Effect of dietary magnesium on calcium utilization by sheep*)

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Zusammenfassung

Um den Einfluß von Mg auf die Utilisation von Ca zu messen, erhielten insgesamt 15 Lämmer drei halbsynthetische Diäten mit verschiedenen Mg-Gehalten (500, 5500 und 10 500 ppm), sowie 0,25 % Ca und 0,20 % PO_4. 52 Tage später erhielten die Tiere einmalig eine orale Dosis von 600 µ ^{45}Ca und intravenös 150 µ ^{47}Ca. Die meßbare Mg-Absorption stieg an bis 5500 ppm Mg im Futter ($P<0,01$), ohne weiteren Anstieg bei noch höherer Zufuhr. Die Mg-Ausscheidung im Urin erhöhte sich ($P<0,01$) und korrelierte ($r=0,74$) mit der meßbaren Mg-Absorption. Die Mg-Retention korrelierte signifikant ($P<0,01$) mit dem absorbierten Mg ($r=0,96$), so daß der Körperpool für Mg erhöht wurde, was auch durch erhöhte Plasma-Mg-Werte zum Ausdruck kam. Die Mg-Konzentrationen im Femur betrugen 0,82; 0,92 und 1,15 %.

In Abhängigkeit vom Mg-Gehalt des Futters nahm die meßbare ^{45}Ca-Absorption ab ($P<0,05$) bei geringer Beeinflussung des stabilen Ca. Das metabolische fäkale ^{47}Ca und das stabile Ca wiesen einen abnehmenden Trend auf bei ansteigender Mg-Konzentration im Futter. Die Gesamt-Ca-Absorption wurde signifikant ($P<0,05$) durch Mg vermindert. Die Urin-Ausscheidung von stabilem Ca und ^{45}Ca sowie Plasma-Ca und Knochen-Ca blieben unbeeinflußt. Die Verluste von ^{47}Ca und stabilem Ca über den Urin in % des gesamten absorbierten Ca wurden signifikant durch Mg erhöht. Die Analyse der ^{47}Ca-Plasma-Clearance ergab, daß das pro Zeiteinheit aus dem Plasma abgegebene Ca nicht durch Mg beeinflußt wurde.

Summary

To measure the effect of dietary magnesium on calcium utilization, fifteen lambs were assigned to three semipurified experimental diets containing three levels of magnesium (500; 5500 and 10 500 ppm) and 0,25 % calcium and 0,20 % phosphorus. Fifty-two days later, the animals were given a single oral dose of 600 µc of ^{45}Ca and injected intravenously with 150 µc ^{47}Ca. Apparent absorption of magnesium increased ($P<0,01$) up to 5500 ppm dietary magnesium, with no further increase at higher intake. Urinary excretion of magnesium was significantly ($P<0,01$) correlated ($r=0,74$) with apparent absorption. Magnesium net retention was significantly ($P<0,01$) correlated with absorbed magnesium ($r=0,96$), thus increasing the body pool of the element, as also indicated by plasma magnesium increments ($P<0,01$). Femur ash magnesium levels were 0,82, 0,92 and 1,15 %.

Dietary magnesium decreased ($P<0,05$) apparent absorption of ^{45}Ca and only numerically of stable calcium. Metabolic fecal ^{47}Ca and stable calcium showed a diminishing trend as dietary magnesium levels increased. True magnesium absorption was significantly ($P<0,05$) diminished by dietary magnesium. Urinary excretion of stable calcium and ^{45}Ca as well as plasma calcium and bone calcium were not affected by dietary magnesium. Urinary loss of ^{47}Ca and of stable calcium as percent of true absorbed calcium were significantly increased by dietary magnesium. Multi-term exponential functions of ^{47}Ca plasma clearance indicated that the amount of inorganic calcium removed per unit time from the plasma were not affected by magnesium levels in the diet.

