EFFECTS OF THE IMBALANCE IN THE DIETARY RATIO CALCIUM TO PHOSPHORUS TO MAGNESIUM ON THE YOUNG RAT

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INTRODUCTION

At the Balcarce Experiment Station, Province of Buenos Aires, Argentine Republic, a team of both Argentine and foreign research workers are studying the factors causing a disease called "enteque seco". The installation of laboratories for Animal Biology, Plant Biology, Soils, Pathology and Fertility, as well as the provision of facilities for experiments with large animals, came up as a result of an agreement between INTA (National Institute for Agricultural Technology) and FAO (Food and Agricultural Organization of the United Nations).

The above mentioned disease chiefly affects the female bovine either at the heifer stage or when four to five years of age. The occurrence of the disease in those herds grazing on low, saline, poorly drained lands, indicates a relationship with the nutritive contents of the pasture. As a result studies have been directed towards a complete survey of the natural grasses, the soils on which they grow and the chemical and pathological condition of the animals. As the condition does not appear to be caused by viruses of paratuberculosis or Johne's disease, efforts are focused on the mineral picture of the problem. The main research effort is now directed to study the mineral contents of grasses, carrying on feeding trials with mineral supplements when a particular deficiency is suspected.

The purpose of this study was to determine the effect of imbalanced ratios of calcium, phosphorus and magnesium upon the laboratory rat.

LITERATURE REVIEW

Enteque Seco

The earliest reference to the existence of enteque seco was 1913, when Lignieres (1913) described lesions in the arteries, heart and lungs of sheep. Affected animals were also found in Matto Grosso (Brazil) by Pardi and Dos Santos (1947). These researchers connected the causes to problems of the soil. Tibirica (1927), Vasconcelos (1916) and Pires (1942) also described similar lesions.

In Jamaica, a disease was described by Arnold and Brass (1956). They called it "Manchester Wasting Disease" and presents similarities with "enteque seco". A similar disease occurring in Hasaii, called "nahalehy", was related to grazing problems in certain areas of the islands by Henderson (1942).

According to the description of "enteque seco" given by Carrillo and Gaggino (1961), the typical symptoms are: arching of the back, extreme emaciation, no diarrhea and the animal presents stiffness of the anterior legs, walking on the tips of the hooves. Furthermore, dyspnea is noted as well as an increase of the cardiac beats and, when the animal is excited, it falls down on the ground with difficulty in recovery. There are some reproductive troubles, but cows have been observed contacting the disease after breeding but calving normally. The calf was also normal. The anatomopathological symptoms can be described as follows: lesions in arteries, veins, heart, lungs, bone and muscle.

As similar lesions have been described as occurring in rats, guinea pigs, rabbits and calves, in this work we have tried to reproduce macro and microscopic lesions in the soft tissues of the young rat, through an imbalance of calcium, magnesium and phosphorus contained in the diet.

Role of Calcium, Magnesium and Phosphorus in Animal Nutrition

Calcium

This element is mainly located in the skeletal tissues, but it also appears throughout all the tissues in functions of control of nerve and muscle action, blood clotting and cell permeability. Choline acetylase catalyzes the formation of acetylcholine from choline, reaction in which Ca^{++} is involved. On the standpoint of muscular activity, two enzymes are involved in the following reaction which makes the energy of ATP available:

$$ATP + H_2O \longrightarrow ADP + HOPO_3^{=} + \text{energy}$$

One of these enzymes is activated by Ca and the other inhibited. In clotting of blood the conversion of fibrinogen to fibrin is catalyzed by thrombin, which in turn has been formed with Ca present as activator:

$$\text{Prothrombin} \xrightarrow[\text{thromboplastin}]{} \text{thrombin}$$

The property exhibited by Ca in forming complexes with many ions and the association of this element with cellular membranes act as a control of the diffussion of soluble electrolytes through those membranes. (Mallette, Althouse and Clagett, 1960).

