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INTERRELATIONSHIPS OF DIETARY MAGNESIUM, POTASSIUM AND PROTEIN IN HYPOMAGNESEMIA OF RATS

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Summary

The purpose of this study was to investigate the effects of dietary magnesium, nitrogen and potassium on the hypomagnesemic syndrom in rats. Two hundred seventy male Spragu-Dawley rats weighing 50–60 g at the beginning were used in a 3x3x3 factorial treatment design. The experiment thus included 27 treatments, with ten rats randomly allotted to each.

The experimental animals were fed diets in which the concentrations of Mg, N (as protein) and K were varied. Mg levels covered the range from deficiency to dietary adequacy.

The symptoms of Mg deficiency, including skin lesions and lowered Mg contents of blood, muscle and bone, occured more quikly or with greater severity on the Mg-deficient diet when the levels of dietary N, supplied as casein, were increased from 2.2 to 3.0 or 3.8 %. These observations indicate that the interference by N is quantitative rather than qualitative and that presence of nitrate or other nonprotein nitrogen is not essential to the depression of Mg availability.

Elevated dietary K levels did not appear to influence the depressed weight gains and feed conversions resulting from low-Mg high-N feeding, but they did aggravate the hypomagnesemia. It is suggested that dietary K influences Mg metabolism separately, rather than as part of the Mg-N interaction.

An increased serum calcium generally occurred coincident to hypomagnesemia in this experiment, indicating that calcium is involved in some way in the Mg-N interaction. Dietary K levels did not influence serum calcium concentrations.

Özet

Farelerde rasyonlarla alınan magnezyum, potasyum ve nitrojenin hipomagnezemi sendromu üzerine etkisi

Bu araştırma rasyonlardaki magnezyum, potasyum ve nitrojenin hipomagnezemik kondisyon üzerine etkisini tesbit etmek amacı ile düzenlenmiştir. Araştırma materyali ola-

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rak Sprague-Dawley kan hattından gelen 270 adet albino erkek fare kullanılmıştır. Araştırmanın başlangıcında farelerin canlı ağırlıkları 55–60 gr. arasında olup tesadüfi örnekleme yolu ile 27 deneme grubuna ayrılmışlardır. Düzeni 3x3x3 faktöriyel tipinde olan bu araştırmada her bir deneme grubu üçer farklı seviyede magnezyum, potasyum ve nitrojen ihtiva eden 27 çeşit rasyondan birisi ile beslenmiştir.

Rasyonlarında 50 mg/kg magnezyum taşıyan gruplarda vasodilatasyon ve hiperemi ile başlayan deri lezyonları görülmüştür. Rasyondaki nitrojen seviyesi ve araştırma süresinin artması ile lezyonların sayı ve şiddeti de artmış, yaygın hematom, pustul ve kıl kayıpları meydana gelmiştir. Rasyondaki nitrojen ve potasyumun artması ve mağnezyumun azalması serum magnezyum konsantrasyonunu önemli derecede düşürmüştür ($P<0.01$). Serum magnezyumunun düşmesi üzerinde araştırma süresi, magnezyum ve nitrojen arasındaki interaksiyonlar yüksek derecede önemli etki göstermiştir. Potasyumun ise yalnız magnezyum ile interaksiyonu %5 seviyesinde önemli bulunmuştur. Rasyondaki nitrojen ve potasyumun artması ve magnezyumun azalması ile kemik magnezyum konsantrasyonu önemli derecede düşmüştür. Serum ve kemik magnezyum konsantrasyonları arasında yüksek derecede önemli bir korrelasyon ($r=0.978$) tesbit edilmiştir.

Sonuç olarak, hayvanlardaki hipomagnezemik durumun basit bir magnezyum noksanlığına değil birçok faktörlerin karıştığı komplike bir sebebe bağlı olduğu kanısına varılmıştır. Bu nedenle, özellikle sığır ve koyunlarda bazı kritik devrelerde meydana gelen ve ot tetanisi olarak bilinen hipomagnezeminin önlenebilmesi için muhtelif potansiyel faktörlere karşı muhteliorf kuyucu tdebirleribn irarada alınması gerekmektedir.

