Animal 15 (2021) 100315

Contents lists available at ScienceDirect

Animal

The international journal of animal biosciences

Meta-analysis-based estimates of efficiency of calcium utilisation by ruminants

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A R T I C L E I N F O

Article history:
Received 16 October 2020
Revised 11 June 2021
Accepted 15 June 2021
Available online 23 July 2021

Keywords:
Cattle
Digestibility
Excretion
Requirement
Small ruminants

A B S T R A C T

The most abundant mineral in the body of animals is Ca, which has crucial importance for the regulation of various processes. The maintenance of Ca balance has become more challenging, especially in lactating ruminants, owing to the increased milk yields and thus Ca requirement. To determine the Ca requirement, factors such as Ca secretion via milk or Ca deposition in body tissues and conception products are summed up to the net Ca requirement. Nevertheless, dietary Ca cannot be completely utilised by the animal to cover the net Ca requirement, therefore a value for the efficiency of Ca utilisation is applied, which is the maximum proportion of Ca from the feed that the animal can use for covering the net requirement. However, current estimates for the efficiency of Ca utilisation are inconsistent. Therefore, the objective of the present meta-analysis was to estimate the efficiency of Ca utilisation for ruminants, considering the Ca supply of the animal. A data set of 223 observations was compiled from 37 studies, including data on cattle and small ruminants. Standardised Ca digestibility was calculated from data on Ca intake and faecal Ca excretion, corrected for faecal endogenous losses. Furthermore, a data subset on only lactating ruminants was created. For this subset, Ca excretion via faeces and urine and standardised Ca digestibility were related to the Ca supply of the animal. An exponential function was fitted to standardised digestibility data in response to Ca concentration in the diet and Ca supply, revealing that standardised Ca digestibility decreased with increasing dietary Ca concentration and Ca supply. The median for standardised Ca digestibility was 40%, with a remarkable variation between 9% and 88%. In response to Ca supply, faecal Ca excretion increased in a strong linear manner (slope = 0.76, R^2 = 0.96). Excretion of Ca via urine was very low even when Ca supply was very high. To conclude, Ca digestibility is a suitable indicator for the efficiency of Ca utilisation, since excessive Ca is almost completely excreted in faeces; however, Ca digestibility has to be determined at a Ca supply level below the requirement of the animal. To date, only very limited number of data have been reported for such supply conditions. Comparative studies using various Ca sources are suggested for future studies but should be conducted using a marginal Ca supply level.

Implications

A meta-analysis was performed in order to obtain an estimate for the efficiency of Ca utilisation in ruminants from literature data. In ruminants, Ca supplied in excess of the net requirement is mainly excreted in faeces, thus Ca digestibility is a suitable indicator for the efficiency of Ca utilisation. An exponential equation was obtained, with which the Ca digestibility can be estimated from Ca concentration in the diet. Comparative studies with various Ca sources are needed to obtain estimates for efficiency of Ca utilisation with respect to different Ca sources and these studies must be conducted with marginal Ca supply.

Introduction

The most abundant mineral in the body of animals is Ca. Approximately 99% of body Ca has structural functions as a constituent of bones and teeth, and only about 1% is found in tissues and extracellular fluids. Ca is crucial for the regulation of various processes in the body such as blood clotting, muscle contraction, cell signalling, membrane permeability, enzyme stabilisation and

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https://doi.org/10.1016/j.animal.2021.100315
1751-7311/

activation, and hormone release (Wilkens et al., 2020). Following its high physiological importance, Ca blood concentration is finely regulated by calciotropic hormones. Owing to the continuous increase of milk yield and growth performance of animals during the last decades, the Ca requirement of ruminant animals has increased and consequently, maintenance of Ca balance has become more challenging.

A common way of determining the Ca requirement of ruminants is the use of the factorial approach, which has been applied by committees such as the Gesellschaft für Ernährungsphysiologie (GfE) (2001), National Research Council (NRC) (2001), and Institut national de la recherche agronomique (INRA) (2018). In this approach, factors such as Ca secretion via milk or Ca deposition in body tissues and conception products are considered when calculating the 'net Ca requirement' of the animals.

Dietary Ca cannot be completely utilised by the animal to cover the net Ca requirement. Therefore, the 'gross Ca requirement' is calculated from the net Ca requirement by the application of a value for the 'efficiency of Ca utilisation'. For the purpose of requirement estimation, the efficiency of Ca utilisation is determined to characterise the potential of feeds, i.e. the maximum proportion of Ca from the feed that the animal can use for covering the net requirement. While factors such as Ca secretion via milk can be determined with high accuracy based on milk yield and Ca concentration in the milk, the efficiency of Ca utilisation is more complex in its determination because homeostatic regulation of animals is involved. This could explain why the estimates of efficiency of Ca utilisation are inconsistent among different committees. While the Agricultural Research Council (ARC) (1980) and the GfE (2001) apply constant values of 68 and 50%, respectively, recommendations of NRC (2001) and INRA (2018) differentiate between feedstuffs. The NRC (2001) suggests values of 30% for forages and 60% for concentrates. The recommendations of the INRA (2018) for the efficiency of Ca utilisation range between 20% for sugar beet pulp and 55% for concentrates, and differentiate between different forages with values ranging between 30 and 40% for legume or grass forages and cereal forages, respectively. When deriving those values, INRA (2018) only considered studies with a Ca supply of the animals that was lower than 150% of the Ca net requirement (Meschy and Corrias, 2005). The other abovementioned recommendations did not directly consider the Ca supply level in the underlying studies. However, it is important to consider the Ca supply of animals because when Ca supply exceeds the requirement, the potential for Ca utilisation will not be reached. Consequently, the efficiency of Ca utilisation may be underestimated when measured with animals that were supplied Ca in excess of their requirement.