As the blood level of this element is so important for the above mentioned functions, there is a control system exerted by the parathyroid gland, whose action is suggested to take place at the level of kidney membranes, reducing or increasing calcium excretion. When not enough calcium is supplied by the diet, this element is mobilized from the reserves stored in the bone trabeculae.

Magnesium

There is a close relationship between this element and phosphorus and calcium. The main contents of magnesium in the body is in teeth and skeleton, but it also plays an important role in enzyme activation. The enzymatic actions most affected by the presence of this element are those of gluconolactonase, transketolase, all the kinases and some of the phosphatases (Mallette, Althouse and Clagett, 1960). The effects of its deficiency (hypomagnesemia) and tetany are widely spread all over the world and there is a voluminous amount of information about this disorder which has been compiled by Rook and Storry (1962).

For farm animals in general, requirements of magnesium are in the order of 0.06 percent of the dry ration. Due to the antagonistic effects of both phosphorus and calcium on the availability of magnesium, the levels of those elements have to be kept at normal amounts in order to prevent the precipitation of a magnesium deficiency (Maynard and Loosli, 1962).

Phosphorus

As in the case of both calcium and magnesium, phosphorus is widely distributed throughout the animal body, namely soft tissues, organic

fluids and bones. But its highest amount is found in the latter, where its concentration is about 70 to 80 percent of the total phosphorus in the body. Its importance is also evident by the formation of ATP, glucose-1-phosphate, phosphoproteins, nuclei acids and others. Its enzymatic action is present in pepsin. The requirement of cattle for phosphorus is fixed between 3 and 20 g./day, depending on size, with special increments during lactation (Mallette, Althouse and Clagett, 1960). These requirements are not always met by the hays or fresh grasses from which the animal feeds; so supplementation is recommended to prevent the consequences of its deficiency. Rickets is among the most serious of these deficiencies, and is closely related to an adequate supply of calcium and vitamin D, showing once more the mineral and vitamin interactions (Maynard and Loosli, 1962).

Orent et al. (1934) observed that animals fed magnesium deficient diets showed a retention of calcium in bones. Duncan et al. (1935) reported that calves with low plasma magnesium presented tetany which could not be differentiated from that of calves with low calcium in blood plasma. Tufts and Greenberg (1936) found that magnesium levels in soft tissues were not altered by a magnesium deficient diet. In similar diets calcium was shown to increase an average of 300 percent, mainly in the kidneys. This fact could indicate that the metastasic calcification is part of a degenerative process of the kidneys, induced by magnesium deprivation. Colby and Frye (1951) reported that the following findings were related with the dietary levels of protein, calcium and magnesium: a very high level of protein aggravates the deficiency of magnesium; the same effect was observed when animals were fed a normal protein diet containing high levels of calcium; however,

high calcium and high protein together did not aggravate the process as much as each of them individually. Vitale et al. (1957) showed that arterosclerosis-producing diets increased the magnesium requirement of the rat: the induced arterosclerosis was reduced or retarded by administration of magnesium through the diet. This was confirmed by Hellerstein et al. (1957).

Phosphorus deficiency symptoms include depraved appetite ("pica"), emaciation, reproductive troubles and others. Wise et al. (1963) studied a wide range of calcium to phosphorus ratios in calves and found that a normal performance was obtained with ratios as high as 7:1. Decreased performance was obtained with ratios below 1:1 and above 7:1. They also found that serum magnesium levels decreased with high phosphorus to low calcium ratios.

The relationship of magnesium to nitrogen in the diet of Merino sheep was studied by Stillings et al. (1964). They reported that high nitrogen-containing forages depressed magnesium utilization 11 to 16 percent; however, high nitrogen favored calcium retention and vitamin D increased availability of both calcium and magnesium in low nitrogen-containing forages. Greater availability and retention of phosphorus was found in animals fed high nitrogen.

