter may not be very different from that during summer.

After distribution in the feed bunk, the silage water changed its isotopic composition rather rapidly. This may require to sample the feed several times during a day depending on the type of offer (*ad libitum* or scheduled feeding), especially if milk is under focus,

which usually is gathered twice a day. The differences in ambient conditions during daytime and nighttime should result in differences in the isotopic composition of the silage and in turn in milk, even though, the difference in day and night feed will be attenuated due to the incomplete turnover of the body water (Cerling, *et al.*, 2007). Also the interpretation of the isotopic composition in other body liquids like blood or urine may require determining the feed water with high temporal resolution. Such a high resolution sampling of the feed may especially become necessary because the kind of change during exposure on the feed bunk is rather variable. Our modeling exercise demonstrated that the silage water can change in any direction depending on the relative humidity and the isotopic composition of the air vapor.

The exsiccator experiment indicated a half life 17 hr. The fast change of the isotopic composition after exposure of the silage to a new environment is not unexpected given that the cell structures of the leaf are weakened due to the mechanical and chemical impact of the production process (Greenhill, 1964). Whether the silage water can be regarded as one pool or whether there are two pools as indicated by Fig. 3.7 is uncertain, given that our experimental design did not include any measurement between 48 hr and 96 hr of exposure. However, under practical aspects, exposure times of this length hardly will occur. Fig. 3.7 shows, that the slower pool contributed about 10% of the total silage water, which would be about 5% of the silage fresh mass. It is likely that this water mainly is stem water and that the structural changes during the production process open the cell structure of the stems less than that of the leaves (Moon and Henk, 1980).

Within the experimental restrictions, the isotopic composition of the silage in all experiments followed to what could be expected from the Craig-Gordon model. Depending on the relative humidity and the isotopic composition of the air vapor and the degree of drying of the silage after removal from the silo, a rather large variation of the silage water is possible that may even span more than 200 ‰ for $\delta^2\text{H}$ (Fig. 3.8). Under realistic scenarios, where the atmospheric vapor does not differ strongly from the equilibrium vapor of silage water and where drying is limited, the variation of silage water after removal from the silo is restricted to about 40 ‰ for $\delta^2\text{H}$ (Fig. 3.3), but this variation is still considerable. In any case, however, the silage water is considerably more depleted than the water in fresh leaves (Table 3.1, Fig. 3.3). This contributed to the fact

that domestic herbivores fed with silage were more depleted in water isotopes than that those grazed on pasture (Boner and Förstel, 2004; Auerswald, *et al.*, 2011). The use of water isotopes as an indicator of the geographical origin, which has been well demonstrated in wildlife (Hobson, 1998; Bowen *et al.*, 2005), may only be useful in domestic animals if their type of feed is known (fresh grass vs silage). In domestic animals, water isotopes are better suited to indicate the type of feeding. Water isotopes will vary pronouncedly with season in the case of summer-grazed animals while there will be only a small seasonal variation caused by the isotopic exchange on the feed bunk in cases where silage is fed throughout the year.

4 Oxygen isotopic composition of total inputs

4.1 Introduction

Oxygen isotopes are applied for tracing the origin and movement of animals and their products because the large isotopic gradients of water isotopes across the globe (Bowen *et al.*, 2005) are imprinted in the animal tissues. However, in addition to the geographical variation of water isotopes there is also a large seasonal variation in rain, which may further be modified by seasonal changes in feed. For domestic herbivores such seasonal changes in feed typically involve fresh grass during the growing season and conserved roughage during the dormant season. It is thus not surprising that the isotopic composition of domestic herbivores varies seasonally in addition to the geographic variation (Boner and Förstel, 2004; Camin *et al.*, 2007). Both sources of variation thus need to be quantified for proper geographical tracing or for identification of the feed source (fresh vs. conserved roughage) depending on the question under focus. Thus, this chapter aims to model the oxygen isotopic composition of the total oxygen input of domestic herbivores, here steers, during the year while its feed, its drinking water demand and the isotopic composition within each feed component vary.

4.2 Materials and methods

4.2.1 Animals

The steers kept and raised on the Gruenschwaige Grassland Research Station were used to predict the isotopic composition of total oxygen input. On average, the body weight of steers was 470 kg. During the winter dormant period (October to April), the steers were maintained on a diet of grass silage in the stall, whereas in grazing season green pasture was the sole feed source.

4.2.2 Quantification of oxygen input components

The components of oxygen input taken into consideration are air oxygen, air vapor, chemically bound oxygen in feed, free water of feed, and drinking water (where the sum of free water of feed and drinking is termed “total liquid water intake”). Except for the components in the total liquid water intake, the quantifications of the other input

components followed Kohn's model for herbivores (1996). The quantities of the inputs are related to four factors: metabolic requirement, diet, the extraction efficiency of oxygen from air, and water economy (the ratio of daily water turnover to energy expenditure).