Résumé

Pour mesurer l'effet du magnésium de régime sur l'utilisation du Ca, 3 régimes expérimentaux semi-purifiés contenant 3 taux de Mg (500; 5500 et 10 500 ppm) et 0,25 % de Ca+ 0,20 % de phosphore ont été attribués à 15 agneaux. 52 jours plus tard, les animaux ont reçu une dose orale unique de 600 µc de ^{45}Ca et ils ont reçu une injection intraveineuse de 150 µc de ^{47}Ca. L'absorption apparente du Mg s'est accrue ($P<0,01$) jusqu'à 5500 ppm de Mg dans la ration, sans accroissement ultérieur pour une absorption plus élevée. L'excrétion urinaire du Mg a présenté une corrélation significative ($P<0,01$) ($r=0,74$) avec l'absorption apparente. La rétention totale du Mg a été en corrélation significative avec le Mg absorbé ($r=0,96$), accroissant de la sorte le pool corporel de l'élément, ce qui est indiqué aussi par les accroissements du Mg plasmatique ($P<0,01$). Les taux de Mg des cendres du fémur ont été de 0,82, 0,92 et 1,15 %.

Le magnésium de la ration a réduit ($P<0,05$) l'absorption apparente de ^{45}Ca et seulement numériquement la valeur du Ca stable. Le ^{47}Ca fécal métabolique et le Ca stable ont présenté une tendance à la réduction quand les taux du Mg de la ration ont été accrus. L'absorption réelle du Mg a été réduite significativement ($P<0,05$) par le Mg de la ration. L'excrétion urinaire du Ca stable et du ^{45}Ca, ainsi que le Ca plasmatique et le Ca osseux n'ont pas été affectés par le Mg de la ration. La perte urinaire du ^{47}Ca et celle du Ca stable en pourcentage du Ca absorbé véritablement ont été accrues par le Mg de la ration. Des fonctions exponentielles à plusieurs termes de la clearance plasmatique de ^{47}Ca ont indiqué que la quantité de Ca minéral éliminé par unité de temps à partir du plasma n'a pas été affectée par les taux de Mg dans la ration.

Introduction

Information on magnesium (Mg) metabolism in ruminants, particularly concerning the hypomagnesemia associated with grass tetany, has increased greatly in recent years, but basic knowledge on its physiology, metabolic role and the influence of calcium (Ca) and phosphorus is still limited. The linear relationship existing between Mg intake and fecal Mg excretion suggest that true absorption of this element is probably independent of intake level (*Chicco* et al. 1973). Mg appears to be a threshold substance within the

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body (*Storry* and *Rook* 1963) with a linear relationship existing between plasma concentration and urinary excretion, which supports the concept that urinary excretion of Mg is a filtration reabsorption mechanism (*Storry* and *Rook* 1963; *Blaxter* et al. 1954).

The existing information on the effect of Mg supplementation on Ca and phosphorus utilization is not consistent. In research with sheep, some authors (*Dutton* and *Fontenot* 1967; *Hjerpe* 1967) have reported no effect of Mg supplementation on Ca and phosphorus balance and blood values, while others obtained some reduction of plasma Ca (*Chicco* et. al 1973; *Dehority* et al. 1961; *Stewart* and *Moodie* 1956), and increased losses of fecal Ca (*Myburgh* and *Du Toit* 1970; *Chicco* et al. 1973)

The objetives of this experiment were to investigate further some nutritional and physiological aspects of Mg utilization and its interaction with Ca in ruminants.

Experimental procedure

Fifteen lambs, averaging approximately 26 kg and housed in individual stalls, were randomly assigned by body weight to three levels of dietary Mg (550, 5500, 10 500 ppm) in a semi-purified diet (table 1), containing 0,25 % calcium as $CaCO_3$, 0,20 % phosphorus as Na_2HPO_4 and 250 ppm Mg, as verified by chemical analysis. Supplemental Mg was added as reagent grade magnesium carbonate to provide the desired dietary levels. Distilled water was offered *ad libitum*. A constant daily feed intake of 600 g was maintained during the experimental period.

After 38 days, lambs were transfered to metabolism crates, and 14 days later, prior to the morning feedings, the animals were given a single oral dose of 600 μc of ^{45}Ca. Fifteen minutes later, each lamb was injected intravenously with 150 μc of ^{47}Ca which had been incubated at room temperature with 5 ml of plasma for 30 minutes.