Forbes (1963) studied the effects of varying the ratios of calcium to magnesium to phosphorus and found that high levels of calcium precipitated a magnesium deficiency only when the phosphorus supply was inadequate. The ratio calcium to magnesium gain in the severely deficient animals was 160:1, as opposed to 40:1 in animals fed the normal diet. Urine analysis did not reveal a predisposition to a deposition of calcium in kidney tissue when the diet was low in magnesium along

with added calcium or phosphorus. Forbes (1964) also studied the restoration of normal tissue levels of magnesium and calcium following a magnesium deficiency and found that after three weeks of adequate magnesium diet, the rats continued to retain the major portion of the calcium which was deposited in their kidneys during the deficiency. Decalcification was enhanced, however, by producing renal tubular acidosis by the use of ammonium chloride. He suggested that restoration could proceed independently of removal of calcium deposits, but this was not demonstrated. Restoration in bones could proceed as rapidly as depletion.

Mineral nutrition is concerned with the interrelationships existing among the various mineral elements and these are incompletely summarized in Mulder's Chart as presented by Schutte (1964). Forbes (1964) studied the effects of calcium, phosphorus, lactose and sources of protein in diets both deficient and adequate in zinc, and found that soybean protein stimulated feed intake and weight gain of rats fed low levels of zinc. Depression in zinc absorption accompanying the presence of both calcium and soybean protein was greater than in the presence of either variable alone. The effect of calcium level was to depress zinc absorption but phytic acid probably is involved in this phenomenon. The inclusion of soybean protein inhibited the percentage utilization of calcium, magnesium, phosphorus, zinc and iron as compared to that exhibited by rats receiving egg white protein. Inclusion of lactose improved absorption and balance of calcium, and the absorption but not the balance of magnesium, and was without a consistent effect on zinc absorption.

Luecker and Lofgreen (1961) worked with sheep and used the isotope dilution technique to measure the effects of calcium to phosphorus ratios

on absorption in animals fed calcium:phosphorus ratios 0.8:1; 2.8:1 and 6.0:1. They found that the excretion of metabolic fecal calcium remained constant. Metabolic fecal phosphorus increased with increasing phosphorus absorption and decreased with increasing calcium absorption.

EXPERIMENT

As calcifications in soft tissues are among the most remarkable characteristic of the 'enteque seco' disease under study in Argentina, it was decided to determine if a mineral imbalance would produce these lesions in rats. The design of the experiment was similar to that of Forbes (1963) except that different levels of magnesium and different protein sources were employed in the present experiment.

Experimental Procedure

Forty male albino rats with an initial average weight of 48.6 gm. were fed a commercial rat pelletted food for one week. Five rats were then assigned at random to each treatment in the 2 x 2 x 2 factorial arrangement of treatments shown in Table I, and fed the experimental rations for 28 days.

TABLE I

EXPERIMENTAL DESIGN

	Ration							
	1	2	3	4	5	6	7	8
Calcium, %	0.8	0.8	0.8	0.8	0.4	0.4	0.4	0.4
Magnesium, ppm.	142	142	71	71	142	142	71	71
Phosphorus, %	0.5	0.2	0.5	0.2	0.5	0.2	0.5	0.2

A metalic frame with four rows of six cages on both fronts was utilized to house the experimental animals, each cage measuring 20 x 18 x 25 cm. All cages were provided with a metallic screen for the collections of feces and a funnel-shaped stainless steel attachment for drainage of urine into a beaker for each rat. During adjustment trials it was observed that the metallic screen allowed the mixture of feces with urine. An attempt was made to remedy this situation by covering the screen with a piece of plastic screen having 30 square holes per cm^2. Eight diets, the composition of which are shown in Table II, were prepared, one for each treatment. The diet for each rat was placed in tightly capped containers and kept under refrigeration during the length of the experiment. The rats were fed ad-libitum, with the feeders refilled as necessary. Glass bottles with attached suckling tubes were used to supply glass-redistilled water. Collection of urine and feces was made during the 28-day feeding period. Weights were recorded at 0, 14 and 28 days, using a tared cage and taking the readings to one-tenth gram. At the termination of the trial all the rats were sacrificed by decapitation; blood samples were collected in tubes with 0.1 ml. of 0.6 percent heparin and kept under refrigeration until analyzed. Hemoglobin and hematocrit were determined immediately after sacrifice. The carcasses were prepared for microscopic examination of tissues.