The objective of the present study was to provide an updated estimate of the efficiency of Ca utilisation by ruminants and how it is affected by Ca supply of animals. Data on Ca intake (**CaIN**) and faecal Ca excretion (**CaFE**) were used to calculate Ca digestibility and evaluated together with urinary Ca excretion (**CaUE**) in relation to the Ca requirement of the animal and the Ca supply. It was hypothesised that Ca digestibility could be used as an estimate for the efficiency of Ca utilisation provided that Ca is supplied below the requirement of the animal.

Material and methods

Data collection and description of the data set

A data set was created using the literature databases, Scopus and CAB Abstracts and combinations of the keywords "calcium", "digestibility", "absorption", "minerals", "ruminants", and "cattle". Only peer-reviewed sources were included in the search. Included studies must have at least reported data on intake and faecal excretion of Ca. Studies that reported only calculated values that describe total tract Ca digestibility, but not CaFE, such as apparent Ca digestibility or Ca absorption coefficients, were not included in the data set. Data had to be measured in ruminating cattle, sheep, or goats disregarding sex, physiological stage, and performance level. In addition to CaIN and CaFE, data on CaUE, DM intake, diet composition, milk yield and Ca concentration of milk and diet, breed, and BW were recorded. A total of 41 studies reporting data on total tract Ca digestibility were initially identified. Three studies, representing 11 observations, were excluded from the meta-analysis because they only reported calculated values that describe total tract Ca digestibility, but not CaFE (Braithwaite, 1979; Liesegang and Risteli, 2005; Kronqvist et al., 2011). Furthermore, one publication (n = 49) using diets with very high Ca concentrations (up to 22 g/kg DM) that were not investigated in any of the other studies (maximal Ca concentration of the included studies was 12.7 g/kg DM) and reporting implausible Ca concentration in milk (>2 g/kg milk) was excluded (Hibbs and Conrad, 1983). In total, 43 experiments, published in 37 studies, were included in this meta-analysis, representing 223 observations. An overview of the distribution of different animal species, physiological stages, and descriptive statistics of the analysed traits is provided in Table 1 and full description of the data set including references is provided in Supplementary Table S1.

The majority of the experiments included in the data set were conducted with cattle (25 experiments, 129 observations) and 14 experiments investigated lactating cows, which represent about one-quarter of the total 223 observations (63 observations in lactating cows). Five experiments, representing 33 observations, investigated growing cattle (including steers and heifers). Pregnant cows and cattle fed on maintenance level (i.e. mature steers, cows and non-pregnant dry cows) were investigated by two and five experiments (representing five and 28 observations), respectively. Small ruminants were investigated in 18 experiments (94 observations) and the majority of those studies used growing animals (34 observations) or animals fed at maintenance level (22 observations; wether sheep and non-pregnant dry ewes). Five experiments were conducted with lactating small ruminants, representing 26 observations. In 36 experiments, data on CaUE were reported in addition to CaIN and CaFE, representing 169 observations.

Calculations

Data in this study were re-calculated and expressed as mg/day per kg BW, because of the marked differences in BW and DM intake of the different animal species and data on CaIN and Ca excretion. For some studies that investigated lactating Holstein cows, information on BW was not provided, thus, we assumed it to be 650 kg. Standardised Ca digestibility (%) was calculated as follows:

$$\text{Standardised Ca digestibility } (\%) = (\text{CaIN} - \text{CaFE} + \text{endogenous faecal Ca loss})/\text{CaIN} \times 100, \quad (1)$$

where endogenous faecal Ca loss was assumed to be 1 g Ca per kg DM intake according to the estimate for inevitable Ca losses suggested by GfE (2001); CaIN and CaFE were expressed as g/day.

A data subset was created using only data of lactating animals. For these animals, Ca net requirement was estimated to be the sum of Ca in milk and inevitable Ca loss; the latter was assumed to be 1 g Ca/kg DM intake (GfE, 2001). In some studies, milk yield but not Ca concentration in milk was reported, thus, 1.25 g Ca/kg milk was assumed for it, which was calculated as the mean of values obtained from literature (Cerbulis and Farrell, 1976; van Hulzen

Table 1
Descriptive statistics of the data set used for the meta-analysis in ruminants.[1,2]

	Number of[3]							
	O	S	E	Mean	SD	Median	Min[4]	Max[4]
Cattle	129	23	25					
Lactating	63	13	14					
Growing	33	5	5					
Pregnant	5	2	2					
Maintenance[5]	28	5	5					
Small ruminants	94	14	18					
Lactating	26	4	5					
Growing	34	5	8					
Pregnant	12	2	3					
Maintenance[5]	22	7	8					
Ca concentration (g/kg DM)	223	37	43	6.19	2.51	6.30	1.20	12.7
Ca intake (CaIN)[6]	223	37	43	161	95.0	146	24.0	461
Faecal Ca excretion (CaFE)[6]	223	37	43	125	73.6	110	21.0	334
Urinary Ca excretion (CaUE)[6]	169	31	36	3.91	5.19	1.94	0.17	28.5
Standardised Ca digestibility (%)[7]	223	37	43	40.4	15.6	40.2	9.12	88.0