For ease of simplicity, it was assumed that energy expenditure (J) can be scaled according to body mass (M_{body} in kg) (Nagy and Peterson, 1988). Because all other parameters were ultimately scaled to energy use based on specific physiological data, exact energy values were not critical,

$$J \text{ [kJ]} = 900 * M_{\text{body}}^{0.73} \quad (4.1)$$

Once a metabolic requirement was assigned, the amount of air oxygen required (M_{O}) can be calculated based on the oxygen conversion factor (0.00216 moles O_2/kJ),

$$M_{\text{O}} \text{ [moles } \text{O}_2] = 0.00216 * J. \quad (4.2)$$

Based on the fraction of O_2 taken up (assumed to be 20%), mole volume of air (22.4 L/mole) and the concentration of oxygen in air (20%), the amount of air (V_{air}) fluxed through the lungs was calculated as

$$V_{\text{air}} \text{ [L]} = 22.4 * M_{\text{O}} / (0.2 * 0.2) \quad (4.3)$$

Saturation concentration of H_2O in the air at ambient temperature (T in $^{\circ}\text{C}$) and the corresponding relative humidity (h) were used to determine the amount of air vapor (M_{vapor}) taken into the lungs

$$M_{\text{vapor}} \text{ [mole]} = h * 10^{(0.686 + 0.027 * T)} * V_{\text{air}} / (760 * 22.4) \quad (4.4)$$

From the daily energy requirement (Eqn. 4.1), the composition of the feed (85% carbohydrate, 5% fat and 10% protein), the food component energy values (17300 kJ/kg for carbohydrate, 39700 kJ/kg for fat and 20100 kJ/kg for protein), the energy extraction efficiency (P_{energy} , 90%) and digestibility (P_{digest} , 70%), the dry mass of feed ingested (M_{feed}) can be estimated as

$$M_{\text{feed}} \text{ [kg]} = J / (P_{\text{energy}} * P_{\text{digest}} * (0.85 * 17300 + 0.05 * 39700 + 0.10 * 20100)) \quad (4.5)$$

Then, the amount of chemically bound oxygen in feed (M_{feedO}) can be determined from the amount of feed ingested, feed composition, digestibility, concentration of oxygen in feed (15.4 mol O_2/kg for carbohydrate, 2 mol O_2/kg for fat and 3 mol O_2/kg for protein) and energy extraction efficiency (P_{energy}):

$$M_{\text{feedO}} \text{ [moles } \text{O}_2] = M_{\text{feed}} * P_{\text{digest}} * P_{\text{energy}} * (0.85 * 15.4 + 0.05 * 2 + 0.10 * 3) \quad (4.6)$$

The amount of chemically bound hydrogen in feed (M_{feedH} in mol H₂), all of which was assumed to be transferred to metabolic water, was calculated similar as bound oxygen except the concentration in feed (30.9 mol H₂/kg for carbohydrate, 60 mol H₂/kg for fat and 11 mol H₂/kg for protein):

$$M_{\text{feedH}} [\text{moles H}_2] = M_{\text{feed}} * P_{\text{digest}} * P_{\text{energy}} * (0.85 * 30.9 + 0.05 * 60 + 0.10 * 11). \quad (4.7)$$

The calculation of the amount of total liquid water intake based on the total water turnover rate, which was computed from the water economy index (0.25 ml/kJ for adult but non-lactating cattle, i.e. the ratio of daily water turnover to energy expenditure). The mass contribution of total liquid water intake (M_{liquid}) was determined simply by subtracting the contributions of air H₂O and metabolic water from the total water turnover. Total liquid water intake was calculated from

$$M_{\text{liquid}} [\text{mole}] = 0.25 * J / 18 - V_{\text{vapor}} - M_{\text{feedH}} \quad (4.8)$$

As the amount of oxygen associated with each input component was assigned, the relative mass contribution of them can be calculated,

$$f_{\text{oxygen component}} = M_{\text{oxygen component}} / (2 * M_{\text{O}} + M_{\text{vapor}} + 2 * M_{\text{feedO}} + M_{\text{liquid}}) \quad (4.9)$$

where $M_{\text{oxygen component}}$ and $f_{\text{oxygen component}}$ respectively represent the oxygen mass of the respective component and its relative contribution to the total oxygen input of corresponding input component under focus, including air oxygen, air vapour, chemically bound oxygen and total liquid water intake.