An autopsy was performed on all animals; samples of kidney, lungs, heart and liver were taken for hispathological examination and fixed in 10 percent formol and sodium acetate. Hematoxilin-eosin and Van Kossa's reagent, which is specific for calcium, were used to stain slices prepared from these tissues.

TABLE II

DIETS

Component	Percent
Soybean protein	20.00
Corn oil	10.00
Starch	35.50
Dextrose	25.00
Cellulose	5.20
B-vitamins	0.05
Vitamins A and D	0.05
Choline chloride	0.20
Minerals[a]	4.00
	100.00

[a]Mineral Mixture	Percent
Calcium, magnesium and phosphorus compounds[1]	77.370
Potassium chloride	12.970
Sodium chloride	8.011
Cupric sulfate	0.008
Ferric ammonium citrate	1.528
Manganese sulfate	0.020
Ammonium alum	0.009
Potassium iodide	0.004
Sodium fluoride	0.051
Molybdic acid	0.002
Zinc chloride	0.019
Cobalt chloride	0.008
	100.000

[1]Levels of calcium, magnesium and phosphorus were supplied in accordance with Table I, by calcium carbonate, magnesium carbonate and monosodium phosphate. These levels were confirmed by subsequent analysis of the diets.

The analytical procedures were as follows:

a) Blood samples. After precipitation of the protein by 10 percent trichloroacetic acid, separation of plasma was accomplished by centrifugation. The supernatant was diluted and aliquots taken for calcium and magnesium determinations by atomic spectrophotometry (Perkin-Elmer, 1964) and for phosphorus by the Fiske-SubaRow method (1925). Using direct methods for the determination of red blood cells, hemoglobin and packed cells, the other values presented below have been obtained from them, utilizing the formulas presented by Wintrobe (1961) as follows:

$$\text{Mean corpuscular hemoglobin, } \mu\mu\text{gm.} = \frac{\text{hemoglobin, gm./1000 ml.}}{\text{RBC, millions/mm}^3}$$

$$\text{Volume Index} = \frac{\text{hematocrit, ml./100 ml. x 2.3}}{\text{RBC x } 10^6/\text{mm}^3 \text{ x } 20}$$

$$\text{Mean corpuscular volume} = \frac{\text{volume packed red cells/1000 ml.}}{\text{RBC, million/mm}^3}$$

b) Feces. After drying this material at 60° C. for four days, it was ground in a No. 3 Wiley Mill with a 1 mm. mesh. Preceded by a complete homogenation an aliquot of about 1 gm. was taken for mineral analysis. This amount was ashed at 550° C. during four hours, then dissolved in 6 N HCl and made up to a volume of 25 ml. with distilled water. With proper aliquots of this solution analyses were run as for blood.

c) Urine. All collections were dried on steam-heated plates, then dissolved with 6 N HCl and made up to a volume of 10 ml. Mineral analyses were run as for the feces.

Data were subjected to analysis of variance (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Table III shows the effects of treatments upon gain, feed consumption and feed efficiency and there were no differences ($P > .05$). In a similar experiment involving two levels of calcium (0.4 and 0.8 percent), magnesium (142 and 420 ppm) and phosphorus (0.19 and 0.50 percent) Forbes (1963) found that a combination of high calcium and low phosphorus caused lower feed intakes and gains. No explanation is offered for this apparent discrepancy in results. There were certain differences in experimental techniques that could bear upon the subject. Forbes used casein as a protein source while isolated soybean protein was used in the present experiment. The calcium and phosphorus levels of the present experiment were the same as used by Forbes; however, lower magnesium levels (71 and 142 ppm) were used. Isolated soybean protein contains a high level of phytic acid (O'Dell and Savage, 1960) and added levels of calcium reduces the availability of phosphorus (Waldroup *et al.*, 1965) and zinc (O'Dell *et al.*, 1964); however, these factors should tend to accentuate the effects of the high calcium levels when isolated soybean protein is fed. Perhaps there are other differences between the basal rations which offer an explanation.