[1] Publications used to build the data set are listed in the Supplementary Table S1.
[2] Values presented in this table are the raw data (not corrected for the experimental effect).
[3] Number of observations (O), studies (S) and experiments (E).
[4] Minimum and maximum values.
[5] Includes mature steers, cows and wethers, as well as non-pregnant dry cows and ewes.
[6] CaIN, CaFE and CaUE in mg/day per kg BW.
[7] Standardised Ca digestibility was calculated as (CaIN − CaFE + endogenous faecal Ca loss (assumed 1 g per kg DM intake)/ CaIN × 100; the term standardised digestibility is explained in the text in detail.

et al., 2009; Bijl et al., 2013; Buitenhuis et al., 2015; Bonfatti et al., 2017; Denholm et al., 2019; Stocco et al., 2019). Studies which used lactating animals but did not report Ca secretion in milk or milk yield were not included in the data subset of lactating animals. Ca retention in the body of lactating animals could not be estimated and was assumed to be zero.

In an attempt to evaluate data in relation to the requirement of animals, 'Ca oversupply' (**CaOS**) was estimated. For the purpose of this study, CaOS was defined as follows:

$$\mathrm{CaOS\ (g/d) = Ca\ intake\ (g/d) - Ca\ net\ requirement\ (g/d)} \quad (2)$$

where Ca net requirement was the sum of Ca in milk and inevitable Ca losses as defined above. However, this definition implies the hypothetical situation that Ca from the feed could be completely used to cover the net requirement of the animal, that is, the theoretical efficiency of Ca utilisation was assumed as 1.0. The CaOS ratio was then calculated as an estimate of Ca supply in relation to Ca net requirement:

$$\mathrm{CaOS\ ratio = CaOS\ (g/d)/Ca\ net\ requirement\ (g/d)} \quad (3)$$

Accordingly, a CaOS ratio of "0" indicates that calculated Ca net requirement was equal to Ca intake; negative values indicate that Ca supply was below the net requirement, while positive values indicate that Ca intake was above the Ca net requirement. A CaOS ratio of "1" means that actual CaIN was twice as high as the net requirement.

Estimation of Ca requirement in non-lactating animals (e.g. growing and pregnant animals) appeared not to be reliable because data on Ca deposition in body tissues and conception products were rarely reported in the included studies. Hence, CaOS and CaOS ratio were calculated only for the data subset of lactating animals.

Statistical analysis

Effects of CaIN and CaOS as independent variables (X) on CaFE and CaUE as dependent variables (Y) were evaluated with linear regressions using the MIXED procedure of the SAS software package for Windows (version 9.4, SAS Institute, Cary, NC, USA). The intercept of each experiment was defined as a random effect, as suggested by St-Pierre (2001). The underlying model was as follows:

$$Y_{ij} = B_0 + a_i + B_1X_{ij} + e_{ij}, \quad (4)$$

where Y_{ij} and X_{ij} are the dependent variable and the continuous predictor variable (independent variable), respectively. B_0 is the overall intercept (inter-study intercept), B_1 is the overall regression coefficient of Y on X as fixed effects, a_i is the individual intercept of the experiment i as a random effect and e_{ij} is the residual error.

Observations were weighted by number of replicates, as suggested by Mikolajewicz and Komarova (2019) for cases when weighting by sample variance was impossible. Weighting by sample variance was impossible in the present meta-analysis because most variables in this meta-analysis were computed from variables stated in the publications. Thus, the studied variables were on a different scale than sample variance stated in the publications, making sample variances for the variables we used unknown for most observations. Eq. (4) was expanded with discrete factor variables as follows:

$$Y_{ij} = B_0 + a_i + \tau_j + B_1X_{ij} + B_jX_{ij} + e_{ij}, \quad (5)$$

where τ_j is the fixed effect of the j^{th} level of the discrete factor τ and B_j is the effect of the j^{th} level of τ on the regression coefficient as fixed effect. The ruminant type (cattle or small ruminants) or the physiological stage of the animals (lactation, growth, pregnancy or maintenance) was assumed for the factor τ (separate models). It was not possible to consider the effect of physiological stage within ruminant type because of insufficient data for some factor combinations.

An exponential model was found to be most suitable when the standardised Ca digestibility (Y) was described in dependence of the CaOS ratio and the Ca concentration in the diet (X). The NLMIXED procedure of SAS was used to calculate these regressions using the following model:

$$Y_{ij} = a_i + (C_0 - P) \times e^{(-K \times X_{ij})} + P + e_{ij,} \quad (6)$$

where C_0 is the overall intercept (inter-study intercept) of the exponential regression, P is the response of Y at an infinite value of X

(plateau) and K is the rate constant of the regression as fixed effects. Dummy variables were used to determine different parameter estimates for C_0, P, and K in Eq. (6) for the discrete factor variables τ described for Eq. (5). The ESTIMATE statement of the MIXED and NLMIXED procedures was used to compare parameter estimates between classes of qualitative traits for statistical significance.

We additionally attempted to investigate the effects of other dietary factors besides the Ca concentration in the diet. However, factors such as the dietary cation–anion difference and the Ca concentration of feed ingredients were rarely reported in the studies and an estimation based on table values appeared not feasible. Other dietary factors, such as supplementation of inorganic Ca and main concentrate or forage type, were very heterogenic in the data set. As the number of observations per dietary factor was very restricted, a differentiation between dietary and experimental effects was not possible. In the lactating animals, impact of feed intake and days in milk on Ca excretion and Ca digestibility were evaluated in addition to the dietary factors, but appeared to be irrelevant in the current data set and were thus not considered in the model.