Total urinary and fecal collections were made at 24-hr. intervals for six days, and heparinized blood samples were taken by jugular puncture at determined intervals after dosing. The animals were slaughtered at the end of the collection period and femur and kidney were removed for chemical analysis and determination of ^{45}Ca and ^{47}Ca. Mg and Ca in urine, feces and bone were determined by atomic absorption spectrophotometry (*Parker* 1963; *Willis* 1961). Phosphorus in the experimental diet was determined as described by *Fiske* and *Subarrow* (1925). Plasma Ca and Mg were assayed by atomic absorption spectrophotometry (*Sunderman* and *Carrol* 1965). Measurements of ^{47}Ca in feces, urine and body tissues were made with a well counter having a sodium iodide crystal. In the same samples, ^{45}Ca was determined with a conventional liquid scintillation spectrometer following ^{47}Ca physical decay. Radioactivity was recorded as disintegrations per minute (dpm).

Tab. 1: Composition of basal diet[a].

Ingredient	International Reference number	Percent
Corn, cobs and shucks, grnd.		35,50
Isolated soy protein[b]		7,50
Urea, crystalline		2,50
Cerelose	4 02 125	18,00
Corn, starch, dehy, grnd[c]	4 02 889	29,90
Corn, oil[d]	4 07 882	3,00
Minerals[e]		2,50
Na_2HPO_4, reagent grade		0,55
$CaCO_3$, reagent grade		0,55
Vitamins[f]		+
		100 00

[a] The basal diet contained 0,25 % Ca; 0,20 % P and 0,025 % Mg.
[b] Assay protein C-1, Skidmore Enterprises, Cincinnati, Ohio, USA.
[c] Variable levels of $MgCO_3$ replaced starch to obtain diets with 500, 5500 and 10,550 ppm Mg.
[d] Santoquin added at 0,0125 % of total diet.
[e] The composition in percent was as follows: K_2CO_3, 28,3; Na_2SO_4, 35,0; NaCl, 35,88; $FeSO_4$, 1,1; $CuCl_2$, $2H_2O$, 0,08; $MnCO_3$, 0,21; $CaCO_3$, 0,02; $ZnCO_3$, 0,38; and KI, 0,03.
[f] Vitamins added per kilogram of diet: 11 mg DL-Alpha Tocopherol; 4400 IU Vitamin A Palmitate; and 1100 IU Vitamin D_2.

The biological utilization of Mg was measured as apparent absorption (total intake minus fecal) and net retention (total intake minus fecal and urinary). Ca utilization was measured as apparent and true absorption, net retention and tissue uptake of stable and labeled Ca. True absorption of calcium was calculated as follows:

True absorption (%) =

$$\frac{\text{Total Ca intake} - (\text{fecal Ca} - \text{metabolic fecal Ca})}{\text{Total Ca intake}} \times 100$$

Fecal metabolic calcium was estimated according to *Comar* et al. (1953) as follows:

Metabolic fecal Ca (%) =

$$\frac{100 - \text{fecal } ^{45}\text{Ca } (\%)}{1 - \text{met. fecal } ^{47}\text{Ca } (\%)} - 100 - \text{Fecal Ca } (\%)$$

The data were treated by analysis of variance, correlation and regression and the treatment means were compared by the multiple range test of *Duncan* (1955).

Results and discussion

Data representing fecal and urinary excretion of Mg, as well as calculated values of apparent absorption and net retention, are summarized in table 2. Increased dietary Mg resulted in an increase (P<0,01) in fecal and urinary as well as in apparently absorbed and net retained Mg. A correlation (P<0,01) existed between apparent absorbed and net retained Mg (r=0,96). Urinary excretion was correlated (P<0,01) with both apparently absorbed (r=0,86) and net retained Mg (r=0,73). The relation between urinary excretion and apparently absorbed and net retained Mg showed a significant (P<0,01) quadratic regression. Earlier reports (*Ammerman* et al. 1972; *Chicco* et al. 1972) indicated a significant linear regression at lower dietary Mg levels. The significant correlation between absorption and urinary excretion of Mg supports the suggestion that the excretion of Mg in the urine reflects the availability of the Mg source consumed (*Ammerman* et al. 1972; *Gerken* and *Fontenot* 1967; *Rook* et al. 1958).

Mg levels for plasma, femur and kidney are presented in table 3. Plasma and kidney Mg increased (P<0,01) with greater dietary Mg, while femur magnesium showed only a numerical increase. Blood Mg was correlated (P<0,01) with absorption (r=0,89), net retention (r=0,77) and urinary excretion of Mg (r=0,84). This corroborates the suggestion by *Blaxter* and *McGill* (1956) that the concentration of plasma Mg is controlled by a homeostatic mechanism in which an important factor is the balance between the Mg entering and leaving the body, particularly in the adult animal, in which the skeletal reserves of Mg are less readily mobilized.

