

ENERGY REQUIREMENTS DURING LACTATION IN LAMAS (*LAMA GLAMA*)

Alexander Riek¹, Annegret Klinkert¹, Martina Gerken¹, Jürgen Hummel^{1,2}, Eva Moors¹ and Karl-Heinz Südekum²

¹*Department of Animal Sciences, University of Göttingen, 37075 Göttingen, Germany*

²*Institute of Animal Science, University of Bonn, 53115 Bonn, Germany*

mgerken@gwdg.de

Abstract

Llamas and alpacas have become popular as companion and farm animals in Europe and North America. However, scientific knowledge on the nutrient requirements for lactating and suckling llamas is limited. Most of the nutrient recommendations for New World Camelids have been derived from other livestock species, such as goats and sheep. Therefore we aimed to investigate the energy needs for llamas during lactation. We measured daily milk output in 5 llama dams at different stages of lactation (i.e. at week 3, 10, 18 and 26 *pp*) using an isotope dilution technique (IDT). The method involved the application of the stable hydrogen isotope Deuterium (²H) to the lactating dam. We also related estimated milk outputs to daily energy intakes. The IDT was validated by measuring total water turnover (TWT) directly and comparing it with values estimated by the IDT. Water intake and TWT decreased significantly with lactation stage, whether estimated by the isotope dilution technique or by calculation from drinking water and water ingested from feeds. The IDT estimated TWT with high accuracy with only small variations. Calculated ME intakes during lactation decreased with lactation stage but remained constant per kg milk output. However, lactation stage had no effect on the milk water fraction, i.e. the ratio between milk water and TWT. Although recommendations for energy requirements in lactating llamas so far have been based on extrapolations from sheep and goat data, the comparison with our measured data shows that these extrapolations seem to be fairly appropriate. However, our more detailed and measured data on ME intakes in lactating llamas could serve as a more accurate basis for further recommendations for the energy requirements in New World Camelids.

Key words: lactation, llama, energy expenditure, growing young

Resumen

Llamas y alpacas se han vuelto populares como animales mascotas y animales de granja en Europa y América del Norte. Sin embargo, el conocimiento científico sobre las necesidades de nutrientes para llamas lactantes y mamandas es limitada. La mayoría de las recomendaciones de nutrientes para los camélidos sudamericanos ha sido derivada de otras especies animales, como cabras y ovejas. Por lo tanto decidimos investigar las necesidades de energía para llamas durante la lactancia. Medimos la producción diaria de leche en 5 llama hembras en diferentes fases de la lactancia (en la semana 3, 10, 18 y 26 *pp*) utilizando una técnica de dilución isotópica (IDT). El método consistió en la aplicación del isótopo de hidrógeno estable deuterio (²H) en la hembra lactante. También se relaciono la estimación de los productos lácteos con el consumo diario de energía. El IDT fue validado mediante la medición directa del volumen total de agua (TWT) y se comparo con los valores estimados por el IDT. El consumo de agua y TWT disminuyeron significativamente con la duración de

la lactancia, ya sea estimado por la técnica de dilución isotópica o mediante el cálculo basado en el agua bebido y el agua de los alimentos ingeridos. IDT estima TWT con alta precisión con sólo pequeñas variaciones. El consumo de ME calculado durante la lactancia disminuye con la duración de la lactancia, pero se mantuvo constante por kg de leche. Sin embargo, la fase de la lactancia no tuvo efecto en la fracción de agua de la leche, es decir, la proporción entre agua de leche y TWT. Aunque las recomendaciones sobre las necesidades de energía en llamas hasta el momento han sido basadas en extrapolaciones a partir de ganado ovino y caprino, la comparación con nuestros datos medidos muestra que estas extrapolaciones parecen ser bastante apropiadas. Sin embargo, nuestra información más detallada y los datos medidos del consumo de ME en llamas lactantes podrían servir como una base más exacta para futuras recomendaciones sobre los requerimientos de energía en Camélidos Sudamericanos.

Palabras claves: lactación, llama, consumo energético, crecimiento

Introduction

In the last 20 yr llamas and alpacas, the two domesticated New World camelids (NWC) became increasingly popular as companion and farm animals in Australia, North America and Europe, i.e. outside their natural distribution range. However, very little scientific information is available on feeding recommendations in general and energy expenditure during lactation in particular compared to other livestock species. The resources transferred from a lactating mother to its suckling young during the course of a lactation period are energetically more costly than the entire prenatal costs for gestation (Cameron, 1998).

Therefore the objective of our study was to measure milk output in llamas using an isotope dilution technique and relate it to the energy intakes during lactation, thus being able to give some recommendations on the energy requirements for lactating llamas based on measured data not available so far. Furthermore we aimed to validate the dilution technique by measuring water turnover directly and compare it with values estimated by the isotope dilution technique.

Materials and methods

Animals and Management

For our study 5 female llamas (aged between 9 and 16 yr) and their suckling young (crias) were involved. All animals involved were bred in Germany and originated from the herd of the Experimental Station Relliehausen of Göttingen University. Animals were transferred 2 months prior to parturition for acclimatisation and were kept at the research facilities of the Department of Animal Sciences, University of Göttingen, Germany, for a lactation period of 27 wk under controlled stable conditions. All dams were pluriparous and gave birth to a single young in May/June 2009 (2 female and 3 male crias). During measurement weeks, each dam was kept with its cria individually in a room measuring 5.8 m by 3.2 m with permanent access to an outdoor pen allowing animals to see their conspecifics. In the stable, light schedule was kept constant (14 h light to 10 h dark). Dams were offered chopped hay from perennial ryegrass-dominated grassland for *ad libitum* intake and 1 kg of a commercial compound feed fed in two portions of 0.5 kg twice a day (0800 h and 1700 h). In order to measure feed and water intakes individually for the dams hay was offered in a hay rack positioned 1.5 m above ground to prevent crias from accessing it. During the milk output trials at wk 3, 10, 18 and 26

(see below) hay consumption was measured daily for one wk by weighing and re-weighing amounts of hay offered. Similarly water was available *ad libitum* in buckets positioned 1.5 m above ground and daily water consumption was determined during the milk output trial wk by weighing and re-weighing water buckets. Two hay samples were taken for each respective measurement wk. All samples were milled by 1 mm sieve in a cutting mill. Chemical analyses were done according to VDLUFA (2007). The ME was estimated using prediction equations for ruminants (GfE, 2009), including values from Hohenheim gas test (Menke et al., 1979).