Goodness-of-fit of the models was evaluated following Rosenfelder-Kuon et al. (2020) based on the descriptions of Létourneau-Montminy et al. (2010) using the R^2 and RMSE between the observed and experiment-adjusted Y values as well as the residual slope plotting residuals against predicted values. Goodness-of fit criteria were computed using the REG procedure of SAS. Figures were created using GraphPad Prism (version 5 for Windows, GraphPad Software Inc., San Diego, CA, USA).

Results and discussion

The Ca concentration of the diets in this data set ranged between 1 and 13 g/kg DM, but the majority of the experiments applied Ca concentrations between 4 and 8 g/kg DM (25th and 75th percentile). CaIN ranged from 24 to 461 mg/day per kg BW and the median was 146 mg/day per kg BW. The medians of CaFE and CaUE were 110 and 1.94 mg/day per kg BW and ranged from 21 to 334 and 0.17 to 29 mg/day per kg BW, respectively (Table 1). While CaFE increased in a linear manner with increasing Ca intake (slope = 0.72, SE = 0.015, R^2 = 0.98), CaUE consistently was on a very low level of about 4 mg/d per kg BW, corresponding to a mean CaUE of approximately 1 g/d, even when CaIN was very high (Table 2, Fig. 1). This is in accordance with Wilkens et al. (2020), who reported that urinary excretion generally ranges between 0.5 and 2 g/d in ruminants. Accordingly, Ca is considered to be almost entirely excreted via faeces but barely in urine. This supports the hypothesis that the determination of efficiency of Ca utilisation can be based on faecal Ca excretion alone.

In lactating animals, CaFE increased in response to CaOS in a strong linear manner. The slope was 0.76 (SE = 0.033, R^2 = 0.96) and showed that CaIN above the net requirement is excreted in faeces to a large extent (Table 2). Nevertheless, we expected the slope of this regression to be close to 1, meaning that all CaIN exceeding the Ca requirement is excreted in faeces. The discrepancy between this assumption and the results indicates that Ca requirement was underestimated. For calculation of the Ca net requirement, we assumed Ca retention in the body of lactating animals to be zero. However, losses of skeletal minerals during lactation include 25–35% decreases in skeletal ash weight and ash Ca content in mammals (Kovacs, 2017). This mobilisation of Ca primarily occurs in the first weeks of lactation and skeletal Ca reserves are replenished during the later phases of lactation (Braithwaite, 1976). As only few of the included data were obtained in animals during the first weeks of lactation, it is likely that most data were obtained after the stages of bone Ca mobilisation and the animals retained some Ca during later lactation to compensate for Ca mobilisation from skeleton in early lactation. This Ca retention can be understood as an additional requirement during lactation, but is impossible to predict and therefore could not be considered in our calculations. This explanation is also supported by a slightly lower slope of the regression, between CaIN and CaFE, for lactating animals (slope = 0.72, SE = 0.02) than animals in other physiological stages (growth, maintenance, pregnancy; slopes between 0.78 and 0.80), indicating a lower faecal Ca excretion in lactating animals (Supplementary Table S2).

As for the entire data set, CaUE remained at a negligible level in lactating animals (intercept = 3.5 mg/d per kg BW; Table 2). This level remained close to constant (slope = −0.001) even when Ca supply largely exceeded the Ca net requirement. The small but significant slope might partly evolve from a systematic bias at high CaIN because the slope of the residual function was significant. A possible explanation for this relationship is very few higher CaUE values between 5 and 20 mg/d per kg BW, which were apparently not related to high CaIN or CaOS (Figs. 1b and 2b). These values were mostly associated with a low dietary cation–anion difference (Oehlschlaeger et al., 2014; Gaignon et al., 2019). This is consistent with other studies that reported elevated urinary Ca excretion when anion-rich diets were provided (Schonewille et al., 1994b; Grünberg et al., 2011; Wilkens et al., 2012; Goff et al., 2014). Schonewille et al. (1994a) stated that urinary excretion of Ca depends on dietary cation–anion difference rather than Ca intake.

Table 2
Parameter estimates (EST) and goodness-of-fit criteria for the linear model (Eq. (4)) fitted to faecal (CaFE) and urinary (CaUE) Ca excretion of ruminants as dependent variables (Y; mg/d per kg BW) and using Ca intake (CaIN) or Ca oversupply (CaOS) as the independent variables (X; mg/d per kg BW).

			Intercept			Slope				
X[1]	Y	n[2]	EST	SE	P[3]	EST	SE	P[3]	R^2	RMSE
CaIN										
	CaFE[4]	223	9.92	3.83	0.013	0.72	0.015	<0.001	0.98	10.5
	CaUE[4]	169	4.05	1.03	<0.001	0.003	0.005	0.533	0.64	3.15
CaOS										
	CaFE[5]	89	58.6	7.43	<0.001	0.76	0.033	<0.001	0.96	15.1
	CaUE[5]	67	3.49	0.98	0.003	−0.001	0.005	0.809	0.66	1.85

[1] Estimation of Ca net requirement and CaOS was only possible for lactating animals. Therefore, regressions included the complete data set and the data subset only with lactating animals for CaIN and CaOS as independent variables, respectively.
[2] Number of observations.
[3] Indicates whether a parameter estimate is significantly different from zero.
[4] Residual function (residuals (w) against predictions (z)) for CaFE w = 0.4 (SE = 1.4; P = 0.753) + 0.0038 (SE = 0.0097; P = 0.693) × z and for CaUE w = −0.8 (SE = 0.4; P = 0.034) + 0.20 (SE = 0.07; P = 0.005) × z.
[5] Residual function (residuals (w) against predictions (z)) for CaFE w = −1.5 (SE = 4.1; P = 0.722) + 0.0063 (SE = 0.0220; P = 0.774) × z and for CaUE w = −0.5 (SE = 0.4; P = 0.166) + 0.20 (SE = 0.10; P = 0.010) × z.

