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Dynamics of body calcium and net calcium requirements for maintenance of Saanen goats

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Abstract

The objectives of the current study were to investigate the dynamics of body calcium (Ca) and to estimate the net Ca maintenance requirements (NCa_m) of Saanen goats, using ^{45}Ca as a radiotracer. Eighteen castrated male Saanen goats (25 ± 2.3 kg body weight (BW)) received a basal diet (ground ear maize, ground maize and vitamin–mineral premix). The treatments consisted of adding limestone to the basal diet to provide Ca content of 0.6, 1.7 and 3.0 g/kg dry matter (DM). The experiment lasted 45 days (i.e. 36 d of adaptation and 9 days of measurements). On day 38, 0.5 ml of 7.4 MBq ^{45}Ca solution was administrated before feeding. From days 39 to 45, samples of faeces, blood and urine were collected, and Ca concentration determined. The Ca intake, Ca in faeces, Ca in urine, faecal endogenous Ca and true absorbed Ca increased linearly as Ca content in the diets increased, while retained Ca increased at a decreasing rate. Dry matter intake decreased at an increasing rate with increased Ca content in the diets. In contrast, Ca content in the diets did not affect biological availability of Ca, or Ca in plasma. The true biological availability of Ca from limestone in Saanen goats was 0.72. The daily NCa_m was 11.6 (±1.3) mg/kg BW. The current results might help to understand Ca dynamics in goats and enhance the formulation of balanced diets to best meet Ca requirements of Saanen goats.

Introduction

Macrominerals are essential components of ruminant metabolism, with calcium (Ca) required principally as a structural element, constituting the bones in a stoichiometric ratio with phosphorus (P) (Suttle, 2010). In addition, Ca participates in several intra- and intercellular processes such as nerve transmissions, muscle contraction and hormone regulation (Buttery *et al.*, 2000). Calcium deficiency can lead to rickets in young animals, osteomalacia in adults and milk fever in lactating ewes (NRC 2007). Thus, the accurate estimation of Ca requirements is necessary to assure the proper functioning of maintenance and growth processes in animals.

The NRC (2007) reports Ca requirements for the maintenance of goats using data obtained partially from sheep, which could be inappropriate since serum and bone Ca metabolism differs between sheep and goats (Liesegang and Risteli, 2005). Previous studies demonstrated that under Ca dietary restriction, sheep decrease plasma Ca and increase phosphate concentrations, while goats compensate for low dietary Ca supply by stimulating vitamin D-dependent intestinal Ca absorption (Wilkens *et al.*, 2012, 2014). Several studies estimating the Ca requirements for maintenance have been conducted in goats (Fernandes *et al.*, 2012; Teixeira *et al.*, 2015; Santos *et al.*, 2016); however, the dynamics of Ca (i.e. true Ca intake, absorption, urine excretion, faeces excretion, retention and endogenous losses) have not been assessed so far. This is necessary to improve the knowledge of Ca metabolism and hence, Ca requirements for maintenance.

The dynamics of Ca can be assessed using the isotopic dilution method, which is a widely accepted technique for mineral dynamic studies in animals (Vitti and Kebreab, 2010). This method estimates the true Ca inputs and outputs in comparison to conventional assays (e.g. feeding trials and comparative slaughter studies), which are only able to obtain the apparent Ca inputs and outputs (Roque *et al.*, 2007; Fernandes *et al.*, 2012). Thus, the objectives of the current study were to investigate the dynamics of body Ca of goats fed diets with different Ca concentrations and to estimate the net Ca requirements for maintenance of Saanen goats, using ^{45}Ca as a radiotracer.

Materials and methods

Humane animal care and handling procedures were conducted in accordance with the Animal Care Committee (Comissão de Ética e Bem Estar Animal) of the Sao Paulo State University and with instructions from the Ministry of Agriculture in Brazil (instruction number 56/2008).