The correlation between urinary Mg and plasma Mg supports the concept that urinary excretion of Mg is a filtration-reabsorption mechanism (*Blaxter* et al. 1954; *Storry* and *Rook* 1963). In the resulting regression equation, $y=23,68x-49,40$, where "y" is the urinary excretion in mg/hr and "x" the plasma Mg in mg/100 ml, the point at which the regression line intercepts the abscissa, 2,08 mg/100 ml, can be defined as the theoretical renal Mg threshold (fig. 1). This is higher than the value of 1,6 mg/100 ml reported previously by *Chicco* et al. (1972) and those reported by other authors (*L'Estrange* and *Axford* 1964; *Storry* and *Rook* 1963) but closer to the value of 2,15 mg/100 ml suggested by *Rook* et al. (1958).

Blood Mg was also correlated (P<0,01) with kidney Mg (r=0,70), and the latter with the net retention of the element (r=0,73). Contrary to *Chicco* et al. (1972), the correlation between plasma and bone Mg did not reach significance (r=0,44); (P>0,05) probably due to the short feeding period used in this trial, which failed to alter bone levels of Mg to a significant degree. Feeding supplemental Mg resulted in a decrease in the apparent absorption of ^{45}Ca (P<0,01) and true absorption of stable Ca (P<.05) (table 4). The apparent absorption and metabolic fecal fraction of stable Ca showed only a decreasing trend with the increase of dietary Mg, while metabolic fecal ^{47}Ca was not affected by dietary treatments. When metabolic fecal stable Ca was related with the absorbed Mg, a negative correlation (P<0,05) was found with the apparent

Tab. 2: Fecal and urinary excretion, apparent absorption and net retention of magnesium in lambs fed different levels of dietary magnesium[a].

Dietary magnesium[b] ppm	Fecal excretion mg/day	Urinary excretion mg/day	Apparent absorption %	Net retention %
500	238[c]	60[c]	20,3[c]	0,3[c]
5500	1890[d]	820[d]	42,7[d]	17,7[d]
10,500	3559[e]	776[d]	43,5[d]	31,2[e]

[a] Each value represents the average for five animals.
[b] The intake of magnesium was 300, 3300 and 6300 mg/day for the 500, 5500 and 10,500 ppm diets, respectively.
[c, d, e] Means in columns with different superscripts are significantly different (P<0,01)

Tab. 3: Plasma and tissue magnesium in lambs fed different levels of dietary magnesium[a].

Dietary magnesium ppm	Plasma mg/100 ml	Femur[b] %	Kidney[c] %
500	2,40[d]	0,82	0,009[d]
5500	3,30[e]	0,95	0,097[e]
10,500	3,62[f]	1,15	0,108[e]

[a] Each value represents the average for five animals.
[b] Expressed as percent of femur ash.
[c] Expressed as percent of kidney dry matter.
[d, e, f] Means in columns with different superscripts are significantly different (p<0,01)

absorbed (r= −0,55) and net retained Mg (r= −0,53). An increased fecal excretion of radioactive Ca has been reported previously by *Chicco* et al. (1973) while in the same study stable Ca was not influenced by Mg intake. *Myburgh* and *Du Toit* (1970) reported a negative Ca balance when an excess of Mg sulphate was fed to sheep. Average values for metabolic fecal Ca, when expressed on a body weight basis, ranged from 8,5 to 15 mg/kg body weight daily, presenting a greater variation than those values reported by *Brathwaite* (1975), which ranged from 9,1 to 11,2 mg/kg body weight daily in sheep. The metabolic fecal ^{47}Ca values, as percent of the injected dose, were lower than 15 % value suggested as a constant by *Hansard* et al. (1952) for 12 to 36 months old cattle.