4.2.3 Quantification of the components in total liquid water intake

The constitution of the total liquid water intake differs between the dormant and the grazing seasons. During the dormant season when the domestic herbivores are kept in stall, the components of the total liquid water are only drinking water and internal feed water (e.g. silage water). In contrast, on pasture the external feed water that induced by wet soil conditions and intercepted rain constitute the additional sources to the liquid water intake (Chapter 2).

During the grazing season the daily drinking water intake of the steer was predicted from Eqn. (2.12) based on the environmental conditions in 2010 and 2011. On pasture the intercepted rain on the leaves decreased the drinking water intake. Thus, the intercepted rain intake ($M_{\text{intercepted rain}}$) during wet days was calculated as the difference of the

predicted drinking water intake between a wet day (D_{wet}) and a dry day (D_{dry}) with all other conditions being identical among days,

$$M_{\text{intercepted rain}} = D_{\text{dry}} - D_{\text{wet}} \quad (4.10)$$

Wet soil conditions promoted dew and guttation formation and increased internal moisture content of the grass. Hence, the drinking water demand decreases if steers graze during wet soil conditions (Chapter 2). Thus, the increased dietary water intake resulting from the wet soil status (M_{dew} because it was expected that this is mainly due to dewrise) was the difference between drinking water intake of steers under certain soil moisture condition (D) to that under dry soil condition when PAW was smaller than 30% PAW capacity while all other were maintained ($D_{<30\%}$)

$$M_{\text{dew}} = D - D_{<30\%} \quad (4.11)$$

The internal dietary water is the water source for both seasons. The amount of internal dietary water (M_{internal}) was calculated from the dry matter intake (M_{feed} , Eqn. 4.5) and the internal relative moisture content (C_{internal}) of fresh grass and silage of 75% and 50% (Chapter 2), respectively,

$$M_{\text{internal}} = M_{\text{feed}} / (1 - C_{\text{internal}}) * C_{\text{internal}} \quad (4.12)$$

The daily DWI during the dormant period was predicted from the equation by Cardot *et al.* (2008), which was developed from the drinking water intake from the cows in the stall over three consecutive winters. These predictions for the grazing season matched well with the measurements for 2010 and 2011 (the predicted average daily drinking water intake was only < 2 L/d higher than the measurements when milk yield was set to 0 L/d in the case of steers),

$$D = 1.54 * M_{\text{feed}} + 1.33 * M_{\text{milk}} + 0.89 * C_{\text{drymatter}} + 0.58 * T_{\text{min}} - 0.3 * M_{\text{rainfall}} - 25.65 \quad (4.13)$$

where M_{feed} , M_{milk} , $C_{\text{drymatter}}$, T_{min} and M_{rainfall} represent dry matter intake [kg/d], milk yield [kg/d], dry matter content of feed [%], daily minimum temperature [$^{\circ}\text{C}$] and rainfall [mm/d], respectively.

Then, the mass proportion of each component in the total liquid water intake can be calculated based on their respective mass contribution. To calculate the mass contribution to the total oxygen input, the mass proportion in the total liquid water intake should be further multiplied with the relative contribution of the total liquid water intake to the total oxygen input,

$$f_{\text{liquid component}} = M_{\text{liquid component}} / (D + M_{\text{internal}} + M_{\text{dew}} + M_{\text{intercepted rain}}) * f_{\text{liquid}} \quad (4.14)$$

where $f_{\text{liquid component}}$ and $M_{\text{liquid component}}$ represent the relative oxygen mass contribution to the total oxygen input and the oxygen mass of corresponding liquid component under focus, including the drinking water, internal feed water, dew and rain, and f_{liquid} is the oxygen relative contribution of total liquid water intake to the total oxygen input (Eqn. 4.9).

4.2.3 Measurements of oxygen isotopic composition of input components

From 2006 to 2011 the air vapor, rain and drinking water were sampled every month throughout the year, while the fresh leaf water and soil water was sampled monthly from April to October. The silage was taken randomly from silo in April from 2011 to 2013. The oxygen isotope composition of the dry matter in feed was assumed to be equal with that of cellulose oxygen, because cellulose contributes the largest share (Barbour and Farquhar, 2000). The oxygen isotope composition in cellulose was assumed to be 27 ‰ more enriched than leaf water (Sternberg *et al.*, 1989). The oxygen isotopic composition of the water intake related to the wet soil status was assumed to be same as the isotopic composition of soil water. Air oxygen isotopic composition is assumed to be 23.5 ‰ worldwide (Kroopnick and Craig, 1972) with no seasonal variation.