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Animal handling, experimental design and sampling

Eighteen castrated male Saanen goats (25 ± 2.3 kg body weight (BW), 180 days old) were housed indoors in metabolic cages (0.6 × 1.2 m^2) designed for isotope studies, which allowed total collection of faeces and urine and the control of feed intake. The goats received a basal diet composed of ground ear maize, finely ground maize grain and vitamin–mineral premix (Table 1). The diets were formulated to meet the daily requirements for maintenance of 438 kJ/$kg^{0.75}$ BW of metabolizable energy, 2.2 g of protein/$kg^{0.75}$ BW and 479 mg/day of dietary P, according to AFRC (1998). All goats had free access to fresh water. The treatments consisted of adding different amounts of calcitic limestone to the basal diet (0.0, 3.0 and 7.0 g/kg dry matter (DM)), resulting in 0.6, 1.7 and 3.0 g Ca/kg DM, respectively (Table 1). These Ca levels were selected to be below Ca maintenance requirement, at Ca maintenance requirement and above Ca maintenance requirement, respectively. A constant P dietary supply (i.e. meeting the net P requirement of goats) was assured across diets to test changes of Ca dietary levels but not of P dietary levels on dynamics of body Ca.

The experiment lasted 45 days, which included a 36-day adaptation period and 9 days for sample collection (i.e. control of feed intake, collection of faeces, urine and blood). To avoid feed orts, the animals received 65 g/day of feed per unit of metabolic BW. Feed was offered twice daily at 08:00 and 16:00 h. After the morning meal of day 38, each animal was injected, via the right jugular vein, with a single dose of 7.4 MBq ^{45}Ca in a 0.5 ml sterile isotonic saline solution (9 g/l sodium chloride (NaCl)). Blood samples from all animals were collected from the right jugular vein at 5 and 60 min after radiotracer injection.

Table 1. Composition and characterization of experimental diets

	Diets[a]		
	0.6	1.7	3.0
Ingredients (g/kg DM)			
Ground ear maize[b]	583	574	570
Ground maize grain[c]	402	408	408
Calcitic limestone[d]	0	3	7
Vitamin–mineral premix[e]	15	15	15
Chemical characterization (g/kg DM)			
DM	891	898	889
CP	81.5	79.9	79.5
ADF	116	136	124
NDF	508	516	508
Lignin	22.3	23.8	26.9
EE	32.9	28.0	26.7
P	2.50	2.50	2.50

DM, dry matter; CP, crude protein; ADF, acid detergent fibre; NDF, neutral detergent fibre; EE, ether extract; P, phosphorus.
[a]Diets: 0.6 = 0.6 g Calcium (Ca)/kg DM; 1.7 = 1.7 g Ca/kg DM; and 3.0 = 3.0 g Ca/kg DM.
[b]Ground ear maize included the cobs, grains and husks.
[c]Ground maize included grains only.
[d]Calcitic limestone (Ca = 367.5 g/kg DM; P = 95.2 mg/kg DM).
[e]Vitamin–mineral premix = Salt, mineral supplement and vitamin supplement. Micro mineral composition of Premix: Iron = 8.73 mg/kg DM; Copper = 7.64 mg/kg DM; Manganese = 44.36 mg/kg DM; Zinc = 58.73 mg/kg DM; Cobalt = 0.1 mg/kg DM; Iodine = 0.2 mg/kg DM; Selenium = 0.03 mg/kg DM.

From days 39 to 45, samples of blood, faeces and urine were collected daily assuming that during this period the Ca-specific activity in plasma and faeces was in a steady-state condition (Lofgreen and Kleiber, 1954). The Ca concentration in plasma was analysed immediately after collection. Total faeces and urine excretion were quantified daily and representative samples (0.10) of each were stored at −20 °C for later analysis.

Chemical analysis

Feed samples were analysed chemically to determine DM (AOAC 1990; method: 930.15), crude protein (AOAC 1990; method: 954.01) and ether extract (AOAC 1990; method: 920.39). Neutral detergent fibre values were determined with amylase and without sulphite (Van Soest, 1994) and acid detergent fibre values were determined according to Goering *et al.* (1972). Phosphorus concentrations were determined using the colorimetric method (AOAC 1990; method: 965.17). Calcium concentrations in feed samples, faeces and urine were determined with atomic absorption spectrometry (AOAC 1990; method: 935.13).

Blood samples were analysed to determine the concentration of inorganic Ca in plasma. Briefly, after blood collection, samples (10 ml) were centrifuged (1800 ***g*** for 10 min) to achieve plasma separation. Nine millilitres of trichloroacetic acid (100 g/l) was added to 1 ml of plasma for protein precipitation. After centrifugation (1000 ***g*** for 10 min), inorganic Ca in plasma was determined by atomic absorption spectrometry (AOAC 1990, method: 935.13).