Increased dietary Mg resulted in a greater loss of ^{47}Ca (P<0,01) and stable Ca in the urine (P<0,05), the latter only when expressed as per-

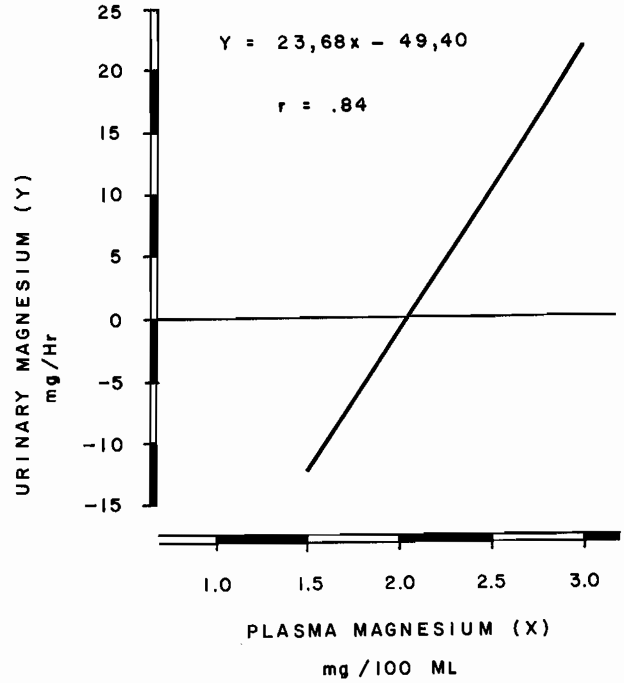

Fig. 1: Relation between plasma and urinary excretion of magnesium.

cent of the true absorbed calcium (table 5). While dietary Mg was shown to increase renal loss of Ca in swine (*Miller* et al. 1965) and in rats (*Clark* 1968), this did not occur with sheep (*Chicco* et al. 1973; *Dutton* and *Fontenot* 1967; *Hjerpe* 1967) and cattle (*O'Kelly* and *Fontenot* 1973).

In this experiment the results on urinary excretion tend to support the findings of a clearance study conducted by *Samily* et al. (1960) who demonstrated that Ca and Mg, each reciprocally, affect the excretion of the other and probably

Tab. 4: Fecal excretion and metabolic fecal calcium and apparent and true absorption of calcium in lambs fed different levels of dietary magnesium[a, b].

Dietary magnesium ppm	Fecal excretion Ca mg/day	Apparent absorption Ca[c] %	Apparent absorption ^{45}Ca[d] %	Metabolic fecal Ca mg/day	Metabolic fecal Ca %	Metabolic fecal Ca[e] %	Metabolic fecal ^{47}Ca[f] %	True absorption Ca %
500	884	43,3	63,1[g]	442	28,4	39,5	12,4	71,7[j]
5500	965	38,2	51,9[h]	309	19,8	33,8	11,5	58,6[k]
10,500	1037	33,5	45,4[i]	271	14,7	34,1	11,1	50,9[l]

[a] Each value represents the average of five animals.
[b] The average intake of calcium per lamb was 1560 mg/day.
[c] Expressed as percent of daily stable calcium intake.
[d] Expressed as percent of oral dose of ^{45}Ca.
[e] Expressed as percent of true absorbed stable calcium.
[f] Expressed as percent of injected dose of ^{47}Ca.
[g, h, i] Means in columns with different superscripts are significantly different (P<0,01).
[j, k, l] Means in columns with different superscripts are significantly different (P<0,05).

compete for a common reabsorptive mechanism.

Plasma and bone Ca was not affected by dietary levels of Mg (table 6). In reasearch with calves and sheep, *Smith* (1957, 1954), *Thomas* (1959) and *Chicco* et al. (1973) reported no change in bone Ca due to dietary Mg. In addition, *Steward* and *Moodie* (1956), *Dehority* et al. (1961) and *Chicco* et al. (1973) indicated that supplemental Mg decreased serum Ca in sheep. *Dutton* and *Fontenot* (1967), *O'Kelley* and *Fontenot* (1973) and *Hjerpe* (1967), however, found no effect of Mg supplementation on blood Ca, and *Parr* (1957) showed an increase in serum Ca after Mg supplementation to calves.