Mineral balance data for calcium, magnesium and phosphorus are shown in Table IV, V and VI, respectively. As feed consumption was not affected by treatments ($P > .05$), the intake of each mineral in general varies with the level in the diet. Level of dietary calcium

TABLE III

EFFECTS OF TWO LEVELS EACH OF CALCIUM, MAGNESIUM AND PHOSPHORUS ON THE WEIGHT GAIN, FEED CONSUMPTION AND FEED EFFICIENCY OF RATS

Item	Diets								Std. Error
	1 +++[a]	2 ++-	3 +-+	4 +--	5 -++	6 -+-	7 --+	8 ---	
Avg. initial wt., gm.	52.1	59.8	57.8	43.3	48.2	49.3	48.9	45.6	
Weight gain, gm.	42.5	50.8	38.8	44.4	46.3	43.8	41.9	48.9	6.20
Feed cons., gm.	251.5	251.0	227.4	227.1	241.7	248.6	224.4	240.9	20.40
Feed efficiency, gm. intake/gm. gain	6.0	5.0	6.2	6.8	5.3	5.8	4.6	5.0	0.84

[a]Calcium, magnesium and phosphorus, respectively. The plus (+) sign refers to the higher level and the minus (-) to the lower level of each element.

TABLE IV

EFFECTS OF LEVELS OF CALCIUM, MAGNESIUM AND PHOSPHORUS UPON EXCRETION AND RETENTION OF CALCIUM

Item	Diets								Standard Error
	1 +++[a]	2 ++-	3 +-+	4 +--	5 -++	6 -+-	7 --+	8 ---	
Intake, gm.[b]	2.012	2.008	1.819	1.817	0.967	0.995	0.898	0.963	0.120
Feces, gm.[c]	0.611	0.538	0.461	0.832	0.517	0.391	0.334	0.721	0.137
% intake[d]	30.37	26.79	25.34	45.79	53.46	39.30	37.19	74.87	11.70
Urine, gm.[e]	0.035	0.099	0.041	0.097	0.047	0.058	0.035	0.048	0.010
% intake[f]	1.74	4.93	2.25	5.34	4.86	5.83	3.90	4.98	0.91
Balance, gm.[g]	0.682	0.735	1.317	0.888	0.403	0.566	0.520	0.194	0.130
% intake[h]	34.10	36.40	74.45	50.26	41.41	56.71	66.79	17.79	8.80
% absorbed[i]	69.32	65.03	76.74	55.49	46.38	60.40	52.42	24.53	11.80

[a]Calcium, magnesium and phosphorus, respectively. The plus (+) sign refers to the higher level and the minus (-) to the lower level of each element.

TABLE IV (Continued)

[b]Intake: High calcium (1.914) > low calcium (0.956)(**). Main effects which were different ($P < .05$) are shown in parentheses.

[c]Feces, gm: magnesium x phosphorus interaction (*).

	High Mg	Low Mg
High P	0.564	0.397
Low P	0.464 *	0.776 *

[d]Feces, % intake: Low calcium (43.71) > high calcium (32.07)(*).

[e]Urine, gm: Calcium x phosphorus interaction (**).

	High Ca	Low Ca
High P	0.038 **	0.041
Low P	0.098 *	0.053

[f]Urine, % intake:

1. Low calcium (4.89) > high calcium (3.57)(*).
2. Low phosphorus (5.27) > high phosphorus (3.14)(*).

[g]Calcium balance, gm:

1. High calcium (9.055) > low calcium (4.208)(*).
2. Calcium x magnesium interaction (**).

	High Ca	Low Ca
High Mg	0.709 **	0.442
Low Mg	1.103 *	0.357

3. Magnesium x phosphorus interaction (*).

	High Mg	Low Mg
High P	0.543 *	0.919 **
Low P	0.651	0.541

[h]Calcium balance, % intake:

1. Calcium x magnesium interaction (*).

	High Ca	Low Ca
High Mg	35.25 **	49.05
Low Mg	62.35 *	43.30

2. Magnesium x phosphorus interaction (**).

	High P	Low P
High Mg	37.75 **	46.55
Low Mg	70.60 *	35.05

[i]Calcium balance, % absorbed:

1. High calcium (66.64) > low calcium (45.93)(**).
2. Magnesium x phosphorus interaction (*).

	High Mg	Low Mg
High P	57.85	64.58 *
Low P	62.71 *	40.01

* $P < .05$.
** $P < .01$.

did not affect ($P>.05$) the amount of calcium excreted in the feces; however, animals receiving the lower level excreted a greater ($P<.05$) percentage of their dietary calcium in the feces, reflecting, in part, differences in intake. The amount of calcium in the feces was affected by a magnesium x phosphorus interaction ($P<.05$): when the phosphorus level was reduced in the low-magnesium rations, fecal calcium loss increased ($P<.05$), while the reduction of the magnesium level in the low-phosphorus rations resulted in increased ($P<.05$) fecal calcium. This interaction was not significant when the fecal calcium was expressed as a percentage of intake.

Dietary calcium level affected the amount and percentage of intake found in the urine; the higher level increased ($P<.01$) urinary calcium loss but when expressed as percentage of intake the lower level caused a greater percentage excretion ($P<.05$). Urinary calcium excretion, when expressed in grams per day, was affected by a calcium x phosphorus interaction ($P<.01$): reduction of dietary phosphorus level in the high-calcium diets caused increased ($P<.01$) calcium excretion, while the reduction of calcium levels in low-phosphorus diets caused a lesser ($P<.01$) urinary calcium loss. The interaction was not significant when urinary calcium was expressed as percentage of intake; however, the low-phosphorus rations caused greater ($P<.05$) urinary calcium loss than did the high-phosphorus ones.

Calcium balance, expressed in grams per day, was affected by calcium x magnesium and magnesium x phosphorus interactions: when the level of magnesium was reduced in high-calcium rations calcium retention was improved ($P<.01$) while the reduction of the calcium level in the low-magnesium rations caused lower ($P<.01$) ratention. When calcium

retention was expressed as a percent of intake, the values were affected by calcium x magnesium ($P < .05$) and magnesium x phosphorus ($P < .05$) interactions and the causes of these interactions were the same as when retention was expressed in grams per day. When calcium retention was expressed as a percentage of the absorbed, the above calcium x magnesium interaction was not significant and animals consuming the high-calcium diets stored more of the absorbed calcium ($P < .01$) than those fed low-calcium diets. A magnesium x phosphorus interaction ($P < .05$) existed and it was caused by a decreased calcium retention ($P < .05$) when the magnesium level was reduced in high-phosphorus diets and when the phosphorus level was reduced in low-magnesium diets. Thus it appears that a low level of magnesium improved calcium retention in rations containing high levels of calcium or phosphorus. The highest retention of calcium (1.317 gram/day) was found in rats receiving the diet containing high levels of calcium and phosphorus and a low level of magnesium and these results are in agreement with those of Forbes (1963). The results of the present experiment indicated that high levels of calcium were detrimental to calcium balance unless a high level of phosphorus was present. This is not in accord with those of Forbes (1963). Differences in rations and magnesium levels make a direct comparison of the results impossible. Closer study of Forbes' results indicate that the effect of high levels of calcium do not appear to be great except in the diet which contained a high level of calcium and low levels of magnesium and phosphorus. Further work in this area is indicated.

Magnesium intake was greater ($P < .01$) for the high-magnesium diets; however, intake was affected by a calcium x magnesium interaction