Radioactivity in plasma, faeces and urine was determined using the liquid scintillation method (Passo and Cook, 1994) within 15 days of radiotracer injection. Plasma and urine samples (1 ml each) were added to 10 ml of scintillation solution in borosilicate vials. Ashes of faecal samples (1 g) were previously dissolved in 5 ml of 18 N sulphuric acid (H_2SO_4) and then 1 ml was placed into borosilicate vials with 10 ml of scintillation solution. The composition of the scintillation solution was 4 g of 2,5-diphenyloxazole and 0.3 g of 1,4-bis-(5-phenyloxazole-2-il) benzene dissolved in a mixture of 500 ml Triton X-100 and 1 litre of toluene. The corrections for decay (^{45}Ca half-life = 165 days) were carried out in relation to the standard prepared at injection.

Calculations

The proportions of injected activity of Ca in faeces (CaIAfc) and plasma (CaIApl) were determined according to Lofgreen and Kleiber (1954) as follows:

$$\text{CaIAfc} = \frac{\text{cpmfc}}{\text{cpmsp}} \quad (1)$$

$$\text{CaIApl} = \frac{\text{cpmpl}}{\text{cpmsp}} \quad (2)$$

where cpmfc, cpmpl and cpmsp are the counts/min in 1 g of faeces, counts/min in 1 ml of plasma and counts/min in 0.5 ml of the standard solution of ^{45}Ca containing 55 000 dpm/0.5 ml, respectively. The standard solution was prepared from the standard $^{45}CaCl_2$ of 62.7 × 10^6 dpm/µl and ultra-pure water.

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The specific activity of Ca in faeces (SAfc) and plasma (SApl) was determined by the following equations:

$$\text{SAfc} = \frac{\text{CaIAfc}}{\text{faeces (mg Ca/g)}} \quad (3)$$

$$\text{SApl} = \frac{\text{CaIApl}}{\text{plasma (mg Ca/ml)}} \quad (4)$$

where faeces and plasma are the Ca in faeces (mg Ca in 1 g faeces) and Ca in plasma (mg Ca in 1 ml plasma), respectively.

Using SAfc and SApl values, the proportion of faecal endogenous Ca (pCa_{fce}) was calculated using the equation:

$$pCa_{fce} = \frac{\text{SAfc}}{\text{SApl}} \quad (5)$$

The faecal Ca output (Ca_{fc}) and Ca_{fce} were used to calculate the total amount of faecal endogenous Ca (Ca_{fce}), according to the equation:

$$Ca_{fce}(g/day) = Ca_{fc}(g/day) \times pCa_{fce} \quad (6)$$

The true absorbed Ca (Ca_{abs}) was determined using the following equation:

$$Ca_{abs}(g/day) = Ca_{int}(g/day) - [Ca_{fc}(g/day) - Ca_{fce}(g/day)] \quad (7)$$

where Ca_{int} is the Ca intake (g/day).

The biological availability of Ca was determined by dividing the Ca_{abs} (g/day) by the Ca_{int} (g/day), according to the equation:

$$\text{Biological availability of Ca} = \frac{Ca_{abs}(g/day)}{Ca_{int}(g/day)} \quad (8)$$

The retained Ca (Ca_{ret}) was determined using the following equation:

$$Ca_{ret}(g/day) = [Ca_{int}(g/day)] - [(Ca_{fc}(g/day) + Ca_{ur}(g/day)] \quad (9)$$

where Ca_{ur} is the Ca in urine (g/day).

The daily net Ca requirements for maintenance were calculated from the intercept of the linear regression between daily excretion of Ca (i.e. sum of Ca_{fc} and Ca_{ur} (g/kg BW)) against daily Ca_{int} (g/kg BW) using the minimum endogenous losses method (MEL).

Statistical analyses

The animals were assigned to one of three dietary treatments (0.6, 1.7 and 3.0 g Ca/kg DM) in a completely randomized design with six replications for each treatment. The statistical model used to analyse the data was:

$$Y_{ij} = \mu + t_i + e_{ij} \quad (10)$$

where Y_{ij} is the observed value in the portion that received treatment i in replication j, μ is the overall mean of the experiment, t_i is the effect of treatment i that was applied on a portion and e_{ij} is the error term for i treatment and j replication.