4.2.4 Prediction of oxygen isotopic composition of total inputs

Once the relative oxygen mass contribution to total oxygen input and the oxygen isotopic composition can be assigned to each input component, the isotopic composition of total oxygen input ($\delta^{18}\text{O}_{\text{total}}$) can be calculated based on a mass balance equation (1.1), in which the dietary water should be modified for application on respective grazing and dormant season according to the different diet strategy,

$$\delta^{18}\text{O}_{\text{total}} = f_{\text{O}} * \delta^{18}\text{O}_{\text{O}} + f_{\text{vapor}} * \delta^{18}\text{O}_{\text{vapor}} + f_{\text{feedO}} * \delta^{18}\text{O}_{\text{feedO}} + f_{\text{drink}} * \delta^{18}\text{O}_{\text{drink}} + f_{\text{internal}} * \delta^{18}\text{O}_{\text{internal}} + (f_{\text{dew}} * \delta^{18}\text{O}_{\text{dew}} + f_{\text{intercepted rain}} * \delta^{18}\text{O}_{\text{intercepted rain}}) \quad (4.15)$$

where f and $\delta^{18}\text{O}$ represent the mass fraction of the respective component to total oxygen input and its oxygen isotopic composition and subscripts O, vapor, feedO and drink, denote the respective components air oxygen, air vapor, chemically bound oxygen and drinking water. During the dormant season the dietary water is solely silage water, which

is represented by subscript internal, while during grazing season wet soil conditions and rain also contribute to the dietary water intake by increasing the internal moisture content of grass and leaf wetness (Chapter 2). The subscript “dew” and intercepted rain represent the increased dietary water intake caused by wet soil conditions and rainfall.

After a diet switch, the body water requires time to reach a new isotopic steady state, depending on the size of the body water pool and the rate of water flows. Water constitutes approximately 60%-70% of an animal’s live weight (Faries *et al.*, 1997). Herbivores obtain water mainly from drinking water and dietary water, while the metabolic water contributes little to the water requirement (NRC, 1988). Thus, the turnover time of body water is assumed to be the time required for replacing the total body water by the total liquid water intake.

In order to detect the most important variables determining seasonal variation in oxygen isotopic of domestic herbivores, the model prediction of the oxygen isotopic composition of the total oxygen input (Eqn. 1.1) was tested for its sensitivity to (i) the variation related to seasonal transition and (ii) to the uncertainties associated with the quantification of these variables. The baseline was set as a steer with body weight of 470 kg consuming the grass with 60% moisture content without the external feed water intake at 70% relative humidity. Sensitivity is expressed as the deviation of isotopic composition of the total oxygen input relative to the baseline.

4.3 Results

Table 4. 1 Sensitivity of the model predicting the oxygen isotopic composition ($\delta^{18}\text{O}$) of total oxygen input

Input	Average relative mass contribution (%)	Range of variation (%)	Deviation relative to baseline due to the variation in mass (‰)	Average $\delta^{18}\text{O}$ (‰)	Range of variation (‰)	Deviation relative to baseline due to variation in isotopic composition (‰)
Air O ₂	24	4	0.9	23.5 [‡]	0.6	0.1
Air H ₂ O	5	3	0.5	-15.0 (<i>n</i> = 126)	10	0.5
Bound O in feed	8	4	1.3	31.5 (<i>n</i> = 122)	13	1.0
Dietary water	39	24	1.1	4.5 (<i>n</i> = 122)	24	9.4
Drinking water	24	39	4	-10.0 (<i>n</i> = 124)	0.8	0.2

[‡] Data from Kroopnick and Craig, 1972 (Science, 175, 54 – 55).

Over the course of the year, the relative oxygen mass contributions derived from air oxygen, air vapor and chemically bound oxygen changed little, while the contributions from the dietary free water (e.g. sum of leaf water, “dew” and rain) and from the drinking water intake varied considerably due to the diet switch from fresh grass to silage (Table 4.1). Regarding to the variations in the amount of input components, the changes in the drinking water intake caused the largest deviation from the baseline, about 4‰. The isotopic composition of the each input component varied during a year, in which the dietary water changed most compared to the others, resulting in 24‰ of deviation from the baseline.