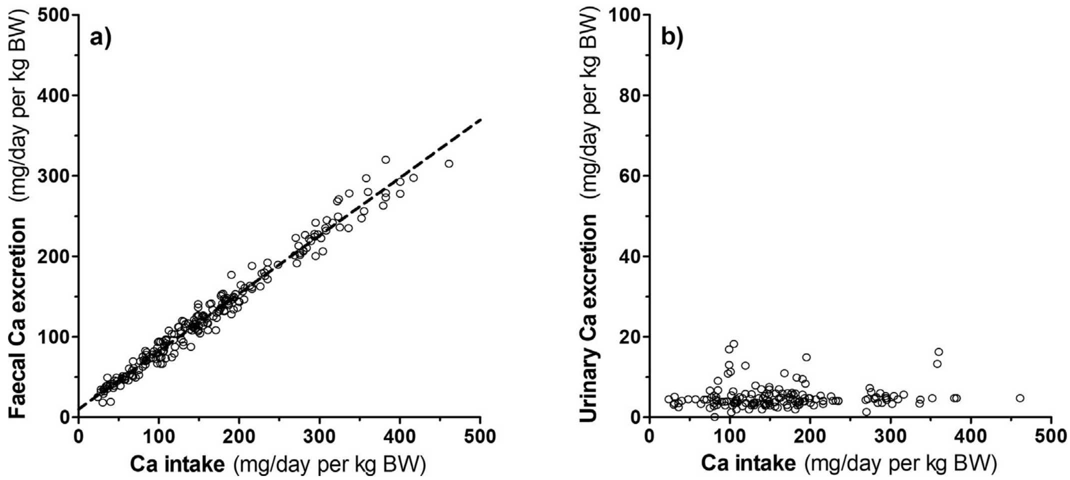

Fig. 1. Relationship between Ca intake and (a) faecal (n = 223) or (b) urinary (n = 169) Ca excretion (mg/day per kg BW) in ruminants. The dashed line and symbols represent estimated regression line and estimated excretion corrected for the experimental effect, respectively (according to Eq. (4) based on parameter estimates shown in Table 2). Note the different scaling of the y-axes.

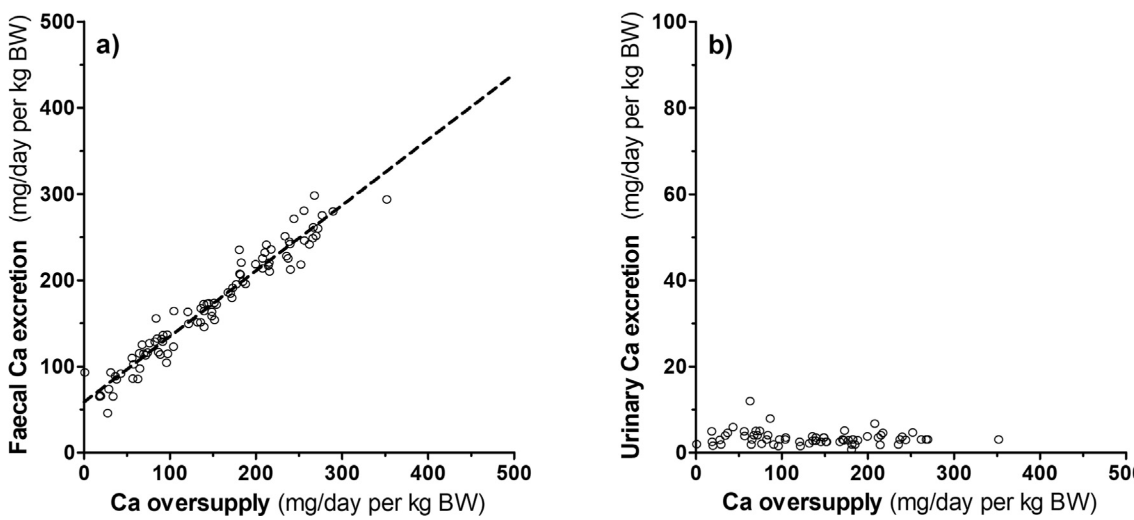

Fig. 2. Relationship between Ca oversupply and (a) faecal (n = 89) or (b) urinary (n = 67) Ca excretion (mg/day per kg BW) in lactating ruminants. The dashed line and symbols represent estimated regression line and estimated excretion corrected for the experimental effect, respectively (according to Eq. (4) based on parameter estimates shown in Table 2). Note the different scaling of the y-axes. Ca oversupply was calculated as difference between Ca intake and Ca net requirement (sum of Ca in milk and inevitable Ca loss), assuming a theoretical efficiency of Ca utilisation of 1.0.

Nevertheless, even in studies which reported elevated excretion of Ca in the urine, the amounts were low, indicating that the intermediary utilisation of Ca absorbed from the gut is almost complete.