The effect of different Ca content in the diets on DM intake (DMI), Ca_{int}, Ca_{fc}, Ca_{ur}, Ca_{fce}, Ca_{abs}, biological availability of Ca, Ca_{ret} and Ca_{pl}, recorded per goat, were analysed using the MIXED procedure of SAS (version 9.4, SAS Inst. Inc. Cary, NC, USA). The statistical model included treatment (i.e. Ca dietary level) as fixed effect and the error as random effect. Orthogonal contrasts were performed to compare treatment means, using the CONTRAST statement and orthogonal polynomial coefficients in the MIXED procedure.

Regression equations to predict Ca_{abs}, Ca_{ret}, Ca_{ur} and excretion of Ca using Ca_{int} as the regressor, to predict Ca_{fce} using DMI as regressor and to predict net Ca requirements for maintenance, were obtained as mixed models considering the treatment as fixed effect and the error as random effect. Linear and quadratic regressions were tested using the MIXED procedure, selecting the most adequate model based on significance of P-value and lowest error. Slopes and intercepts of each equation were estimated using the ESTIMATE statement of the MIXED procedure. Significance level was declared at $P < 0.05$.

Results

As Ca content in the diet increased from 0.6 to 3.0 g/kg, Ca_{int}, Ca_{fc}, Ca_{ur}, Ca_{fce} and Ca_{abs} showed linear increases of 309, 154, 77, 68 and 342%, respectively ($P < 0.05$; Table 2). Conversely, Ca_{ur} as a percentage of Ca_{int} decreased from 4.1 to 1.8% as dietary Ca content increased. In addition, the Ca_{fc} as a percentage of Ca_{int} decreased from 80 to 50% as Ca content in the diet increased from 0.6 to 3.0 g/kg DM, indicating that Ca is excreted mainly via faeces (Table 2).

Dry matter intake reduced at an increasing rate ($P < 0.01$; Table 2) and Ca_{ret} increased at a decreasing rate ($P < 0.05$; Table 2) with rising dietary Ca content. In contrast, Ca content in the diet did not affect BW (25 ± 4.6 kg), biological availability of Ca (0.66 ± 0.026) from diets or Ca_{pl} (17 ± 4.6 mg/dl). The biological availability of Ca from limestone in Saanen goats was 0.72 ± 0.026 (Fig. 1; $P < 0.01$).

It was found that Ca_{abs}, Ca_{ret} and Ca_{ur} were associated linearly with Ca_{int}, which allowed the development of regression equations to predict Ca_{abs} (root mean square of error (RMSE) = 3.23; $P < 0.01$; Fig. 1), Ca_{ret} (Eqn (11)), Ca_{ur} (Eqn (12)) and Ca_{fc} (Eqn (13)) as a function of Ca_{int}:

$$Ca_{ret} = -12\,(\pm 1.6) + 0.61\,(\pm 0.025) \times Ca_{int} \quad (\text{RMSE} = 2.96,\ P < 0.01) \quad (11)$$

$$Ca_{ur} = 0.8\,(\pm 0.24) + 0.01\,(\pm 0.004) \times Ca_{int} \quad (\text{RMSE} = 0.39,\ P = 0.022) \quad (12)$$

$$Ca_{fc} = 10\,(\pm 1.3) + 0.39\,(\pm 0.022) \times Ca_{int} \quad (\text{RMSE} = 2.52,\ P < 0.01) \quad (13)$$

Additionally, it was found that Ca_{fce} was associated linearly with DMI (RMSE = 1.51; $P < 0.01$; Fig. 2). The daily Ca requirement for maintenance, estimated from the intercept of the linear

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Table 2. Mean values of variables related to daily calcium (Ca) dynamics in Saanen goats fed diets containing different content of Ca