Introduction

Hypomagnesemic tetany or "grass tetany" continues to present a serious problem in the production of cattle and sheep in many parts of the world. Although the symptoms have been recognized for some years (12), it remains a disease of economic significance and increasing concern is generated by the observation that it frequently occurs on "improved" grassland (18) which suggests reevaluation of fertilization or seeding practices. Recent findings of Burau and Stout (1) have added significantly to the understanding of the physiological mechanism of hypomagnesemia, by their implication of *trans*-aconitic acid; the conditions in soil and forage which allow this mechanism to operate still require study. Sjollema(13) who pioneered in this area of investigation, reported that protein, potassium and nitrate levels were consistently high in pastures on which grazing cattle manifested symptoms of tetany.

It is generally accepted that some degree of dietary magnesium deficiency is associated with hypomagnesemia, although it is becoming increasingly evident that the deficiency is induced, rather than a simple one (14). A number of investigations have related high dietary nitrogen and potassium levels to hypomagnesemia in cattle, sheep and laboratory animals (2,5,7,9,16). This paper reports on the effects of feeding graded levels of supplementary magnesium, nitrogen (as protein) and potassium, in varying combinations, to laboratory rats on parameters including blood chemistry, bone and muscle magnesium contents and general animal health and weight gains.

Materials and Methods

Two hundred seventy male Spragu-Dawley rats weighing 50–60 g were allocated to a 3x3x3 factorial treatment design. Three levels of magnesium were fed, on which were superimposed three levels each of dietary nitrogen and potassium. The experiment thus included 27 treatments, with ten rats randomly allotted to each. The animals were individually housed in galvanized iron cages and were given their respective diets and water, *ad libitum.* Weight gains and feed consumption were recorded weekly. One half the animals selected at random from each treatment group were decapitated at the end of the fourth week and blood, skeletal muscle (right leg), heart muscle and femurs were obtained for chemical analyses. The remainder were similarly sampled at eight weeks, when the experiment was terminated.

The experiment was organized in three trials, characterized by the three different dietary nitrogen levels used: Trial one :2.2 % N, Trial two: 3.0%, and Trial three:3.8 % N. The nitrogen levels were supplied by purified vitamin-free casein. Composition of the basal diet is given in Table 1. At each level of nitrogen, three levels of magnesium (50, 200, and 400 mg/kg) and three levels of potassium (1.8, 3.6 and 7.2 g/kg diet) were incorporated, as $MgSO_4 . 7H_2O$ and K_2SO_4, respectively. The salts listed in Table 1. were weighed, appropriate magnesium and potassium variables added, and the resulting mix made up to 4 % of the total diet with glucose. Variations in the casein content of the rations, to accomodate the nitrogen variables, were compensated for by addition or deletion of cornstarch.

Skin lesions associated with hypomagnesemia were arbitrarily classified, according to severity, using a scale from one to five. Rank 1 was given those rats showing only limited erythematous conditions, while rank 5 indicated extensive hematomatous and pustulous skin lesions accompanied by extensive hair loss.

Table 1. The basal rat diet

Ingredient	g/kg	
Starch	643.0	
Casein	180.0	
Cottonseed oil	100.0	
Glucose	5.9	
Vitamin mixture	11.1	
Cellulose	20.0	
Salts:	40.0	
$Ca_3(Po_4)_2$		15.4700
KH_2Po_4		6.2648
Na Cl		5.2930
$Fe\ So_4.7H_20$		1.0001
$Mn\ SO_4.H_20$		0.1520
$ZnCl_2$		0.0098
$Cu\ SO_4.5H_20$		0.0180
$Co\ Cl.6H_20$		0.0008

An atomic absorption spectrophotometer (a combination of Techtron Model AA-3 Atomic absorption unit with a Carl Zeiss Model PMQ II spectrophotometer) was used for the determination of Mg, Ca and K in serum, bone and soft tissue samples. Methods developed by Willis (17) were used for serum Ca, Mg and K determinations. Magnesium concentrations in bone, heart and skeletal muscle were determined on freeze-dried, ether-extracted samples by the method of Parker (11).