TABLE V

EFFECTS OF LEVELS OF CALCIUM, MAGNESIUM AND PHOSPHORUS UPON EXCRETION AND RETENTION OF MAGNESIUM

Item	Diets 1 +++[a]	2 ++-	3 +-+	4 +--	5 -++	6 -+-	7 --+	8 ---	Standard Error
Intake, gm.[b]	0.0357	0.0357	0.0163	0.0161	0.0343	0.0274	0.0159	0.0171	0.0199
Feces, gm.[c]	0.0233	0.0221	0.0223	0.0337	0.0245	0.0170	0.0174	0.0304	0.0051
% intake[d]	65.27	61.90	136.81	209.32	71.43	62.04	109.43	177.78	23.90
Urine, gm.[e]	0.0075	0.0152	0.0062	0.0099	0.0059	0.0075	0.0055	0.0073	0.0010
% intake[f]	21.01	42.58	26.29	61.49	17.20	27.37	34.59	42.69	8.48
Balance, gm.[g]	0.0049	-.0016	-.0121	.0274	0.0040	0.0029	-.0069	-.0206	0.0530
% intake[h]	13.66	-6.16	-64.93	-161.72	11.46	5.64	-66.08	-119.30	39.50
% absorbed[i]	34.80	37.72	-27.91	-101.95	32.78	33.88	-21.49	-82.61	23.68

[a]Calcium, magnesium and phosphorus, respectively. The plus (+) sign refers to the higher level and the minus (-) to the lower level of each element.

TABLE V (Continued)

[b]Intake:

1. High magnesium (0.0333) > low magnesium (0.0163)(**).
2. Calcium x magnesium interaction (*).

	High Ca	Low Ca
High Mg	0.0357 **	0.0308 **
Low Mg	0.0162	0.0165

[c]Feces, gm: Magnesium x phosphorus interaction (*).

	High Mg	Low Mg
High P	0.0239	0.0198 *
Low P	0.0196 *	0.0321

[d]Feces, % intake: Low magnesium (158.33) > high magnesium (65.16)(**).

[e]Urine, gm:

1. High calcium (0.0097) > low calcium (0.0065)(**).
2. High magnesium (0.0090) > low magnesium (0.0072)(*).
3. Low phosphorus (0.0100) > high phosphorus (0.0063)(**).

[f]Urine, % intake:

1. Low magnesium (41.26) > high magnesium (27.04)(**).
2. Low phosphorus (43.53) > high phosphorus (24.77)(*).

[g]Magnesium balance, gm:

1. High magnesium (0.0004) > low magnesium (-0.0670)(**).
2. High phosphorus (-0.0101) > low phosphorus (-0.0467)(*).

[h]Magnesium balance, % intake: High magnesium (6.18) > low magnesium (-103.0)(**).

[i]Magnesium balance, % absorbed:

1. Low calcium (-9.35) > high calcium (-57.34)(*).
2. High magnesium (34.80) > low magnesium (-58.49)(**).
3. High phosphorus (4.55) > low phosphorus (-28.24)(**).

* $P < .05$.
** $P < .01$.

($P<.05$) in which reduced intake ($P<.01$) was obtained when the level of magnesium was reduced in either the high- or low-calcium diets.

Fecal magnesium values were not different ($P<.05$) but were affected by a magnesium x phosphorus interaction ($P<.05$): reduction of the phosphorus level in low-magnesium diets or the reduction of the magnesium level in low-phosphorus diets caused increased fecal magnesium excretion. As most of the animals receiving the low-magnesium diets excreted more fecal calcium than was found in the diet, expression of fecal magnesium on a percent of intake is meaningless.

The higher levels of calcium or magnesium caused greater ($P<.01$ and $P<.05$, respectively) losses of urinary magnesium, while the lower level of phosphorus caused the greater ($P<.01$) loss. These results are in agreement with those of Forbes (1963). When the urinary magnesium values were expressed as a percent of magnesium intake, the effects of different intakes were apparent; however, the low-phosphorus diets caused a greater ($P<.05$) percentage of the dietary magnesium to be excreted via the urine.

Magnesium balance, expressed in grams per day, was affected by dietary magnesium and phosphorus levels. The higher magnesium level caused greater ($P<.01$) magnesium retention than did the lower level; however, the higher phosphorus level caused a less ($P<.05$) negative balance than did the lower phosphorus level. When the magnesium balance was expressed as a percent of intake, the high level of magnesium caused greater retention ($P<.01$) than the lower level. When expressed as a percent of the absorbed magnesium, the higher levels of magnesium and phosphorus caused greater storage ($P<.01$), while the higher level of calcium caused lower ($P<.05$) storage. These results are in accord with those of Forbes (1963).