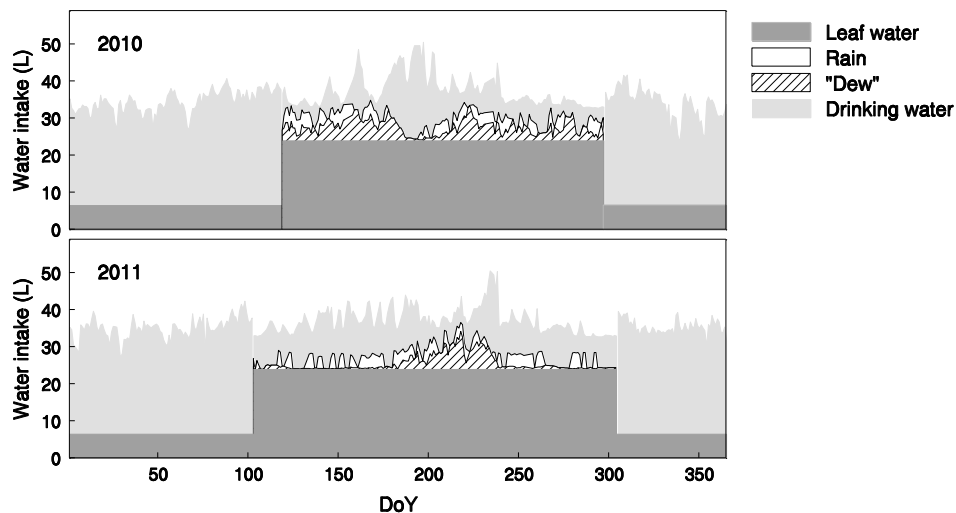


Fig. 4. 1 Mass contribution of different water components to the total liquid water intake of a steer fed on fresh grass during the grazing season and on silage during the dormant season, respectively, modelled according to the meteorological conditions in 2010 and 2011. “Dew” includes dew, guttation on the grass surface, and the increased grass internal moisture caused by a wet soil status.

The air oxygen, air vapor, chemically bound oxygen and total liquid water intake remained nearly constant at 24%, 5%, 8% and 63%, respectively, and contributed little to the seasonal variation of oxygen isotopic composition of the total inputs (Table 4.1). However, the relative mass contribution of each component to the total liquid water intake differed significantly between the grazing and the dormant seasons. Drinking water accounted for 24 % and 82 % of the total liquid water intake, respectively, during the grazing season and the dormant period.

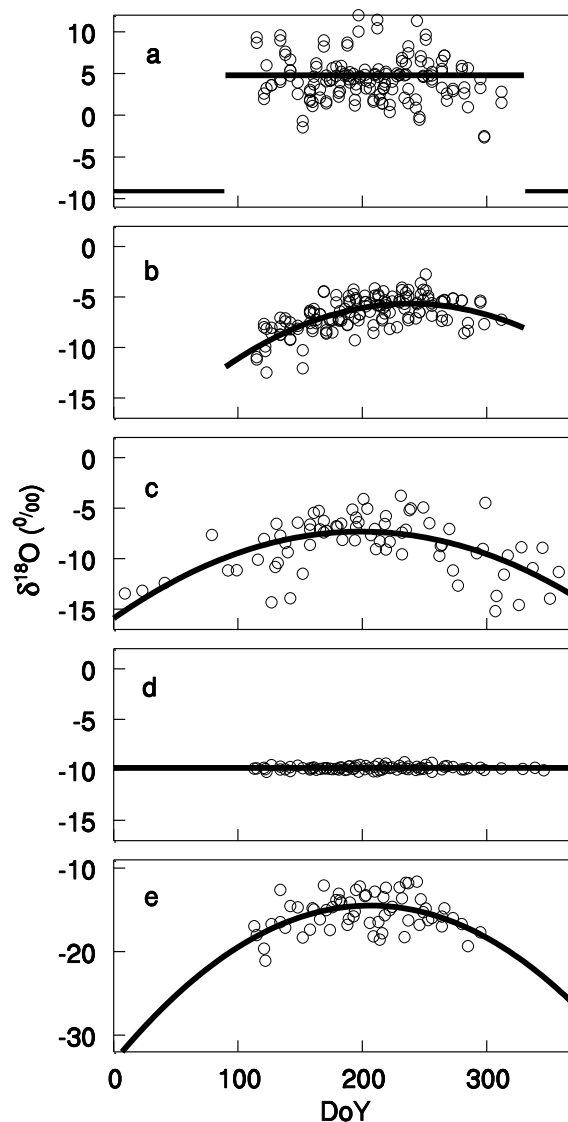


Fig. 4. 2 Oxygen isotopic composition ($\delta^{18}\text{O}$) of oxygen input components of a steer during a year when fed on silage during winter period and grazing on pasture during summer season: a) internal dietary water, leaf water for grazing season ($n = 122$), silage water for dormant season (see chapter 3); b) soil water, which was assumed to be isotopically identical to “dew”; c) rain ($n = 73$); d) drinking water ($n = 124$), and e) air vapor ($n = 127$). Solid lines represent the oxygen isotopic variation for respective oxygen input component. The data for air oxygen and chemically bound oxygen in feed which remains constant at 23.5‰ and 27‰ more enriched than fresh leaf water, respectively are not shown in Fig. 4.2.

The air vapor, rain and soil water (“dew”) showed significant seasonal variation in oxygen isotopic composition (Fig. 4.2). In contrast, drinking water maintained a constant

oxygen isotopic composition of -10 ‰. The oxygen isotopic composition of leaf water fluctuated largely every day during grazing season, ranging from -1.5 ‰ to 12.6 ‰, without a seasonal trend. On average, the dietary water during the grazing season (e.g. leaf water) was 13 ‰ more enriched relative to that during dormant season (e.g. silage water).