Results

Weekly weight gains of the experimental animals are presented in Figure 1. in relation to the nine dietary variables. Differences between treatment groups in terms of weight gains and feed conversions were subjected to analysis of variance.

Rats on the low protein diet (2.2 % N) gained significantly slower ($P<0.01$) than those on the other two protein levels. There was no significant difference in growth response to the two higher levels of protein. Similarly, rat growth on the 50 mg/kg magnesium diet was significantly less than on the other two Mg levels ($P<0.01$). No significant differences in growth occured among the three levels of K applied. The interaction between N and Mg was also significant for weight gains ($P<0.01$) and for feed conversion efficiency ($P<0.05$) indicating that low Mg and high N, within the ranges studied, tended to depress growth and feed conversion.

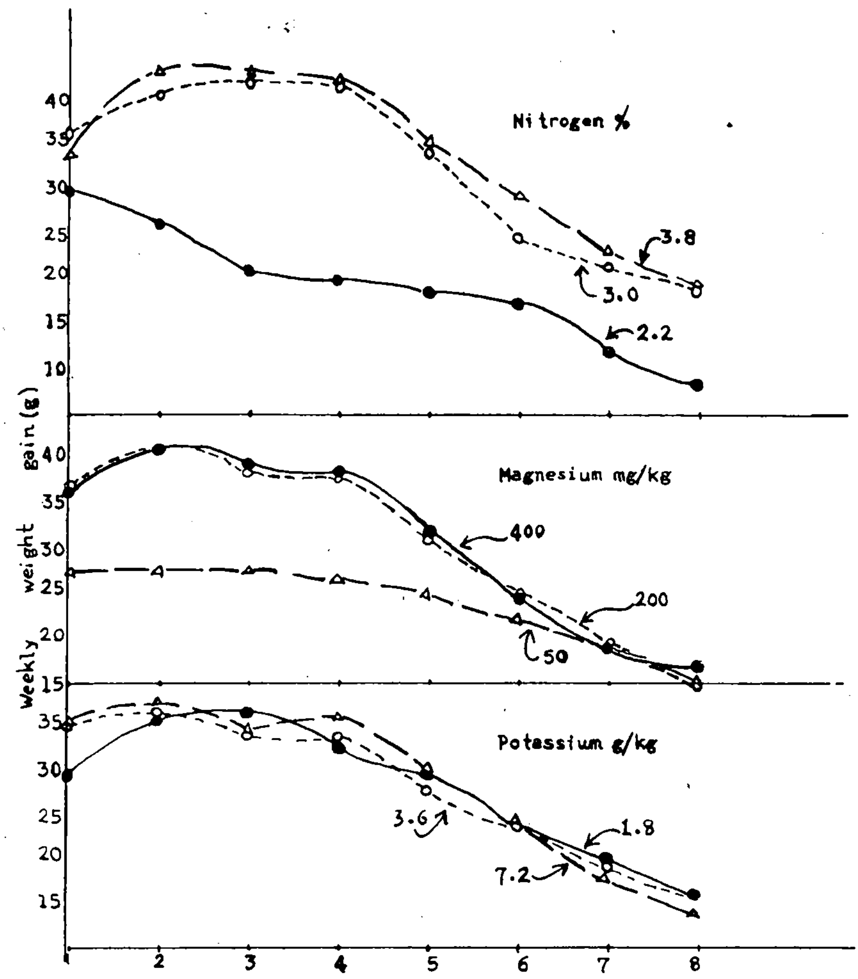

Figure 1. The weekly weight gain of rats with different dietary treatments[a]