Plasma clearance patterns of ^{47}Ca are shown in figure 2. Plasma clearance of ^{47}Ca for all treatments could be characterized by a 3-term exponential function as follows:

$$Y = A_1e^{-k_1t} + A_2e^{-k_2t} + A_3e^{-k_3t}$$

Data for the compartmental parameters and rate constants of ^{47}Ca plasma clearance for the sheep fed different levels of dietary Mg are summarized in table 7. The multi-term exponential functions

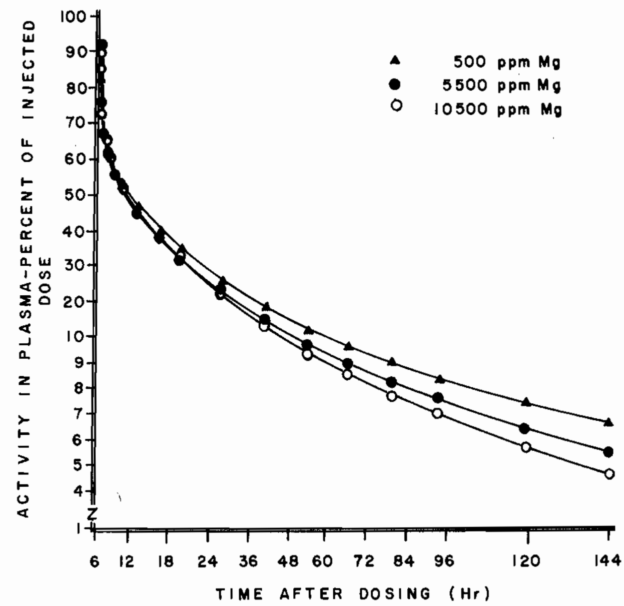

Fig. 2: Plasma clearance of ^{47}Ca of Lambs fed different levels of dietary Mg.

Tab. 5: Urinary excretion and net retention of calcium in lambs fed different levels of dietary magnesium[a].

Dietary magnesium ppm	Urinary Ca mg/day	Urinary Ca[b] %	Urinary ^{45}Ca[c] %	Urinary ^{47}Ca[d] %	Net retention %
500	12,7	1,36[e]	0,299	0,858[g]	42,5
5500	18,2	1,99[f]	0,235	1,275[h]	37,6
10,500	15,4	1,94[f]	0,336	1,515[i]	32,6

[a] Each value represents the average for five animals.
[b] Expressed as percent of true absorbed stable calcium.
[c] Expressed as percent of absorbed oral dose of ^{45}Ca.
[d] Expressed as percent of injected dose of ^{47}Ca.
[e, f] Means in columns with different superscripts are significantly different ($P<0{,}05$).
[g, h, i] Means in columns with different superscripts are significantly different ($P<0{,}01$).

Tab. 6: Plasma and femur calcium in lambs fed different levels of dietary magnesium[a].

Dietary magnesium ppm	Plasma Ca mg/100 ml	Ca[b] %	Femur Calcium			
			^{45}Ca		^{47}Ca	
			dpmx 10[3c]	%x 10 – [3d]	dpmx 10[3c]	%x10 – [3c]
500	9,52	35,9	224,4	51,5	89,9	83,8
5500	10,26	35,9	208,4	58,8	96,5	90,0
10,500	9,96	35,3	189,6	76,7	94,3	87,9

[a] Each value represents the average for five animals.
[b] Expressed as percent femur ash.
[c] Expressed as dmp/g dry fat-free femur.
[d] Expressed as percent of absorbed oral dose of ^{45}Ca.
[e] Expressed as percent of injected dose of ^{47}Ca.

Tab. 7: Equations describing removal of intravenously administered ^{47}Ca from the circulating plasma in sheep receiving different levels of dietary magnesium[a, b].

Supplemental magnesium	Compartment	Compartmental Coefficients			Biological half life hr[c]
		A	k	r^2	
500	A1	44,02	−2,40	0,99	0,29
	A2	24,44	−0,095	0,92	7,30
	A3	21,05	−0,008	0,99	87,82
5500	A1	45,70	−3,27	0,98	0,21
	A2	29,86	−0,092	0,93	7,55
	A3	18,64	−0,009	0,98	74,50
10,500	A1	47,25	−3,08	0,99	0,23
	A2	27,61	−0,098	0,95	7,07
	A3	20,46	−0,010	0,98	67,20

[a] Compartmental analysis based on generalized model:
$Y = A_1 e^{-k_1 t} + A_2 e^{-k_2 t} + A_3 e^{-k_3 t}$

[b] Five animals per treatment.

[c] No significant differences within similar compartments due to treatment.

of ^{47}Ca plasma clearance indicate that the amount of inorganic Ca removed per unit time from the plasma was not affected by dietary levels of Mg.

The data presented suggest that dietary Mg levels influenced the mechanisms involved in Ca absorption and excretion while the increased Mg body pool apparently did not affect Ca uptake at the tissue level.

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