Measurements

Milk output was measured in 5 dams by an isotope dilution technique (Coward et al., 1979; Haisma et al., 2003, Riek and Gerken, 2009). The method depends on measuring the transfer of ^2H labelled water from the mother to the young via milk. The 4 measurement periods were at wk 3, 10, 18 and 26 postpartum, lasting 7 d each. Averages were calculated for the midpoint of each study period i.e., 25, 73, 129 and 186 d PP. A detailed description of the technique and the calculations is given in Haisma et al. (2003) and Riek and Gerken (2009). In brief, a background blood sample was taken prior to the isotope administration from both dams and crias. The dams were then injected with a known amount of D_2O (99.90% purity, Euriso-top GmbH, Saarbrücken, Germany) determined by the weight of the animals, recorded with a scale to the nearest 0.5 kg. Body mass of dams and crias was also recorded at the end of each measurement period to determine weight changes. The mean (\pm SD) D_2O amounts administered to the dams per kg body weight were 0.201 ± 0.005 g, 0.200 ± 0.003 g, 0.198 ± 0.001 g and 0.199 ± 0.002 g, at wk 3, 10, 18 and 26 postpartum, respectively. The actual dose given was gravimetrically measured by weighing the syringes before and after the administration to the nearest 0.001 g. During the trials at the respective measurement wk blood samples (5 mL) were taken from both dams and crias every 1 to 2 d for determination of D_2O concentrations. Blood samples were centrifuged for plasma and kept at -20°C until the determination of the ^2H concentration. Analyses were carried out at the Competence Center of Stable Isotopes (KOSI, Göttingen University, Göttingen, Germany). Isotope ratios of ^2H were measured using an on-line high temperature reduction technique in a helium carrier gas described previously (Gehre et al., 2004) and expressed relative to the Vienna standard mean ocean water (VSMOW), which is the international reference standard for D_2O . Isotope equilibration concentration and fractional water turnover of the isotope was computed for each dam by extrapolating the regression of D_2O concentrations (C_t) on time: $C_t = C_0 \times e^{-k \times t}$, where C_0 is the equilibration concentration (intercept) for D_2O , k is the fractional water turnover (slope) and t is the time elapsed since tracer administration (Holleman et al., 1982). The dilution space for D_2O in the dam was calculated after Schoeller (1983) and Schoeller et al. (1986). The body mass of dams was constant over the measuring periods, therefore no corrections for changing pool size were necessary. Total water turnover (TWT) of the dams estimated by the D_2O dilution technique (TWT_d) was then calculated as the product of TBW and k . Total water turnover was also calculated from conventional measurements (TWT_m) as the sum of drinking water intakes (measured by re-weighing water buckets), the water ingested from feed stuff (i.e. hay and concentrate), metabolic water (i.e. water released from the oxidation of fat, protein and carbohydrates) and milk water (see below). For the calculation of metabolic water it was assumed that fat and protein from ingested feed stuff produce 1.07 and 0.42 g of water per gram of material oxidized (Van Es, 1969), respectively. To measure milk output of llama dams and thus milk intake in crias we fitted the measured D_2O concentrations in crias to the equation given in Haisma et al. (2003).

Daily ME intakes during the measurement periods were calculated from ingested daily amounts of hay, concentrate and their respective analysed ME contents. Daily ME required

for lactation was calculated from milk output data and the respective GE content of milk. It was assumed that the efficiency of utilisation of ME for lactation is 0.63 (NRC, 2007).

Milk samples from each dam for the corresponding measurement wk (i.e., wk 3, 10, 18 and 26 postpartum) were taken and analyzed for fat, protein, lactose and fat-free DM determined by infrared absorption using an infrared spectral-photometer adjusted for llama milk as described in detail previously (Riek and Gerken, 2006). Gross energy in milk (GE) was estimated using the equation (Perrin, 1958): $GE \text{ (MJ/100 g)} = 39.8 \times \text{fat (\%)} + 23.9 \times \text{protein (\%)} + 16.7 \times \text{lactose (\%)}$. DM concentration of milk was calculated by adding the fat concentration of the milk to the fat-free DM. The output of GE was calculated by combining the present milk output results with the respective milk GE concentration.

Statistical analyses

Statistical analyses were performed with the software package SAS version 9.2 (SAS, 2008; SAS Institute Inc., Cary NC). A nonlinear regression procedure (PROC NLIN) in SAS was used to fit data to equations. A two-way ANOVA was performed including the effects of lactation stage and animal on various parameters, using the General Linear Model procedure (PROC GLM), with animal as random effect, to account for repeated measures on the same animals, and lactation as fixed effect: $Y_{ijk} = \mu + LS_i + A_j + e_{ijk}$; where: Y_{ijk} = observation value; μ = overall mean; LS_i = fixed effect of the i_{th} lactation stage (i : 1 = 3, 2 = 10, 3 = 18, 4 = 26 wk); A_j = random effect of the animal; e_{ijk} = random error. An integrated multiple range test (Student-Newman-Keuls) was used to detect differences between means with a 5% significance level.

Results and discussion

Body weight, drinking water intake, TWT and DMI for the four measurement periods during lactation (i.e. wk 3, 10, 18 and 26) are presented in Table 1. Body mass of dams did not differ between the different lactation stages. Similarly, no differences could be detected for DMI. Contrarily, lactation stage had an effect ($P < 0.001$) on drinking water intake and TWT, whether estimated by the D₂O dilution technique or by calculation from ingested drinking water and water ingested from feeds (see Material and Methods for details). In both cases water turnover decreased significantly with increasing lactation stage. The ratios between TWT_d and TWT_m did not differ with lactation stage and were close to 100% at all measurement wk, indicating that the D₂O dilution technique estimated TWT with high accuracy with only small variations (Fig. 1). Similar results on TWT have been reported for sheep and goats (Al-Ramamneh et al., 2010). While water excreted via milk decreased with lactation ($P < 0.001$) the milk-water fraction did not change, indicating a fairly constant relationship between milk-water secretion and TWT during lactation. Total body water did not change with lactation stage, whether expressed as kg or as percentage of BW.