The median of standardised Ca digestibility was 40% but varied remarkably between 9 and 88% (Table 1). With an increasing Ca concentration in the diet, standardised Ca digestibility decreased and levelled off at 34% (SE = 2.7, P < 0.001; Table 3). In accordance with this, van Leeuwen and de Visser (1976) previously observed an inverse relationship, in balance studies with Holstein cows, between Ca concentration in the diet and total tract Ca

Table 3
Parameter estimates (EST) and goodness-of-fit criteria for the exponential model (Eq. (6)) fitted to standardised Ca digestibility of ruminants as the dependent variable (Y; %) and using Ca concentration in the diet (g/kg DM) or Ca oversupply (CaOS) ratio or as the independent variables (X).

		Intercept			Rate constant			Plateau				
X[1,2]	n[3]	EST	SE	P[4]	EST	SE	P[4]	EST	SE	P[4]	R^2	RMSE
Ca concentration	223	97.8	18.3	<0.001	0.47	0.13	<0.001	33.7	2.67	<0.001	0.75	7.83
CaOS ratio	89	57.2	6.02	<0.001	0.91	0.39	0.032	38.4	3.93	<0.001	0.80	6.48

[1] Estimation of Ca net requirement and CaOS ratio was only possible for lactating animals. Therefore, regressions included the complete data set and the data subset only with lactating animals for Ca concentration and CaOS ratio as independent variables, respectively.
[2] Residual function (residuals (w) against predictions (z)) for Ca concentration w = −3.2 (SE = 1.8; P = 0.075) − 0.08 (SE = 0.04; P = 0.063) × z and for CaOS ratio w = −2.7 (SE = 2.6; P = 0.294) + 0.06 (SE = 0.06; P = 0.276) × z.
[3] Number of included observations.
[4] Indicates whether a parameter estimate is significantly different from zero.

digestibility, expressed as absorption coefficient. In diets containing between 1.9 and 2.5 g Ca/kg DM, these authors observed mean Ca absorption coefficients ranging from 63 to 73%; however, it decreased to 34% when Ca concentration of the diet was increased to 4.4 g/kg DM.

The increase in CaFE at increasing CaIN was higher in small ruminants (slope = 0.77, SE = 0.02) than in cattle (slope = 0.69, SE = 0.02). No further significant differences of Ca excretion in response to CaIN and CaOS were observed between cattle and small ruminants (Table 4). The slightly higher slope of CaFE in response to CaIN in small ruminants than in cattle indicates a lower Ca digestibility for this ruminant type. This is consistent with the significantly lower plateau of standardised Ca digestibility in response to Ca concentration in the diet of small ruminants (26%) than of cattle (39%) (Supplementary Fig. S1). Nevertheless, concerning the distribution of the physiological stages, the data set was unbalanced with, for instance, more lactating cattle than lactating small ruminants (Table 1). We attempted to evaluate the aforementioned relationships differentiating between the physiological stages within each ruminant type. However, this was impossible because of lacking data for some combinations of physiological stage and ruminant type. Therefore, the effect of physiological stage on Ca excretion in response to CaIN was evaluated separately from the ruminant type (Supplementary Table S2). The slightly lower slope of the lactating animals compared to the other physiological stages indicates higher Ca digestibility in lactating ruminants. Because of the higher percentage of lactating cattle than small ruminants, higher digestibility in cattle might rather be an effect of the physiological stage of the animals than of the ruminant type or vice versa. However, the unbalanced data distribution does not allow to clarify this issue and the respective results have to be viewed cautiously. Nevertheless, the principal relationships were consistent for all categories so that it is appropriate to consider these categories together for further considerations.

Standardised Ca digestibility in response to the CaOS ratio showed a very similar relationship as observed for the Ca concentration in the diet. Estimated maximal standardised Ca digestibility at an oversupply ratio of zero (i.e. the intercept of the regression) was 57% (SE = 6.0, $P < 0.001$) and levelled off at 38% (SE = 3.9, $P < 0.001$, $R^2 = 0.80$, Table 3). With regard to Ca absorption, the importance of Ca supply in relation to the requirement was highlighted by van den Top (2005) who found that Ca absorption was reduced with increasing Ca supply. Thus, when Ca supply exceeds the requirement, the animal absorbs Ca below its absorption capacity from the digestive tract, which is related to the regulation loop involving hydroxylation of 25-hydroxyvitamin D_3 into 1,25-dihydroxyvitamin D_3. This regulation is important to consider when digestibility or utilisation efficiency of different feedstuffs and Ca sources are studied. If the animal is in a supply status that does not provoke maximal absorption, i.e. the supply exceeds the requirement of the animal, the potential for Ca utilisation is underestimated. Therefore, Ca supply has to be maintained below the Ca requirement to obtain reliable estimates for the efficiency of Ca utilisation of different Ca sources, although this approach might be limiting for feeds with intrinsically high Ca concentrations (e.g. lucerne, sugar beet pulp). Accordingly, in order to obtain estimates for the efficiency of Ca utilisation from the current data set, observations were restricted to those obtained below the Ca gross requirement. For this choice, the efficiency of Ca utilisation was assumed 57%, corresponding to the maximal standardised Ca digestibility at marginal Ca supply. Under the assumption of Ca utilisation of 57%, the Ca gross requirement is met when the 1.75^{th}-fold Ca net requirement (1 divided by 0.57) is supplied, which reflects a threshold for the CaOS ratio of 0.75 (1.75–1). Based on this assumption, only 14 of the included observations were below the Ca gross requirement (Fig. 3). The median value of the standardised Ca digestibility for these data corrected for experimental effects was 50%, with values ranging between 39 and 66%. This shows that efficiency of Ca utilisation can be considerably higher under marginal Ca supply than the aforementioned plateau of standardised Ca digestibility of 38%, confirming that the values for standardised digestibility do not reflect the maximal potential for Ca utilisation when Ca intake exceeds the Ca requirement.