	Diets[a]					Contrast[d]	
Item	0.6	1.7	3.0	SEM[b]	*P*-value[c]	L	Q
Body weight (BW, kg)	25	25	25	2.0	0.983	0.993	0.861
Dry matter intake (g/kg BW)	38.0	31.4	31.1	0.62	<0.01	<0.01	<0.01
Ca Intake (mg/kg BW)	23	53	93	1.1	<0.01	<0.01	0.210
Ca in faeces (mg/kg BW)	18.2	32.4	46.2	0.90	<0.01	<0.01	0.262
Ca in urine (mg/kg BW)	1.0	1.5	1.7	0.18	0.049	0.021	0.303
Faecal endogenous Ca (mg/kg BW)	10	13	17	1.0	<0.01	<0.01	0.711
True absorbed Ca (mg/kg BW)	15	34	65	1.6	<0.01	0.011	0.060
Biological availability of Ca	0.642	0.632	0.694	0.244	0.189	0.141	0.280
Retained Ca (mg/kg BW)	3	18	47	1.1	<0.01	<0.01	<0.01
Ca in plasma (mg/dl)	20	17	14	4.6	0.670	0.383	0.990

[a]Diets: 0.6 = 0.6 g Ca/kg DM; 1.7 = 1.7 g Ca/kg DM; and 3.0 = 3.0 g Ca/kg DM.
[b]SEM = standard error of mean.
[c]*P*-value refers to differences in parameters between different diets.
[d]*Contrast*: Orthogonal contrast: L = Linear effect; Q = Quadratic effect.

regression between excretion of Ca and Ca_{int}, was 12 (±1.3) mg/kg BW (RMSE = 2.44; $P < 0.01$; Fig. 3)

Discussion

The goals of the present study were to evaluate the dynamics of body Ca in goats using different content of Ca in diets and estimate the net Ca requirements for the maintenance of Saanen goats, using the isotopic dilution method.

Increasing Ca content in diets from 0.6 to 3.0 g/kg DM resulted in a decrease of the ratio between Ca_{fc} and Ca_{int} from 0.79 to 0.49, indicating that within the range of Ca content studied, Saanen goats improved their Ca use as the content of Ca in the diet increased, which is in agreement with the increase of Ca_{abs} observed with the increase of Ca content in diets. Since Ca availability did not change with the increase of Ca content in diets, the amount of true Ca absorbed was directly proportional to the dietary Ca content. In addition, because the P content in the diet did not change across treatments, the increase in Ca_{abs} with increase of Ca content was associated with an increase in the Ca:P ratio of diets from 0.24 in the low Ca diet (0.6 g Ca/kg DM) to 1.2 in the high Ca diet (3.0 g Ca/kg DM). This is related to an antagonistic relationship between Ca and P during Ca absorption processes in the intestine (Field *et al.*, 1975; Schneider *et al.*, 1985). Phosphate, which is the principal dietary form of P, binds more easily to Ca and decreases its intestinal availability (Favus *et al.*, 2009). Conversely, mechanisms of Ca and P absorption are mediated by both transcellular and passive cellular processes, with P absorption being more efficient than Ca absorption (Suttle, 2010). Active absorption of both minerals is regulated by 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) metabolism. When Ca requirements are fulfilled, if P concentration is greater than Ca concentration in the diet, $1,25(OH)_2D$ promotes P absorption, which decreases Ca absorption in the intestine (DiMeglio and Imel, 2013). Thus, Ca absorption is regulated not only by its concentration but also by its ratio with P concentration in the diet. In addition, the ratio between Ca_{ret} and Ca_{abs} increased from 0.21 to 0.72 as Ca content in the diets increased from 0.6 to 3.0 g/kg DM, indicating that a greater amount of Ca was incorporated into tissues with greater dietary Ca content

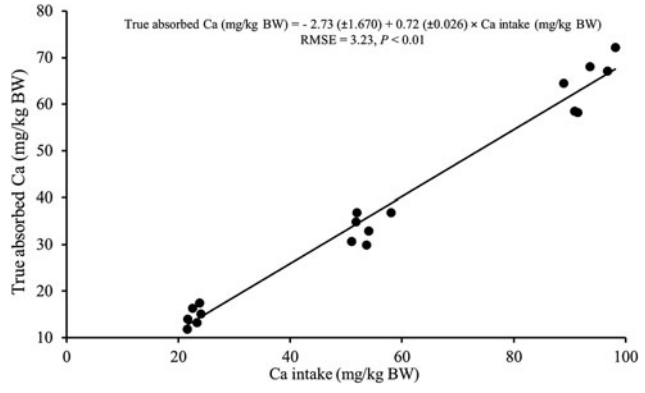

Fig. 1. Relationship between daily calcium (Ca) intake (mg/kg body weight (BW)) and daily true absorbed Ca (mg/kg BW) for Saanen goats fed diets containing different content of Ca. RMSE = root mean square of error.