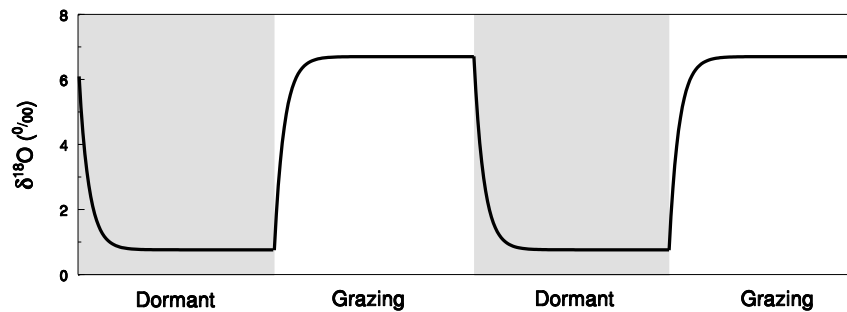


Fig. 4. 3 Predicted oxygen isotopic composition ($\delta^{18}\text{O}$) of total oxygen input of a steer with weight of 470 kg during dormant and grazing season when steer is fed exclusively on silage and fresh grass, respectively.

Model predictions for the isotopic composition of total oxygen input were 0.76 ‰ during the dormant season, approximately 6 ‰ more depleted as compared to the grazing season (Fig. 4.3). The daily amount of total liquid water intake accounted for 12 % of body water of a steer with 470 kg body weight. Because of this low exchange rate, it took almost two months for the body water to reach a new isotopic composition after a diet switch.

4.4 Discussion

The relative mass contribution from air oxygen, air vapor, chemically bound oxygen and total liquid water intake remained approximately constant throughout the year, while the mass proportion of the components in total liquid water intake changed dramatically after diet switch. Based on the relative mass contribution and average isotopic composition of each input component, the total oxygen input for a steer with 470 kg during the grazing season was predicted to be 6 ‰ more enriched than that during the dormant season. This agreed well with the observed seasonal variation in the oxygen isotopic composition of hair of cattle which were raised on the same research site

(Auerswald *et al.*, 2011). This is mainly due to the altered isotopic composition and relative mass contribution of dietary water. Specifically, the oxygen isotopic composition of drinking water was considerably more depleted than fresh leaf water. The moisture content of silage (50%) was much lower than that of fresh grass (75%), resulting in more drinking water requirement during the dormant season to compensate the lack from dietary water relative to the grazing season. Further, compared with dietary water during the grazing season (e.g. fresh leaf water) the silage water was more depleted, aggravating the depletion in ^{18}O of total inputs after diet switch from fresh grass to silage.

5 General and summarizing discussion

Silage had lower moisture content than fresh grass, which will cause a higher drinking water intake of domestic herbivores during the dormant period than during the grazing season because the total water requirement remains fairly constant throughout the year. This is evident from the calculation of the total liquid water intake via the energy intake, which does not necessarily vary with season (Eqn. 4.8; Kohn, 1996). The same conclusion follows from Eqn. 4.13 by Cardot *et al.* (2008) despite its completely different structure and different basis. It indicated that total water requirement of cattle is higher when fed on feed with higher dry matter intake. Thus, the decreased total water requirement caused by low temperatures during the dormant season is compensated by the increased water intake induced by the higher dry matter content of silage compared to that of fresh grass. Also, silage water was more depleted in ^{18}O compared to fresh grass water, further aggravating the depletion in ^{18}O of total oxygen input. The difference in isotopic composition between silage water and fresh grass water has been attributed to the higher enrichment of fresh grass caused by evapotranspiration (Boner and Förstel, 2004). However, the comparison of the isotopic composition of water of silage and of fresh grass grown during the time of silage production suggests that a process depleting water after mowing grass for silage production must occur. The water may become increasingly depleted in both ^{18}O and ^2H during the drying process due to a back flux of air vapor to the drying silage (Chapter 3; Helliker and Griffiths, 2007). Thus, wilting on the field under high relative humidity may cause depletion in ^{18}O of water in mowed grass water relative to water in growing grass.

Among the oxygen input components, air oxygen and total liquid water intake (e.g. drinking water and free dietary water) were the main sources, accounting for 24% and 63% (Table 4.1) of total oxygen input of steer, respectively. In contrast, air oxygen, air vapor and chemically bound oxygen together contributed only about 15% to total oxygen input (Table 4.1). The proportion of each input component was scaled to energy expenditure, which is simply derived from body weight (Eqn. 4.1). Thus, exact energy values are not critical. A change of body weight from 100 kg to 1000 kg only varied the proportion of oxygen in liquid water intake by 10% (Bryant and Froelich, 1995), which in consequence has only minor effects on the oxygen isotopic composition of total inputs.