64 th EAAP Annual meeting, 25-30 August, 2013, Nantes, France
Symposium on South American Camelids and other Fibre Animals

Table 1. Summary of drinking water intakes, DMI, total body water (TBW), total water turnover (TWT) and the accuracy of measuring TWT by an isotope dilution technique (TWT_d) compared to the conventional measurement of TWT (TWT_m) in llamas (*Lama glama*) at different stages of lactation (numbers are means ± SE; n = 5)

Lactation stage (wk)	3	10	18	26
Parameter				
BW, kg	140.8 ± 10.2	139.2 ± 9.0	137.0 ± 7.7	137.6 ± 7.0
Water drunk ¹ , mL/(kg ^{0.75} x d)	216 ± 18 ^a	196 ± 13 ^b	184 ± 14 ^b	141 ± 13 ^c
DMI, g/(kg ^{0.75} x d)	71 ± 5	75 ± 7	68 ± 3	68 ± 5
Water drunk/DMI, L/(kg x d)	3.15 ± 0.41 ^a	2.66 ± 0.27 ^b	2.71 ± 0.20 ^b	2.15 ± 0.29 ^c
Milk-water output ² , L/d	2.52 ± 0.40 ^a	2.49 ± 0.26 ^a	1.67 ± 0.24 ^b	1.46 ± 0.21 ^b
TBW, kg	91.6 ± 6.5	96.8 ± 4.1	97.3 ± 5.3	93.9 ± 5.9
TBW, % of BW	65.3 ± 2.1	70.1 ± 2.7	71.2 ± 2.7	69.1 ± 1.2
TWT _d ³ , L/d	11.8 ± 0.9 ^a	10.8 ± 0.7 ^b	9.6 ± 0.8 ^c	7.6 ± 0.7 ^d
TWT _d ³ , mL/(kg ^{0.75} x d)	289 ± 17 ^a	267 ± 13 ^a	241 ± 16 ^b	191 ± 15 ^c
TWT _m ⁴ , L/d	11.8 ± 0.9 ^a	10.7 ± 0.7 ^b	9.5 ± 0.7 ^c	7.7 ± 0.8 ^d
Milk-water fraction ⁵ , %	21.3 ± 2.7	21.7 ± 2.0	18.3 ± 2.1	19.4 ± 2.4
TWT _m /TWT _d , %	99.9 ± 0.5	99.5 ± 1.5	98.7 ± 1.3	100.2 ± 1.2

^{a-c}Means within a row with different superscript differ ($P < 0.05$).

¹Measured by re-weighing water buckets.

²Measured by an isotope dilution technique (see text for details).

³TWT measured by an isotope dilution technique (including drinking water, preformed water from feed, metabolic water and milk water).

⁴TWT calculated by the sum of drinking water, measured by re-weighing water buckets preformed water from feed, metabolic water (calculated from feed composition data) and milk water.

⁵Relationship between milk-water output and TWT.

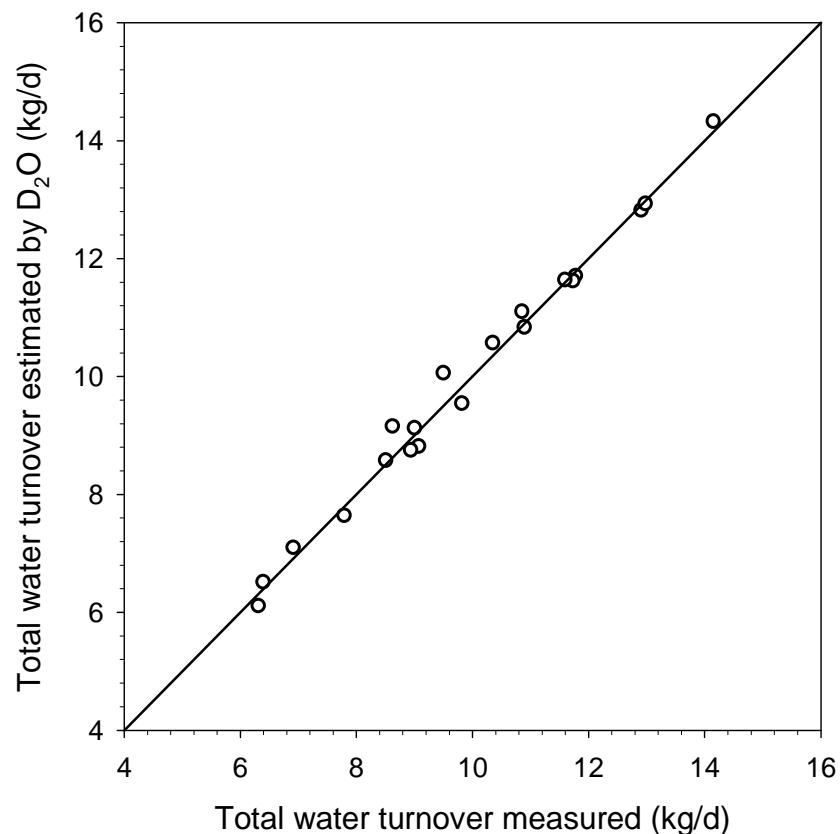


Figure 1. Relationship between measured (calculated as the sum of measured drinking water intakes, water ingested from feed stuff, metabolic water and milk water) and estimated (by the D₂O dilution technique) total water turnover in llama dams during lactation ($n = 20$). The straight line depicts the 100% line, i.e. total water turnover estimated by D₂O = total water turnover measured.

ME intakes, milk output and GE in milk for the four measurement periods during lactation are presented in Table 2. While milk output decreased ($P < 0.001$) with lactation stage, the GE content of milk remained relatively stable during the course of lactation, except for wk 3 and 10, where the GE content in milk differed ($P < 0.001$). Similar to DMI, ME intakes did not differ between different lactation stages, whether expressed as total amount ingested per day or on a metabolic body size basis (Table 2). However, calculating the required ME intakes for lactation from milk output data and GE content of milk, showed that with increasing lactation stage ME requirements per day for lactation decreased ($P < 0.001$) but remained constant per kg milk output (Table 2) which is in agreement with sheep and goat data (NRC, 2007). Furthermore, our results on milk output are comparable to earlier studies at similar stages of lactation and representative for the nutrient demands of suckling llamas (Riek et al., 2007).

Table 2. Summary of ME intakes, milk output and gross energy (GE) content of milk in llamas (*Lama glama*) at different stages of lactation (numbers are means \pm SE; n = 5)

Lactation stage (wk)	3	10	18	26
Parameter				
ME intake ¹ , MJ/d	26.3 \pm 2.4	27.5 \pm 2.6	25.1 \pm 1.7	24.8 \pm 2.2
ME intake ¹ , kJ/(kg ^{0.75} x d)	643 \pm 38	678 \pm 55	625 \pm 23	621 \pm 38
ME _{lac} ² , MJ/d	18.5 \pm 2.4 ^a	15.1 \pm 1.7 ^b	12.2 \pm 1.8 ^c	10.2 \pm 1.3 ^c
ME _{lac} ² , MJ/(kg milk x d)	6.9 \pm 0.4	6.1 \pm 0.2	6.7 \pm 0.3	6.5 \pm 0.2
Milk output ³ , kg/d	2.74 \pm 0.73 ^a	2.49 \pm 0.26 ^a	1.81 \pm 0.26 ^b	1.58 \pm 0.22 ^b
Milk output ³ , kJ/(kg ^{0.75} x d)	66 \pm 8 ^a	61 \pm 5 ^a	45 \pm 5 ^b	39 \pm 4 ^b
GE in milk, MJ/kg	4.34 \pm 0.24 ^a	3.82 \pm 0.11 ^b	4.24 \pm 0.17 ^{ab}	4.10 \pm 0.01 ^{ab}

^{a-c}Means within a row with different superscript differ ($P < 0.05$).