Skin lesions, as shown in Figure 2, were observed in rats fed the 50 mg/kg level of Mg and appeared characteristic of Mg deficiency. Again, the interaction between dietary Mg and N was apparent. Vasodilation and hyperemia were visible by two weeks in rats fed the low level of Mg and 2.2 % N; they were notable in nine days in rats on the 3.0 and 3.8 % N, low Mg rations. Among the rats which remained on the experiment throughout the eight weeks, there was a significant increase in severity of skin lesions ($P < 0.05$) at the 3.0 and 3.8 % dietary N levels over the 2.2 % level, according to the nume-

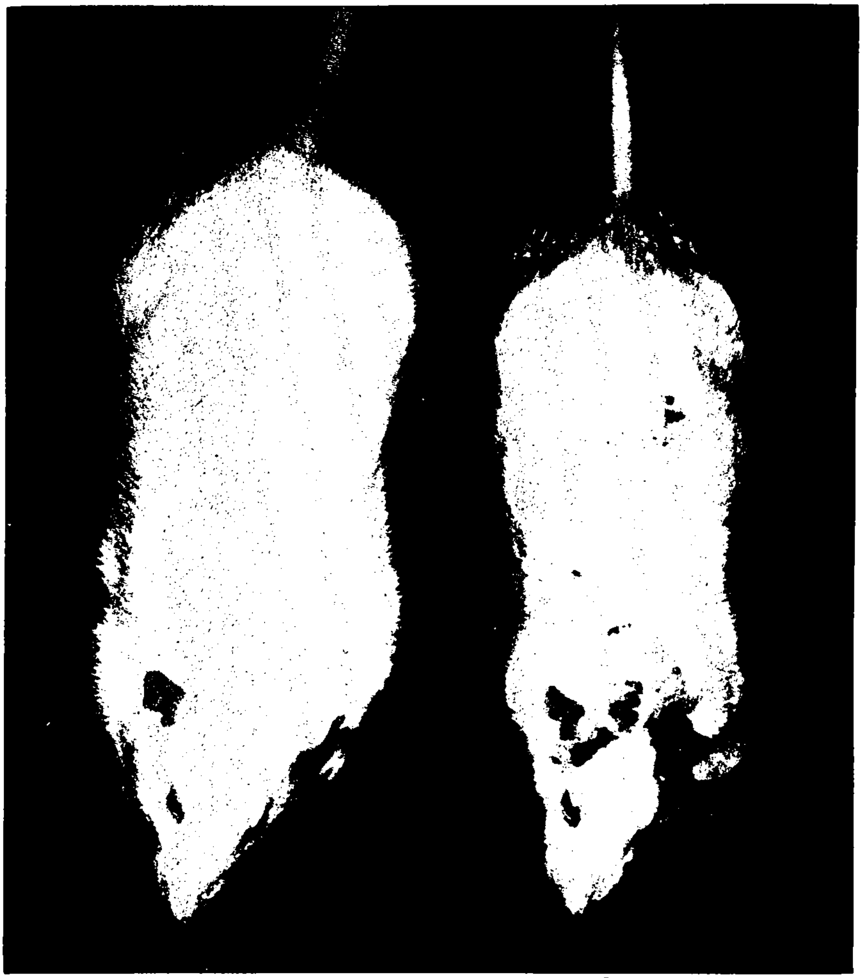

Figure 2. Skin lesions of magnesium deficiency (right) as compared to a normal rat (left)

rical rating scale, previously described. Variations in dietary K level did not exert significant effects on skin lesion development.

Concentrations of Mg, Ca and K in blood serum are listed for various dietary treatments in Table 2 and more detailed data for serum Mg are given in Table 3.