Differentiation of the efficiency of Ca utilisation among specific Ca sources or types of feedstuffs, as suggested by NRC (2001) and INRA (2018), would allow a more precise evaluation of Ca supply of animals. Nevertheless, because only 14 of the included observations were below the Ca gross requirement, differentiation of the efficiency of Ca utilisation between different types of feedstuffs does not seem feasible as the current database is too limited. This would require more comparative studies conducted with deficient Ca supply and various Ca sources. We therefore suggest using a constant value of 50% for Ca utilisation when Ca requirements are predicted. This suggestion is based on the estimated maximal standardised Ca digestibility of 57% at marginal Ca supply, i.e. the mean potential for Ca utilisation, considering the variation of

Table 4
Parameter estimates (EST) and goodness-of-fit criteria for the linear model (Eq. (5)) fitted to faecal (CaFE) and urinary (CaUE) Ca excretion as dependent variables (*Y*; mg/d per kg BW) and using Ca intake (CaIN) or Ca oversupply (CaOS) as the independent variables (*X;* mg/d per kg BW) differentiated by the ruminant type.

			Cattle						Small ruminants							
			Intercept			Slope			Intercept			Slope				
X[1]	*Y*	*n*[2]	EST	SE	*P*[3]	EST	SE	*P*[3]	EST	SE	*P*[3]	EST	SE	*P*[3]	R^2	RMSE
CaIN																
	CaFE[4]	223	6.33	4.48	0.159	0.69[b]	0.019	<0.001	12.9	5.56	0.022	0.77[a]	0.024	<0.001	0.98	10.3
	CaUE[4]	169	3.59	1.35	0.009	−0.001	0.006	0.857	4.54	1.57	0.005	0.010	0.008	0.205	0.64	2.13
CaOS																
	CaFE[5]	89	54.2	8.22	<0.001	0.76	0.036	<0.001	74.8	18.0	<0.001	0.75	0.083	<0.001	0.96	15.1
	CaUE[5]	67	3.53	1.12	0.003	−0.002	0.005	0.659	1.88	2.93	0.523	0.012	0.017	0.487	0.67	1.31

[1] Estimation of Ca net requirement and CaOS was only possible for lactating animals. Therefore, regressions included the complete data set and the data subset only with lactating animals for CaIN and CaOS as independent variables, respectively.
[2] Number of included observations.
[3] Indicates whether a parameter estimate is significantly different from zero.
[4] Residual function (residuals (w) against predictions (z)) for CaFE w = −0.2 (SE = 1.4; $P = 0.906$) − 0.00005 (SE = 0.00952; $P = 0.996$) × z and for CaUE w = −0.8 (SE = 0.4; $P = 0.032$) + 0.20 (SE = 0.07; $P = 0.007$) × z.
[5] Residual function (residuals (w) against predictions (z)) for CaFE w = −1.8 (SE = 4.1; $P = 0.669$) + 0.0061 (SE = 0.0220; $P = 0.783$) × z and for CaUE w = −0.4 (SE = 0.3; $P = 0.221$) + 0.14 (SE = 0.10; $P = 0.155$) × z.
[a,b] Indicates significant difference between estimated slope of cattle and small ruminants ($P \leq 0.05$); Non-tagged slopes were not significantly different between cattle and small ruminants ($P \geq 0.05$).

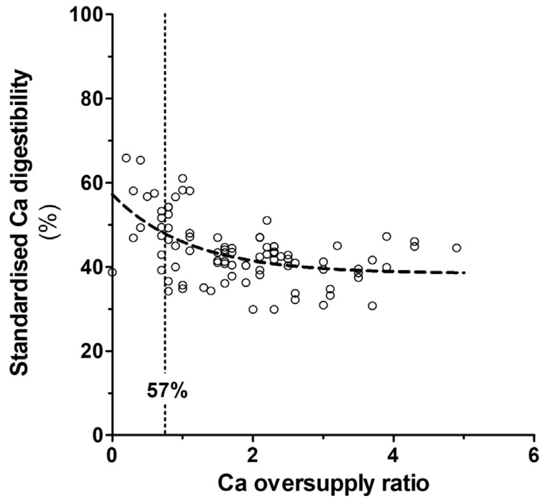

Fig. 3. Relationship between Ca oversupply ratio and standardised Ca digestibility (%) in lactating ruminants (n = 89). The dashed line and symbols represent estimated regression line and estimated standardised Ca digestibility corrected for the experimental effect, respectively (according to Eq. (6) based on parameter estimates shown in Table 3). The dotted line indicates the estimated gross Ca requirement when the estimated maximal standardised Ca digestibility of 57% is assumed (≙ a Ca oversupply ratio of 0.75).