¹Sum of ME intakes from hay (8.2 MJ ME/kg DM) and concentrate (11.2 MJ ME/kg DM).

²Calculated assuming an efficiency of utilisation of ME for lactation of 0.63 (NRC, 2007).

³Measured by an isotope dilution technique (see text for details).

In our study lactating llamas ingested on average 643, 678, 652 and 621 kJ ME / (kg^{0.75} x d) (Table 2) at wk 3, 10, 18 and 26 postpartum, respectively. Calculating ME intakes for the same animals from recently published recommendations for energy requirements in llamas (NRC, 2007; Van Saun, 2009) resulted in slightly different values (i.e. 708, 685, 621 and 598 kJ ME / (kg^{0.75} x d) at wk 3, 10, 18 and 26 postpartum, respectively). Although these recommendations for energy requirements in lactating llamas are based on extrapolations from sheep and goat data, the comparison to our measured data shows that they seem to be fairly accurate. However, our more detailed and measured rather than extrapolated data from sheep and goats on ME intakes in lactating llamas could serve as a basis for further recommendations for the energy requirements in NWC.

Acknowledgement

We thank the German Research Foundation (DFG, Deutsche Forschungsgemeinschaft, Bonn, Germany) for financial support. We thank J. Langel for organizing the deuterium oxide analyzes. The technical assistance of A. Oppermann and K. Salzmann of the Experimental Station Relliehausen and J. Dörl and the technical staff of the Department of Animal Sciences of the University of Göttingen is highly appreciated.

References

- Al-Ramamneh, D., A. Riek, and M. Gerken. 2010. Deuterium oxide dilution accurately predicts water intake in sheep and goats. *Animal* 4: 1606-1612.
- Cameron, E. Z. 1998. Is suckling behaviour a useful predictor of milk intake? A review. *Anim. Behav.* 56: 521-532.
- Coward W. A., M. B. Sawyer, R. G. Whitehead, A. M. Prentice, and J. Evans. 1979. New method for measuring milk intakes in breast-fed babies. *Lancet* 2: 13-14.

64 th EAAP Annual meeting, 25-30 August, 2013, Nantes, France
Symposium on South American Camelids and other Fibre Animals

- Gehre, M., H. Geilmann, J. Richter, R. A. Werner, and W. A. Brand. 2004. Continuous flow 2H/1H and 18O/16O analysis of water samples with dual inlet precision. *Rapid Comm. Mass Spect.* 18: 2650-2660.
- Gesellschaft für Ernährungsphysiologie (2009): New equations for predicting metabolisable energy of compound feeds for cattle. *Proc. Soc. Nutr. Physiol.* 18: 143-146.
- Haisma, H., W. A. Coward, E. Albernaz, G. H. Visser, J. C. K. Wells, A. Wright, and C. G. Victoria. 2003. Breast milk and energy intake in exclusively, predominantly, and partially breast-fed infants. *Eur. J.Clin. Nutr.* 57: 1633-1642.
- Holleman, D.F., R. G. White, and J. R. Luick. 1982. Application of the isotopic water method for measuring total body water, body composition and body water turnover. Pages 9-32 in *Use of Tritiated Water in Studies of Production and Adaptation in Ruminants*. International Atomic Energy Agency, Nairobi, Kenya.
- Menke, K.H., L. Raab, A. Salewski, H. Steingass, D. Fritz, and W. Schneider. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro. *J. Agric. Sci.* 93: 217-222.
- National Research Council. 2007. *The Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids and New World Camelids*. Natl. Acad. Press, Washington, DC.
- Perrin, D. R. 1958. The calorific value of milk of different species. *J. Dairy Res.* 25: 215-220.
- Riek, A., and M. Gerken. 2006. Changes in llama (*Lama glama*) milk composition during lactation. *J. Dairy Sci.* 89: 3484-3493.
- Riek, A., M. Gerken, and E. Moors. 2007. Measurement of milk intake in suckling llamas (*Lama glama*) using deuterium oxide dilution. *J. Dairy Sci.* 90: 867-75.
- Riek, A., and M. Gerken. 2009. Milk intake studies in llamas (*Lama glama*) using the “dose-to-the-mother” technique. *Small Rum. Res.* 82: 105-111.
- SAS. 2008. User’s guide Release 9.02. SAS Inst., Cary, NC, USA.
- Schoeller, D. A. 1983. Energy-expenditure from doubly labeled water – some fundamental considerations in humans. *Am. J. Clin. Nutr.* 38: 999-1005.
- Schoeller, D. A., E. Ravussin, Y. Schutz, K. J. Acheson, P. Baertschi, and E. Jequier. 1986. Energy-expenditure from doubly labeled water – validation in humans and proposed calculation. *Am. J. Physiol.* 250: R823-R830.
- Van Es, A. J. H. 1969. Report to the subcommittee on constants and factors: constants and factors regarding metabolic water. Pages 513-514 in *Energy Metabolism of Farm Animals*. K. L. Blaxter, J. Kielanowski, and G. Thorbek, ed. Oriel Press, Newcastle-upon-Tyne, UK.
- Van Saun, R. J. 2009. Nutritional requirements and assessing nutritional status in camelids. *Vet. Clin. North Am. Food Anim. Pract.* 25: 265-279.
- Van Soest, P. J., and J. B. Robertson. 1985. *Analysis of Forages and Fibrous Foods. A Laboratory Manual for Animal Science* 613. Dept. Anim. Sci., Cornell Univ., Ithaca, NY, USA.
- VDLUFA, 2007. *Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten. Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch), Bd. III. Die Chemische Untersuchung von Futtermitteln*. VDLUFA-Verlag, Darmstadt, Germany.