The mean concentration of serum magnesium decreased significantly ($P<0.01$) over all treatments from 2.04 mg/100 ml at four weeks to 1.73 mg/100 ml at eight weeks. This depression may be attributed partly to aging of the rats but probably also reflects the

Table: 2. Mean values of blood serum analyses for different periods and treatments

		Period or level	Number of rats	Serum Magnesium mg/100ml	Serum Calcium mg/100ml	Serum Potassium No. of rats	Serum Potassium mg/100ml
Period		4 Weeks	135	2.04[a]	10.8[a]	90	24.2[a]
		8 Weeks	135	1.73[b]	10.7[a]	90	22.6[b]
Diet	Nitrogen %	2.2	90	2.14[a]	10.5[a]		
		3.0	90	1.84[b]	10.8[ab]	90	24.6[a]
		3.8	90	1.66[b]	11.1[b]	90	22.2[b]
	Magnesium mg/kg	400	90	2.51[a]	10.8[a]	60	23.1
		200	90	2.18[b]	10.4	60	24.1
		50	90	0.95[c]	11.1[a]	60	23.1
	Potassium g/kg	1.8	90	2.06[a]	10.7	60	22.6[a]
		3.6	90	1.83[b]	10.9	60	22.9[a]
		7.2	90	1.75[b]	10.7	60	23.7[b]

a,b,c Mean values with unlike superscripts within each group are significantly $(p<0.05)$ different.

Table: 3. Mean serum magnesium values (mg/100 ml) of rats for the different cross treatments*

Diet	Level	Magnesium mg/kg diet 400	200	50	Potassium g/kg diet 1.8	3.6	7.2
Nitrogen %	2.2	2.88	2.46	1.09	2.36	2.07	2.00
	3.0	2.39	2.22	0.91	2.02	1.78	1.72
	3.8	2.28	1.86	0.84	1.79	1.64	1.64
Potassium g/kg	1.8	2.76	2.38	1.03			
	3.6	2.45	2.14	0.90			
	7.2	2.33	2.02	0.91			

* Each mean value represents 30 serum samples from 30 rats.

severe depression caused by feeding the low Mg diet. As dietary N, in protein form, increased from 2.2 to 3.0 and % 3.8 serum Mg values decreased, at all levels of Mg intake, from 2.14 to 1.84 and 1.66 % (Table 2). Moreover, increasing dietary K levels from 1.8 to 3.6 g/kg tended to decrease serum Mg values from 2.06 to 1.83 mg % respectively, although increasing dietary K further did not cause additional depression of serum Mg.

Levels of serum Ca were apparently affected by both dietary N levels $(P<0.01)$ and dietary Mg $(P<0.01)$. Potassium content of the diets apparently did not affect serum Ca concentration within the range studied.

Mean values from the analyses of bone, heart and skeletal muscle are listed in Table 4. The Mg content of bone and of bone ash was

Table: 4. Mean values for fat-free dry matter analyses of bone, heart and skeletal muscle

		Period or level	Number of rats	Bone: Ash %	Bone: Magnesium mg/100 g	Bone: Magnesium % of ash	Heart: Ash %	Heart: Magnesium mg/100 g	Skeletal muscle: Ash %	Skeletal muscle: Magnesium mg/100 g
Period		4 weeks	135	51.2[a]	281.5[a]	0.55[a]	4.76[a]	100.8[a]	4.82	114.9[a]
		8 weeks	135	56.5[b]	274.1[b]	0.49[b]	4.88[b]	98.0[b]	4.79	112.3[b]
Diet	Nitrogen %	2.2	90	53.0[a]	291.2[a]	0.55[a]	4.96[a]	101.8[a]	4.64[a]	114.9[a]
		3.0	90	53.1[a]	276.7[b]	0.52[b]	4.74[b]	97.8[b]	4.85[b]	111.8[b]
		3.8	90	55.5[b]	265.5[c]	0.48[c]	4.76[b]	98.6[b]	4.92[c]	114.1[a]
	Magnesium mg/kg	400	90	53.8[a]	373.1[a]	0.69[a]	4.69[a]	99.8	4.83	115.2[a]
		200	90	54.7[b]	336.9[b]	0.62[b]	4.87[b]	99.6	4.77	116.2[a]
		50	90	53.1[c]	123.4[c]	0.25[c]	4.90[b]	98.8	4.81	109.4[b]
	Potassium g/kg	1.8	90	53.4[a]	292.8[a]	0.55[a]	4.86[a]	99.8[a]	4.76[a]	114.0
		3.6	90	54.1[b]	275.8[b]	0.51[b]	4.87[a]	100.0[a]	4.86[b]	113.3
		7.2	90	54.1[b]	265.0[c]	0.49[c]	4.72[b]	98.3[b]	4.79[a]	113.6