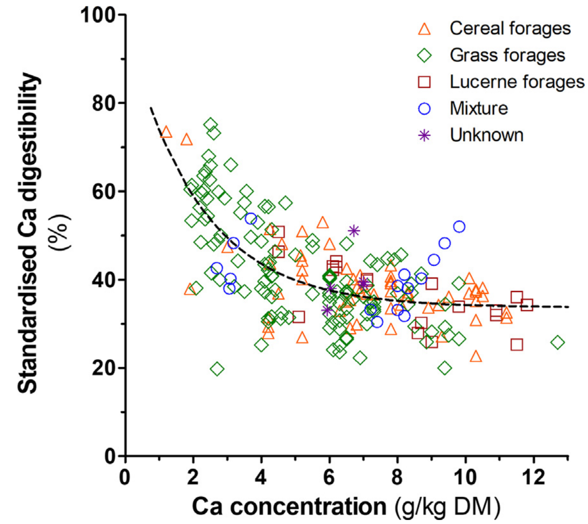

Fig. 4. Relationship between Ca concentration (g/kg DM) and standardised Ca digestibility (%) in ruminants (n = 223). The dashed line and symbols represent estimated regression line and estimated standardised Ca digestibility corrected for the experimental effect, respectively (according to Eq. (6) based on parameter estimates shown in Table 3). Different symbols represent the main forage in the diet. Cereal forages include whole-crop silages from maize, barley, oat, and triticale or cereal straw. Grass forages include fresh grass, grass hay or grass silage. Lucerne forages include lucerne hay, lucerne haylage or lucerne silage. Mixtures include diets with an equal mixture of two or more forages. Unknown represents an unknown diet composition.

the estimate and some safety margin. This choice might become flexible or replaced by a different value once adequate experimental data are available.

For lucerne and other legume forages, a generally low Ca utilisation by ruminants is suspected because Ca mostly occurs in the form of Ca oxalate, which is only little available to ruminants (Ward et al., 1979). Rahman et al. (2013) reported that availability of Ca oxalate varies depending on oxalate solubility and decreased with increased Ca concentrations following the formation of oxalate crystals. Furthermore, these authors compiled data indicating an adaptation to oxalate in the diet. This aspect was considered by the NRC (2001) and INRA (2018) by assuming a low value of 30% for the efficiency of Ca utilisation for this type of feedstuffs. Nevertheless, legumes contain intrinsically high Ca concentrations, impeding the formulation of diets low in Ca concentration including high proportions of legumes. Therefore, when feedstuffs with high Ca concentration shall be evaluated for their Ca availability, they have to be diluted in components with low Ca concentration and evaluated in a difference trial, ensuring a marginal Ca supply. Nevertheless, to the best of our knowledge, no systematic studies have assessed lucerne or other legume forages in such a way. Consequently, it is difficult to conclude whether the assumed low Ca availability of legumes such as lucerne is linked to their high Ca concentration or the chemical form of the Ca. It was therefore attempted to isolate the effect of Ca concentration and the effect of the diet by examining the effect of the main forage on the relationship between Ca concentration in the diet and standardised Ca digestibility. Nevertheless, it was impossible in the present analysis to obtain parameter estimates for the effect of the main forage because of unbalanced distribution of data. This restricts the following discussion to visual examination of the relationship between Ca concentration and standardised Ca digestibility corrected for the experimental effect (Fig. 4). Diets including lucerne as the main forage mostly contained more than 6 g Ca/kg DM and estimated standardised Ca digestibility was relatively low (25–40%). For the few diets including lucerne as the main forage containing less than 6 g Ca/kg DM, estimated standardised Ca digestibility was higher (about 50%). A similar trend was observed for diets including grass products as the main forage. This indicates that the Ca concentration of the diet, i.e. the Ca supply, is crucial for the Ca utilisation from the feed rather than the included feedstuff per se, i.e. the chemical form of Ca. For practical diet formulation, it is therefore suggested to consider the Ca concentration of the diet to predict Ca utilisation from the feed instead of using feed specific values. Inclusion of Ca-rich feedstuffs, such as legumes, in the diet appear to reduce Ca utilisation by increasing the likelihood of oversupplying Ca. Reduction of Ca utilisation with high Ca concentration might also be related to the increased formation of insoluble Ca oxalate under such conditions (Rahman et al., 2013), but the causal relationship cannot be clarified. It may be advisable to combine such feedstuffs with feedstuffs that are low in Ca to avoid high dietary Ca concentrations. The suggested equation of standardised Ca digestibility in response to Ca concentration can be used to predict Ca digestibility when Ca-rich feedstuffs are used.

Conclusions

It is concluded that CaIN by ruminants, which exceeds net requirement, is excreted in faeces to a large extent. Excretion of Ca in urine plays only a very minor quantitative role. Thus, Ca digestibility is a suitable measure of the efficiency of Ca utilisation of ruminants; however, this is only valid when the Ca supply by the diet is below the requirement. It is suggested that this is taken into account when the Ca requirement of ruminants is estimated applying a factorial approach. Based on the data presented in this study, a constant value of 50% is suggested as the estimate for efficiency of Ca utilisation. Ca digestibility can be predicted from the suggested equation depending on the Ca concentration in the diet. A differentiation between different Ca sources on the basis of reported studies is not possible at this time.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2021.100315.

Ethics approval

This meta-analysis did not require any ethical approval.

Data and model availability statement

None of the data were deposited in an official repository. The underlying data are available in the supplementary materials.

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KJW, **WMW**, **KHS**, and **MR** initiated the study and interpreted the results. **KJW** created the data set. **KJW** and **WS** conducted statistical data evaluation. **KJW** drafted the manuscript. **WS**, **WMW**, **KHS**, and **MR** revised the manuscript. **MR** and **KHS** obtained the funding for the work.

Declaration of interest

No potential conflict of interest is reported by the authors.

Acknowledgements

None.

Financial support statement

This work was funded by the German Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany, granted by the Federal Office for Agriculture and Food (BLE; grant number 2818HS013).

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