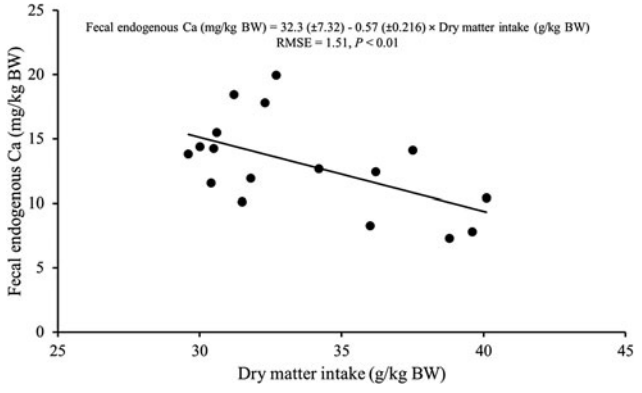

Fig. 2. Relationship between daily dry matter intake (g/kg body weight (BW)) and daily faecal endogenous calcium (Ca) (mg/kg BW), for Saanen goats fed diets containing different content of Ca. RMSE = root mean square of error.

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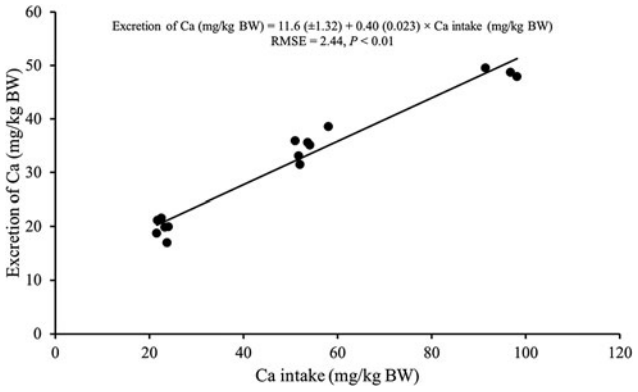

Fig. 3. Relationship between daily calcium (Ca) intake (mg/kg body weight (BW)) and daily excretion of Ca (mg/kg BW), for Saanen goats fed diets containing different content of Ca. RMSE = root mean square of error.

and increased Ca_{int}. The Saanen goats used in the current study were in the growing phase, when Ca retention is directed principally to bone deposition (Suttle, 2010). Therefore, the evaluation of higher Ca contents in the diet of growing Saanen goats is recommended for future studies to estimate the maximum Ca_{ret} achievable.

Although Ca_{ret} and Ca_{abs} increased with increasing Ca in the diet, the biological availability of Ca and Ca_{pl} were not affected by Ca content in the diets. The restriction of dietary Ca or excessive Ca supply is nutritional conditions that may alter the Ca_{pl} and its biological availability in goats (NRC 2007; Suttle, 2010). In addition, the biological availability of Ca and Ca_{pl} are principally associated with animal species, age, nutritional requirements, the chemical form of Ca in feed and dietary P concentration (Suttle, 2010; Wilkens *et al.*, 2014). Therefore, it is suggested that since the Ca content of diets in the current study still met maintenance Ca requirements, the Ca_{pl} and Ca biological availability did not change with the treatments and the amount that exceeded the maintenance requirements was designated to Ca body retention, which was clearly observed in the current study.

Values for biological availability of Ca between 0.30 and 0.55 and for Ca_{pl} between 8 and 12 mg/dl have been accepted by the NRC (2007) for goats. Despite this, the NRC (2007) does not discriminate between the values of biological availability of Ca and the Ca_{pl} between different ruminant species. In the current study, the average values for biological availability of Ca from diets and Ca_{pl} were 0.66 and 17.3 mg/dl, respectively, which were greater than those reported by the NRC (2007). This difference might be due to the mineral concentrations and metabolism in tissues, blood and milk being different between ruminant species (Haenlein, 1980; Herm *et al.*, 2015). In addition, the biological availability of Ca from limestone in Saanen goats (0.72) was greater than reported in lambs (0.62; Roque *et al.*, 2007). As demonstrated by Wilkens *et al.* (2012, 2014), the Ca absorption and active transport in jejunum processes are more efficient in goats than sheep. Herm *et al.* (2015) suggested subsequently that differences in the expression of sodium/Ca exchanger type 1, vitamin receptors and 24-hydrolase may explain the difference in Ca absorption between sheep and goats. Therefore, differences between the findings of the current study and those in the literature highlight the importance to identify the mineral's biological availability for each ruminant species and further studies are needed.