a,b,c The means with unlike superscripts within each group are significantly ($P<0.05$) different.

significantly increased ($P<0.01$) with increasing levels of dietary N and K and, as expected, with decreasing dietary Mg Increasing dietary N and K similarly decreased heart muscle Mg but did not significantly change skeletal muscle Mg The correlation coefficient between serum and skeletal muscle Mg was found to be low and non-significant, suggesting that bone, rather than muscle, serves as a storage site for mobile Mg.

Discussion

This work lends support to the concept that other dietary factors in addition to the well-recognized Mg deficiency *per se*, contribute to the etiology of hypomagnesemia. The Mg defiecincy syndrome, as identified by skin lesions in the area of the head and neck, and the low serum Mg levels was obviously affected by elevated dietary levels of N. This antagonism between dietary N and Mg was suggested by the work of Sjollema (13). More recently Kemp, et *al.* (8), in studying intake and utilization of Mg from herbage by lactating cows, reported that availability of Mg increased as the forage matured. The cause for this phenomenon could have involved either qualitative or quantitative aspects of the dietary N content, since, in addition to a decrease in the quantity of protein in maturing forage, there is a shift from a high to relatively low level of non-protein nitrogen. The work reported here, involving variations in protein nitrogen only, suggests that it is the quantitative aspect (i.e. the total N present) that is the more important. It should be observed that the problems of nitrate interference with Mg metabolism observed in ruminants would probably be mediated through protein formed as a result of microbial synthesis.

It is interesting that variations in dietary K did not significantly affect the growth responses of the rats, althoug they did have some influence on serum Mg values, and hence on incidence of hypomagnesemia, at the higher end of the range of K levels administered. Others have reported such hypomagnesemic effects of increased K intakes in rats (5), sheep (9) and cows (15). Since all interactions among variables other than K for hypomagnesemia were highly significant but when K was included the significance was lost, it appears that K acts on Mg independently of the other items studied. It has been suggested (3) that high dietary K has a negative effect on the absorption of Mg, or that it increases re-excretion of Mg into the gut (4).

The finding that hypomagnesemia was accompanied by an increase of serum calcium was in agreement with those of previous reports (2, 16). Association of blood Ca and Mg has been also reported in cattle and sheep (6). It has been previously implied that K intake did not appear to affect serum Ca values.

Analytical data gathered on bone support the multiple-cause concept for Mg deficiency in that the simultaneous effects of dietary variables on bone Mg was greater than the separate constituent effects. The rather wide variations noted in Mg content of bone and bone ash suggest the mobility of bone Mg and its probable usefulness in compensating for serum magnesium deficiency. The correlation coefficient between Mg in bone ash and serum magnesium in this study, r=0.978, was higly significant. Others have also speculated on the mobility of bone Mg (5, 10).

These data support the hypothesis that the hypomagnesemia condition in animals is not related to a simple dietary deficiency, but rather a complex interaction. The findings suggest that cropping practices that give rise to high N and K levels in feed herbage may aggravate the problem of Mg deficiency in domestic species of animals.

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