Energy requirements during lactation in llamas

*Alexander Riek¹, Annegret Klinkert¹, Martina Gerken¹,
Jürgen Hummel^{1,2}, Eva Moors¹, and Karl-Heinz Südekum²*

¹Department of Animal Sciences, University of Göttingen, Germany

²Institute of Animal Science, University of Bonn, Germany

Introduction



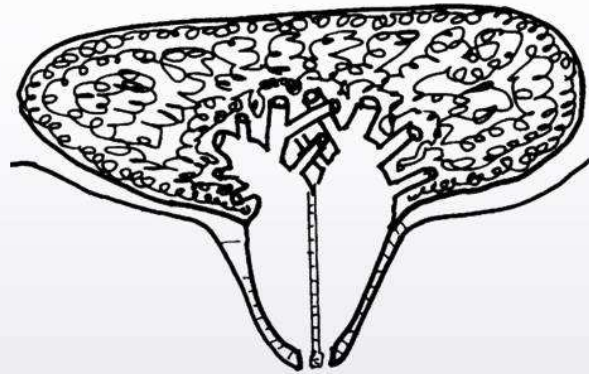
Limited scientific knowledge on feeding recommendations:

- in particular for lactating llamas
- based on extrapolations from sheep and goats (NRC, 2007; Van Saun, 2009)
- measurement of energy requirements during lactation in llamas



Lactation and udder morphology

- 6 - 9 months lactation
- 4 teats
- Teat length: 1 - 2 cm
- 8 Cisternes
- 2 streak canals/nipple



Llama udder (Fowler 1998. pp. 254)



Animals



- Animals: 5 lactating llamas and 5 crias
- Research station of the Department of Animal Sciences, Göttingen, Germany
 - Animals were transferred 2 months prior to parturition
 - 1 pen for dam and cria





Feeding

Feeding

- Hay and water *ad libitum*
- Dams: concentrate (1 kg/d; 16 % CP, 12 % CF, 10.2 MJ/kg)



6th European Symposium on South American Camelids and 2nd European Meeting on Fibre Animals

Nantes, France, August 2013

Measurements



- Milk output measured by an isotope dilution technique at week 3, 10, 18 and 26 pp
- Daily direct measurements of feed and water intakes of the dams at week 3, 10, 18 and 26 pp



6th European Symposium on South American Camelids and 2nd European Meeting on Fibre Animals

Nantes, France, August 2013



Isotope-dilution-technique

- Application (i.m.) of D₂O to the lactating dam
(Coward et al., 1979)
- Measurement of transfer of ²H labelled water from the mother to the young via milk:
 - analyses of blood samples from dams and crias
 - calculation of milk output and water turnover
- Technique so far mainly applied to humans
(Coward et al., 1979; Butte et al., 1988; Haisma et al., 2003)

Isotope-dilution-technique

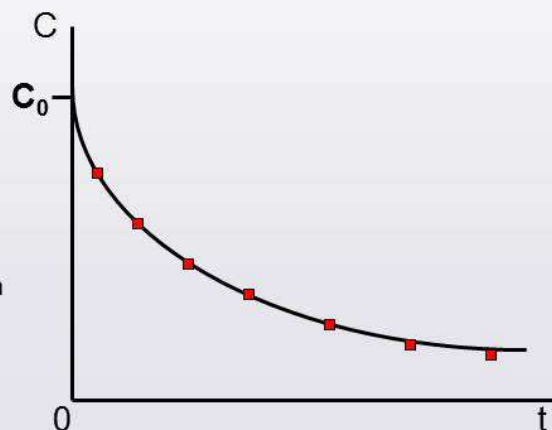


Isotope equilibration concentration and fractional water turnover of the isotope:

extrapolation of the regression of D₂O concentrations (C_t) on time: $C_t = C_0 \times e^{-k \times t}$

where:

- C₀ = equilibration concentration (intercept) for D₂O
- k = fractional water turnover (slope)
- t = time elapsed since tracer administration



Measurements



TWT_m = Total water turnover calculated from conventional measurements, sum of:

drinking water intakes + the water ingested from feed stuff + metabolic water (i.e. water released from the oxidation of fat, protein and carbohydrates) + milk water

Milk output of llama dams and thus milk intake in crias:

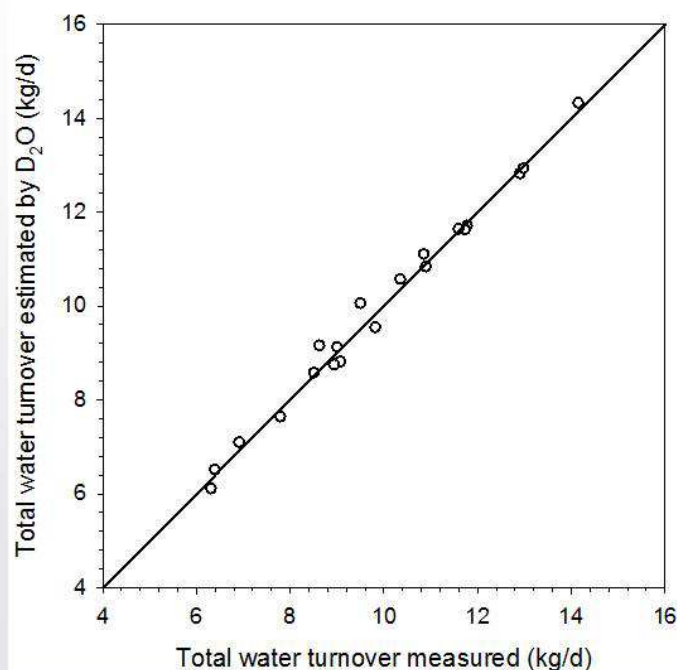
measured D_2O concentrations in crias were fitted to the equation given in Haisma et al. (2003)

Water turnover in llama dams (n = 5)



Time post partum (week)	Body mass (kg)	Water drunk (L · d ⁻¹)	Milk water output (L · d ⁻¹)	Total water turnover (L · d ⁻¹)	Total body water (% of BM)
3	140.8 ± 9.2	8.8 ± 0.7 ^a	2.52 ± 0.40 ^a	11.8 ± 0.9 ^a	65.3 ± 2.1
10	139.2 ± 9.0	7.9 ± 0.6 ^b	2.49 ± 0.26 ^a	10.8 ± 0.7 ^b	70.1 ± 2.7
18	137.0 ± 7.7	7.3 ± 0.6 ^b	1.67 ± 0.24 ^b	9.6 ± 0.8 ^c	71.2 ± 2.7
26	137.6 ± 7.0	5.6 ± 0.6 ^c	1.46 ± 0.21 ^b	7.6 ± 0.7 ^d	69.1 ± 1.2
Two-way analysis of variance					
Animal	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.01	<i>P</i> < 0.001	<i>n.s.</i>
Age	<i>n.s.</i>	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>n.s.</i>

Validating the D₂O dilution technique



6th European Symposium on South American Camelids and 2nd European Meeting on Fibre Animals

Nantes, France, August 2013

Energy turnover in llama dams (n = 5)



Time post partum (week)	ME intake [kJ/(kg ^{0.75} · d)]	ME for lactation		Milk output (kg · d ⁻¹)	GE in milk (MJ · kg ⁻¹)
		(MJ · d ⁻¹)	[MJ/(kg milk · d)]		
3	643 ± 38	18.5 ± 2.4 ^a	6.9 ± 0.4	2.74 ± 0.73 ^a	4.34 ± 0.24 ^a
10	678 ± 55	15.1 ± 1.7 ^b	6.1 ± 0.2	2.49 ± 0.26 ^a	3.82 ± 0.11 ^b
18	625 ± 23	12.2 ± 1.8 ^c	6.7 ± 0.3	1.81 ± 0.26 ^b	4.24 ± 0.17 ^{ab}
26	621 ± 38	10.2 ± 1.3 ^c	6.5 ± 0.2	1.58 ± 0.22 ^b	4.10 ± 0.01 ^{ab}