However, quantification of relative mass contributions of input components based on energy expenditure cannot be applied to herbivores that are lactating or are not weaned because physiological strategies are extremely variable (Smith, 1959). Except for air oxygen, all other oxygen input components are related to surface water (Gammons *et al.*, 2006; Barbour, 2007). Thus, it has been suggested that herbivores' oxygen isotopic composition can track surface water isotopic composition. The additional external feed water (e.g. rain and "dew") is identical to the surface water. The correlation between animal and surface water compositions is primarily due to the fact that food components track surface water, and not because of direct drinking water input. In contrast, the isotopic composition of air oxygen is independent of surface water. Thus, animal tissue should never track surface water perfectly. The slope will always be one minus the fraction of air oxygen in total oxygen input (Ehleringer *et al.*, 2008).

The isotopic composition of the total oxygen input is subject to both seasonal and short term variation. Some components show a seasonal pattern in isotopic composition (air vapour, rain) which is in agreement to variations caused by the global water cycle (Gat, 1996). Dietary water of fresh grass showed the largest variation (Fig. 4.2); however there was no clear seasonal pattern. This is due to the fast turnover of leaf water (Allison *et al.*, 1985; Yakir *et al.*, 1990) and the strongly fluctuating conditions responsible for leaf water enrichment (Föstel, 1987; Bariac *et al.*, 1989). However, short term variations in the isotopic composition of total oxygen input will only translate into the animal products with a turnover rate that is comparable to the time scale of the fluctuations like CO₂. Animal products, which are suitable for tracking the origin or the production system (like hair or bone), have considerably longer turnover rates. Thus, their isotopic composition does not reflect day-to-day variation in the total oxygen input, as these variations are strongly extenuated. Hence, short-term variations in the isotopic composition of input components can be neglected. Using the average isotopic compositions calculated for periods when the relative mass contribution of different input components are constant will give a sufficiently precise estimate of the isotopic composition of total input.

The results show that the seasonal patterns in the oxygen isotopic composition of domestic herbivores exist, mainly due to the seasonality in animal feeding practices modulated by tissue turnover rates (Fig. 4.3). Comparing the oxygen isotopic

composition without reference to the seasonal variation may confound or fail the geographical tracing applications. For instance, the oxygen isotopic difference of 6‰ in total inputs between summer and winter periods reaches the isotopic gradient of surface water between two geographical sites, such as Sicily and Scotland. On the other hand, the seasonal variations in O isotope ratios provide a hint to refer the feed that the herbivores fed. The herbivores fed on conserved roughage in stall should be more depleted in ^{18}O than that grazing on pasture. In conclusion, it is strongly suggests that there is a need to understand the underlying causes for seasonal variation, including tissue turnover, when applying the stable oxygen isotopes on domestic herbivores.

Acknowledgements

I would like to express the deepest appreciation to my supervisor, Prof. Auerswald, who gave me constant advice and help. Without his patient guidance this dissertation would not have been possible.

I would also like to thank Prof. Schnyder for his encouragement and help to my research.

Rudi Schäufele, Richard Wenzel, Anja Schmidt, Monika Michler and Hans Vogl provided essential and indispensable help in conducting experiment.

Many thanks to Melitta Sternkopf and all the colleagues in Lehrstuhl für Grünlandlehre for the uncountable help and creating a pleasant working environment.

List of Abbreviations and Variables

$\delta^{18}\text{O}$	oxygen isotopic composition
$\delta^{18}\text{O}^{\text{“dew”}}$	oxygen isotopic composition of increased dietary water caused by wet soil condition
$\delta^{18}\text{O}_{\text{dietary water}}$	oxygen isotopic composition of dietary water
$\delta^{18}\text{O}_{\text{drink}}$	oxygen isotopic composition of drinking water
$\delta^{18}\text{O}_{\text{feedO}}$	oxygen isotopic composition of chemically bound oxygen of feed
$\delta^{18}\text{O}_{\text{internal}}$	oxygen isotopic composition of internal dietary water
$\delta^{18}\text{O}_{\text{O}}$	oxygen isotopic composition of air oxygen
$\delta^{18}\text{O}_{\text{intercepted rain}}$	oxygen isotopic composition of intercepted rain intake
$\delta^{18}\text{O}_{\text{total}}$	oxygen isotopic composition of total inputs
$\delta^{18}\text{O}_{\text{vapor}}$	oxygen isotopic composition of air vapor
$\delta^2\text{H}$	hydrogen isotopic composition
δ_{cond}	isotopic composition of condensed water on the silage
δ_{E}	isotopic composition of evaporated water
δ_{eq}	isotopic equilibrium with air vapor
δ_{s}'	measured isotopic value of extracted water from silage
δ_{s}	isotopic composition of silage water
δ_{s0}	the initial isotopic composition of silage water
δ_{steady}	isotopic steady state
$C_{\text{drymatter}}$	relative dry matter content of feed
C_{internal}	relative internal moisture content of diet
C_{s}	saturation concentration of vapor
$D_{<30\%,\text{dry}}$	drinking water intake on dry days with soil below 30% plant available water capacity
$D_{<30\%,\text{wet}}$	drinking water intake on wet days with soil below 30% plant available water capacity
$D_{>95\%}$	drinking water intake when PAW>95% plant available water capacity