Faecal endogenous Ca increased as Ca content in the diet increased. Additional studies evaluating the relationship between faecal endogenous Ca and its content in the diet have not been conducted in goats, which makes comparison of the current results with those in the literature difficult. However, Carvalho *et al.* (2003), using the isotopic dilution method, found that castrated Saanen goats of 25 kg BW showed increased faecal endogenous P as P content in the diet increased. This evidence is in accordance with the current study, considering that Ca and P are metabolically related. Conversely, there is a consensus in the literature that Ca_{fce} only increases with higher DMI but not with an increase of Ca_{int} (Braithwaite, 1982; AFRC 1998; NRC 2007). However, the current study does not support those findings. This physiological response was associated with the negative correlation between DMI and dietary Ca content. In comparison to other ruminant species, when subjected to dietary Ca restriction, goats prioritize active Ca absorption over bone Ca resorption (Wilkens *et al.*, 2014). Considering that goats in the current study decreased DMI with increasing of dietary Ca and decreased Ca_{fce} with increasing DMI, goats possibly prioritize Ca absorption over Ca intake.

The current study is the first to evaluate endogenous Ca responses to different Ca content intakes in goats using isotopic dilution, the majority of previous studies related to this subject in the literature were performed with sheep and cows. Therefore, it is possible that there are differences in Ca digestion and metabolism between ruminant species and more studies with goats are needed to better elucidate these mechanisms for this species. This will be important for enhancing diet formulation for goats, because the majority of Ca recommendations for goat feeding systems (AFRC 1998; NRC 2007) are based mainly on DM intake.

The current result for daily Ca requirement for maintenance was lower than that reported for goats by the NRC (2007) using feeding trials (20 mg Ca/kg BW), whereas it was closer to that reported for sheep by the ARC (1980) using the isotopic dilution method (15.7 mg Ca/kg BW). Comparative slaughter studies with male crossbreed Boer × Saanen (Fernandes *et al.*, 2012; Teixeira *et al.*, 2015) and with Saanen goats of different sexes (i.e. castrated males, females and intact males) (Santos *et al.*, 2016) have also reported greater values of daily Ca requirements for maintenance (27.4, 38.3 and 35.4 mg Ca/kg BW, respectively) than those found in the current study.

The differences between the current results and the reports mentioned previously, especially the study using Saanen breed at a similar BW (Santos *et al.*, 2016), may be related to the method used. Comparative slaughter estimates apparent Ca inputs and outputs, in comparison to the isotopic dilution method that is able to obtain true estimates of Ca inputs and outputs (Vitti and Kebreab, 2010). Thus, additional studies should be conducted to improve the accuracy of the estimation of Ca requirements for the maintenance of goats trying to cover more different stages of growth.

The current study seems to be the first to evaluate the dynamics of Ca and Ca requirements for maintenance with different content of Ca in diets in goats using the isotopic dilution method. The current findings might help understand Ca dynamics in goats and promote the formulation of diets adequately balanced in terms of Ca for growing Saanen goats. In conclusion, the ratio between retained Ca and true absorbed Ca increased as Ca content in the diets increased from 0.6 to 3.0 g/kg DM, suggesting that additional studies to evaluate Ca content in diets greater than 3.0 g/kg DM are required to estimate the maximum retained

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Ca in growing Saanen goats. It was also concluded that Ca true absorption and plasma Ca remain similar if dietary Ca supply is above of maintenance requirements of Ca. In addition, daily Ca requirements for maintenance was 12 (±1.3) mg/kg BW.

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Declaration of interest. Authors declared no conflicts of interest.

Ethical standards. Humane animal care and handling procedures were conducted in accordance with the Animal Care Committee (Comissão de Ética e Bem Estar Animal) of the Sao Paulo State University and with instructions from the Ministry of Agriculture in Brazil (instruction number 56/2008).

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