Two-way analysis of variance

Animal	<i>P</i> < 0.01	<i>P</i> < 0.001	<i>n.s.</i>	<i>P</i> < 0.001	<i>n.s.</i>
Age	<i>n.s.</i>	<i>P</i> < 0.001	<i>n.s.</i>	<i>P</i> < 0.001	<i>P</i> < 0.05

6th European Symposium on South American Camelids and 2nd European Meeting on Fibre Animals

Nantes, France, August 2013

Energy requirements for lactating dams



Week post partum		3	10	18	26
Parameter					
Body mass	(kg)	140.8 ± 9.2	139.2 ± 9.0	137.0 ± 7.7	137.6 ± 7.0
GE in milk	(MJ · kg ⁻¹)	4.34 ± 0.24	3.82 ± 0.11	4.24 ± 0.17	4.10 ± 0.01
Milk yield	(kg · d ⁻¹)	2.74 ± 0.73	2.49 ± 0.26	1.81 ± 0.26	1.58 ± 0.22
ME requirement					
For maintenance ¹	(MJ · d ⁻¹)	12.5 ± 0.2	12.4 ± 0.2	12.2 ± 0.3	12.3 ± 0.3
For milk production ²	(MJ · d ⁻¹)	18.5 ± 2.4	15.1 ± 1.7	12.2 ± 1.8	10.2 ± 1.3
Total calculated	(MJ · d ⁻¹)	31.0 ± 2.1	27.6 ± 1.0	24.4 ± 1.4	22.5 ± 1.8

¹ Calculated as 305 kJ ME/kg^{0.75} (Carmean et al. 1992)

² Calculated assuming the efficiency of utilisation of ME for lactation to be 0.63 (NRC 2007)

6th European Symposium on South American Camelids and 2nd European Meeting on Fibre Animals

Nantes, France, August 2013

Energy requirements for lactating dams



Week post partum		3	10	18	26
Parameter					
ME requirement					
For maintenance ¹	(MJ · d ⁻¹)	12.5 ± 0.2	12.4 ± 0.2	12.2 ± 0.3	12.3 ± 0.3
For milk production ²	(MJ · d ⁻¹)	18.5 ± 2.4	15.1 ± 1.7	12.2 ± 1.8	10.2 ± 1.3
Total calculated	(MJ · d ⁻¹)	31.0 ± 2.1	27.6 ± 1.0	24.4 ± 1.4	22.5 ± 1.8
Total measured	(MJ · d ⁻¹)	26.3 ± 2.4	27.5 ± 2.6	25.1 ± 1.7	24.8 ± 2.2
Discrepancy	(MJ · d ⁻¹)	4.7 ± 0.2	0.1 ± 0.0	-0.7 ± 0.0	-2.3 ± 0.1
	(%)	15.1 ± 1.2	0.4 ± 0.0	2.7 ± 0.1	10.2 ± 1.3

6th European Symposium on South American Camelids and 2nd European Meeting on Fibre Animals

Nantes, France, August 2013

Summary / Conclusion

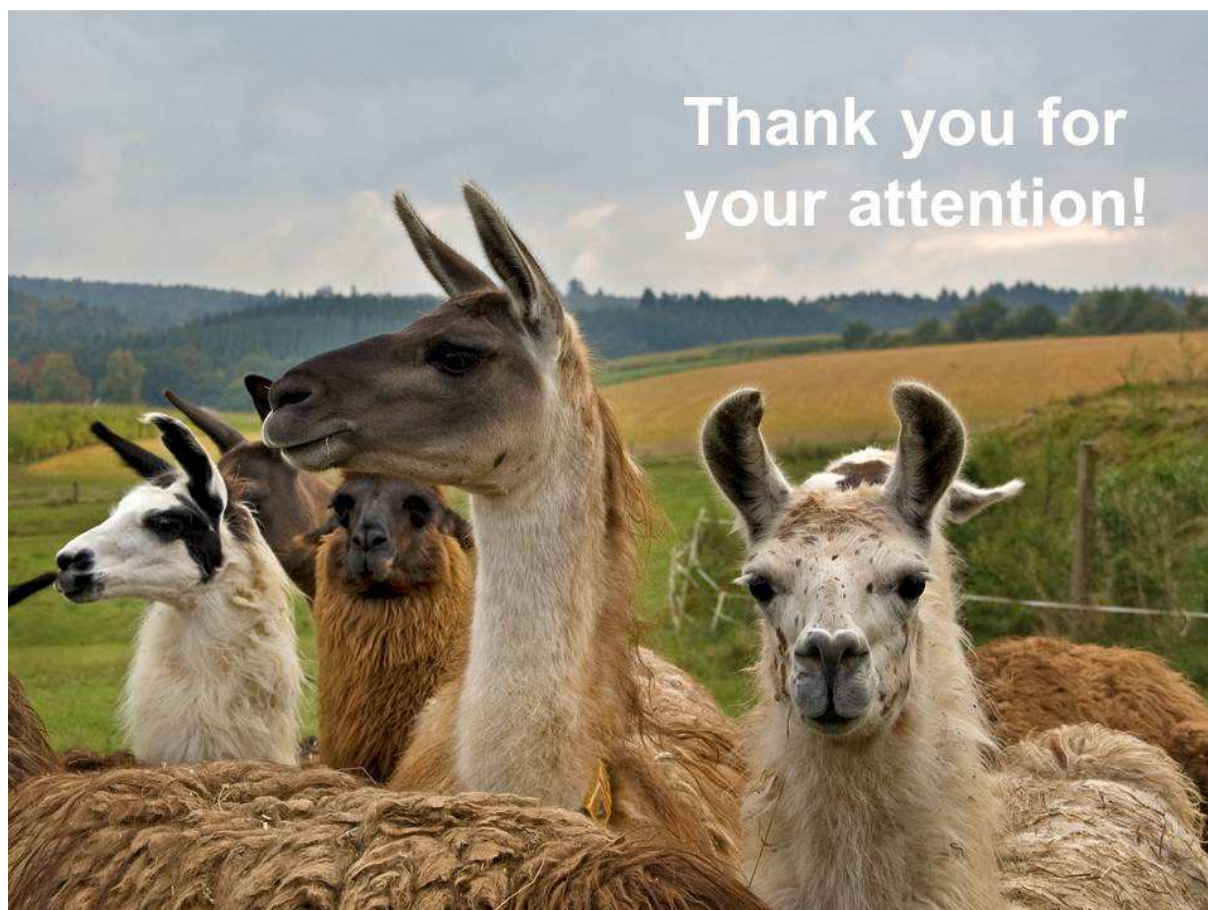


- Energy ingestion per kg milk did not change during different lactation stages
- Measured energy intakes during lactation for llamas are comparable to calculated recommendations derived from sheep and goat data
- Isotope dilution technique gives very accurate results