D_{dry}	drinking water intake on dry days
$df_{\text{remaining}}$	change in the remaining fraction of the initial amount of water
dM_{area}	change in water-mass per surface area
dt	an increment of time
D_{wet}	drinking water intake on wet days
DWI.....	drinking water intake
$d\delta_s$	change in isotopic composition of the remaining silage water
E	rate of water evaporation, actual evapotranspiration
E_i	actual evapotranspiration of corresponding day
E_{pot}	potential evapotranspiration
F	reaction progress variable
$f^{\text{“dew”}}$	relative oxygen mass contribution to the total oxygen input of increased dietary water caused by wet soil condition
$f_{\text{dietary water}}$	relative mass contribution of dietary water to the total oxygen input
f_{drink}	relative mass contribution of drinking water intake to the total oxygen input
f_{feedO}	relative mass contribution of chemically bound oxygen in feed to the total oxygen input
f_{internal}	relative oxygen mass contribution of internal dietary water to the total oxygen input
$f_{\text{liquid component}}$	relative oxygen mass contribution of component to the total liquid water intake
f_{O}	relative mass contribution of air oxygen to the total oxygen input
$f_{\text{oxygen component}}$	relative mass contribution of input component to the total oxygen input
$f_{\text{intercepted rain}}$	relative oxygen mass contribution of the intercepted rain intake to the total oxygen input
$f_{\text{remaining}}$	the remaining fraction of the initial amount of water
f_{vapor}	relative mass contribution of inhaled air vapour to the total

	oxygen input
GR.....	global radiation
h	relative humidity
I	rate of water inflow
i	corresponding day
$i-1$	previous day
J	energy expenditure
LMWL.....	local meteoric water line
M_{s0}	initial water-mass per surface area
M_{area}	water-mass per surface area
M_{body}	body weight
M_{cond}	increased mass resulted from condensation
$M^{\text{“dew”}}$	increased dietary water intake caused by wet soil condition
M_{feed}	feed dry matter intake
M_{feedH}	amount of chemically bound hydrogen in feed
M_{feedO}	amount of chemically bound oxygen in feed
$M_{\text{intercepted rain}}$	intercepted rain intake
M_{internal}	internal dietary water
$M_{\text{liquid component}}$	oxygen mass contribution of component in liquid water intake under focus
M_{liquid}	total liquid water intake
M_{O}	amount of air oxygen required
$M_{\text{oxygen component}}$	oxygen mass of input component
M_{milk}	milk yield
$M_{\text{rainfall},i}$	rainfall of corresponding day
M_{vapor}	amount of air vapour
n	number of samples
PAW.....	plant available water
P_{digest}	digestibility
P_{energy}	energy extraction efficiency
PET.....	potential evapotranspiration

$P_{rel,i}$	ratio of plant available water of corresponding day to the plant available water capacity
R	atom ratio between the heavy and the light isotopes
R^2	coefficient of determination
R_E	atom ratio between the heavy and the light isotopes of evaporated water
RH.....	relative humidity
RMSE.....	root mean square error
R_s	atom ratio between the heavy and the light isotopes of silage water
R_{vapor}	atom ratio between the heavy and the light isotopes of vapor
SD.....	standard deviation
SH.....	sunshine hours
t	time
T_{max}	daily maximum ambient temperature
T_{mean}	daily mean ambient temperature
T_{min}	daily minimum temperature
V_{air}	amount of air fluxed through the lungs
$W_{capacity}$	plant available water capacity
W_i	plant available water of corresponding day
W_{i-1}	plant available water of previous day
WS.....	wind speed
α^*	temperature dependent fractionation factor
α_k	kinetic fractionation
$\Delta\epsilon$	kinetic enrichment
ϵ^*	equilibrium enrichment
λ	first-order rate constant
ρ	resistance coefficient of water substance
ρ_i	resistance coefficient of isotopic water molecules (either HDO or H ₂ ¹⁸ O)

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