Acknowledgement

This project was supported by the DFG (German Research Foundation)





Daily ME intakes during the measurement periods:
ingested daily amounts of hay, concentrate and their respective analysed ME contents

Daily ME required for lactation:
calculated from milk output data and the respective GE content of milk (efficiency of utilisation of ME for lactation is 0.63 (NRC, 2007)

Milk samples:
analyzed for fat, protein, lactose and fat-free DM

Gross energy in milk (GE):
estimated using the equation (Perrin, 1958): $GE \text{ (MJ/100 g)} = 39.8 \times \text{fat (\%)} + 23.9 \times \text{protein (\%)} + 16.7 \times \text{lactose (\%)}$.

DM concentration of milk:
adding the fat concentration of the milk to the fat-free DM.

Output of GE:
combining the present milk output results with the respective milk GE concentration

Formula: Milk intake



Milk intake (MI)

$$MI \text{ (kg)} = \frac{WI + 1.07 \cdot F_d + 0.42 \cdot P_d}{(100 - \% DM) + (\% P \cdot 0.42) + (\% F \cdot 1.07) + (\% L \cdot 0.58)} \cdot 100$$

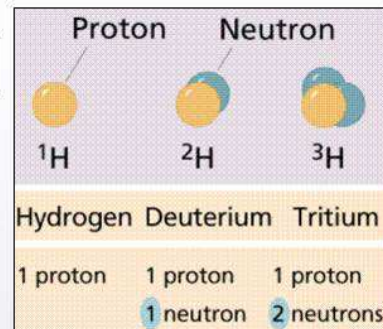
WI	= Water intake in kg
F _d	= Fat deposit in kg
P _d	= Protein deposit in kg
DM	= Dry matter in milk in %
P	= Protein in milk in %
F	= Fat in milk in %
L	= Lactose in milk in %

Deuterium oxide D₂O



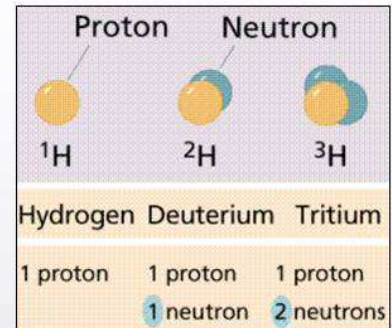
1 ml D₂O = 1.1067 g

- Heavy water
- Stable isotope
- Non toxic
- Uniformly distributed in TBW
- Cross body barriers at the same rate as body water
- Easy measurement in any body fluid



Source: Speakman, 1997; Holleman et al., 1982

**Isotopes are variants of a chemical element:
share same number of protons in each atom, but differ in neutron numbers**



Técnica nuclear: uso de isótopos estables

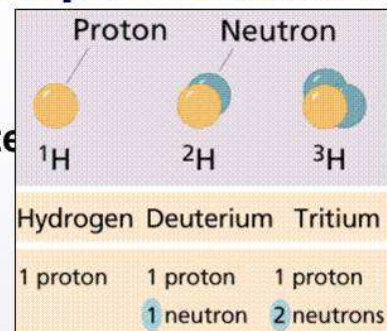
Isótopos estables pesados son especies atómicas menos abundantes en la naturaleza.

Deben sus propiedades a una mayor masa atómica

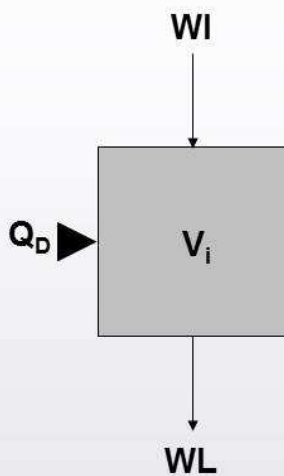
(un mayor número de neutrones en su núcleo).

Se pueden distinguir de la especie más abundante por su mayor masa con equipos de espectrometría de masas (IRMS).

***Ejemplo:* agua pesada o agua deutereada ($^2\text{H}_2\text{O}$) versus el agua común ($^1\text{H}_2\text{O}$)**



Measurement of milk intake: D₂O dilution



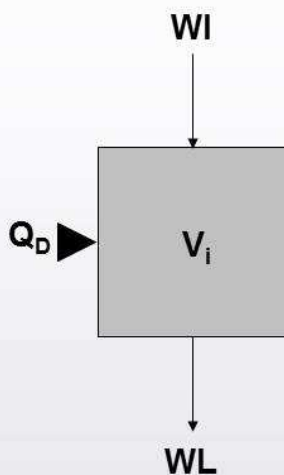
1. Derivation of C_0 :

- Continuous sampling over a short time period
- Least-square regression:

$$C_t = C_0 \times e^{-k \times t}$$

$$V_i = \frac{Q_D}{C_0}$$

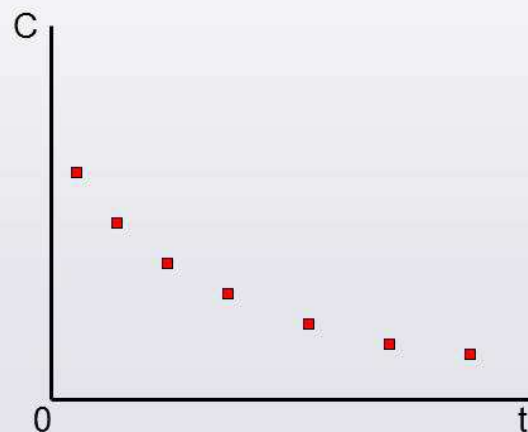
Measurement of milk intake: D₂O dilution



1. Derivation of C_0 :

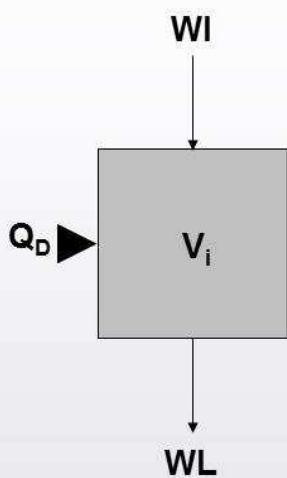
- Continuous sampling over a short time period
- Least-square regression:

$$C_t = C_0 \times e^{-k \times t}$$



$$V_i = \frac{Q_D}{C_0}$$

Measurement of milk intake: D₂O dilution

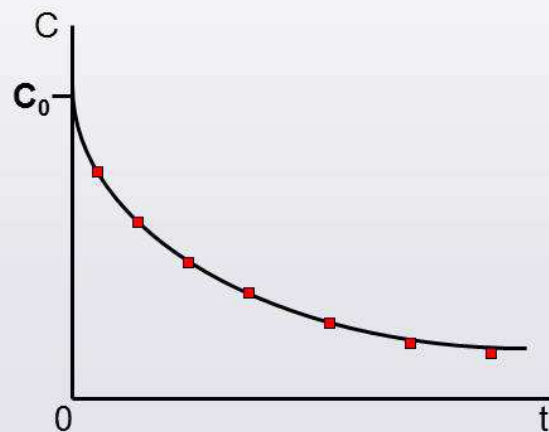


$$V_i = \frac{Q_D}{C_0}$$

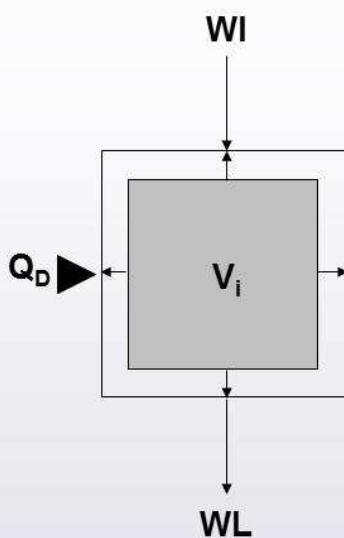
1. Derivation of C_0 :

- a) Continuous sampling over a short time period
- b) Least-square regression:

$$C_t = C_0 \times e^{-k \times t}$$



Measurement of milk intake: D₂O dilution



$$V_i = \frac{Q_D}{C_0}$$

1. Derivation of C_0 :

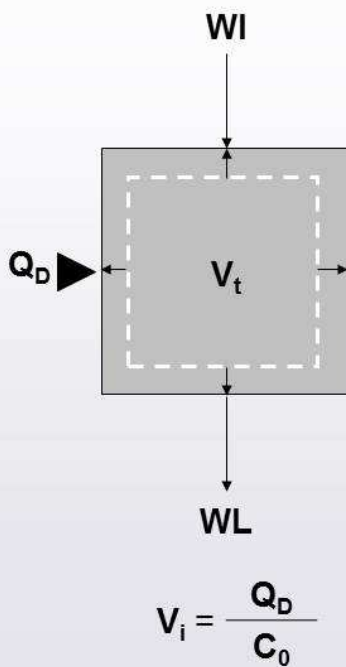
- a) Continuous sampling over a short time period
- b) Least-square regression:

$$C_t = C_0 \times e^{-k \times t}$$

2. Growth: V increases with body weight

$$K_{BW} = \frac{V_i}{BW_i}$$

Measurement of milk intake: D₂O dilution



1. Derivation of C₀:

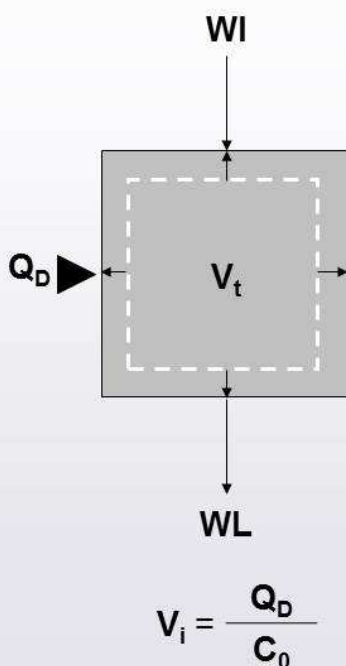
- Continuous sampling over a short time period
- Least-square regression:

$$C_t = C_0 \times e^{-k \times t}$$

2. Growth: V increases with body weight

$$K_{BW} = \frac{V_i}{BW_i} \quad \longrightarrow \quad V_t = BW_t \times K_{BW}$$

Measurement of milk intake: D₂O dilution



1. Derivation of C₀:

- Continuous sampling over a short time period
- Least-square regression:

$$C_t = C_0 \times e^{-k \times t}$$

2. Growth: V increases with body weight

$$K_{BW} = \frac{V_i}{BW_i} \quad \longrightarrow \quad V_t = BW_t \times K_{BW}$$

3. Corrected concentration:

$$C_t^* = C_t \times \frac{V_t}{V_i} \quad \longrightarrow \quad C_t^* = C_0^* \times e^{-k \times t}$$

4. Water intake (WI):

$$WI = WL + G = k^* \times V_{avg} + V_{\Delta}$$

Isotope equilibration concentration and fractional water turnover of the isotope:
extrapolation of the the regression of D_2O concentrations (C_t) on time: $C_t = C_0 \times e^{-k \times t}$
were:

C_0 = equilibration concentration (intercept) for D_2O ,

k = fractional water turnover (slope)

t = time elapsed since tracer administration

TWT_d = Total water turnover (TWT) of the dams estimated by the D_2O dilution technique, calculated as the product of TBW and k

TWT_m = Total water turnover calculated from conventional measurements, sum of: drinking water intakes + the water ingested from feed stuff + metabolic water (i.e. water released from the oxidation of fat, protein and carbohydrates) + milk water (see below). For the calculation of metabolic water it was assumed that fat and protein from ingested feed stuff produce 1.07 and 0.42 g of water per gram of material oxidized (Van Es, 1969), respectively. To measure milk output of llama dams and thus milk intake in crias we fitted the measured D_2O concentrations in crias to the equation given in Haisma et al. (2003).

Daily ME intakes during the measurement periods were calculated from ingested daily amounts of hay, concentrate and their respective analysed ME contents. Daily ME required for lactation was calculated from milk output data and the respective GE content of milk. It was assumed that the efficiency of utilisation of ME for lactation is 0.63 (NRC, 2007).

Milk samples from each dam for the corresponding measurement wk (i.e., wk 3, 10, 18 and 26 postpartum) were taken and analyzed for fat, protein, lactose and fat-free DM determined by infrared absorption using an infrared spectral-photometer adjusted for llama milk as described in detail previously (Riek and Gerken, 2006). Gross energy in milk (GE) was estimated using the equation (Perrin, 1958): $GE \text{ (MJ/100 g)} = 39.8 \times \text{fat (\%)} + 23.9 \times \text{protein (\%)} + 16.7 \times \text{lactose (\%)}$. DM concentration of milk was calculated by adding the fat concentration of the milk to the fat-free DM. The output of GE was calculated by combining the present milk output results with the respective milk GE concentration.