TABLE VI

EFFECTS OF LEVELS OF CALCIUM, MAGNESIUM AND PHOSPHORUS UPON EXCRETION AND RETENTION OF PHOSPHORUS

Item	Diets 1 +++[a]	2 ++-	3 +-+	4 +--	5 -++	6 -+-	7 --+	8 ---	Standard Error
Intake, gm.[b]	1.258	0.502	1.137	0.454	1.208	0.497	1.122	0.482	0.077
Feces, gm.[c]	0.359	0.229	0.357	0.199	0.206	0.256	0.164	0.128	0.035
% intake[d]	28.54	44.02	31.40	43.83	17.05	51.51	14.62	26.56	4.59
Urine, gm.[e]	0.112	0.010	0.087	0.040	0.268	0.131	0.359	0.037	0.051
% intake[f]	8.90	1.99	7.65	8.81	22.19	26.36	32.00	7.68	4.99
Balance, gm.[g]	0.786	0.231	0.692	0.215	0.734	0.110	0.599	0.318	0.61
% intake[h]	62.12	52.64	61.60	48.06	60.24	25.30	56.70	65.60	12.90
% absorbed[i]	71.09	54.89	68.89	56.67	83.00	47.75	85.96	73.22	4.55

[a]Calcium, magnesium and phosphorus, respectively. The plus (+) sign refers to the higher level and the minus (-) to the lower level of each element.

TABLE VI (Continued)

[b]Intake: High phosphorus (1.181) > low phosphorus (0.484)(**).

[c]Feces, gm:
1. High calcium (0.284) > low calcium (0.189)(**).
2. High phosphorus (0.272) > low phosphorus (0.201)(**).
3. Calcium x phosphorus interaction (**).

	High Ca		Low Ca
High P	0.358	**	0.185
		**	
Low P	0.210		0.192

[d]Feces, % intake:
1. High calcium (36.95) > low calcium (27.43)(**).
2. High magnesium (35.28) > low magnesium (29.10)(*).

[e]Urine, gm:
1. Low calcium (0.199) > high calcium (0.062)(**).
2. High phosphorus (0.207) > low phosphorus (0.071)(**).
3. Calcium x phosphorus interaction (*).

	High Ca		Low Ca
High P	0.100	*	0.314
			**
Low P	0.025		0.084

[f]Urine, % intake:
1. Low calcium (22.06) > high calcium (6.84)(**).
2. Calcium x phosphorus x magnesium interaction (*).

[g]Phosphorus balance, gm:
1. High phosphorus (0.702) > low phosphorus (0.219)(**).
2. Magnesium x phosphorus interaction (*).

	High Mg		Low Mg
High P	0.760	**	0.646
		*	**
Low P	0.171		0.267

[h]Phosphorus balance, % intake:
1. High phosphorus (60.15) > low phosphorus (47.90)
2. Calcium x phosphorus interaction (*). (*).

	High Ca		Low Ca
High Mg	57.35	*	42.75
			*
Low Mg	54.85		61.15

3. Calcium x magnesium x phosphorus interaction (*).

[i]Phosphorus balance, % absorbed:
1. Low calcium (72.48) > high calcium (62.89)(**).
2. Low magnesium (71.18) > high magnesium (64.18)(*).
3. High phosphorus (77.23) > low phosphorus (58.13).
4. Calcium x magnesium interaction (*). (**).

	High Ca		Low Ca
High Mg	62.99		65.77
			*
Low Mg	62.78	*	79.59

5. Magnesium x phosphorus interaction (*).

	High Mg		Low Mg
High Mg	77.05		77.42
	*		**
Low P	51.32	*	64.95

* $P < .05$.
** $P < .01$.

Fecal phosphorus excretion was increased ($P < .01$) by the higher levels of calcium and phosphorus. Also the interaction of calcium x phosphorus was significant; it was caused by the reduction ($P < .01$) in level of fecal phosphorus when the level of calcium was reduced in high-phosphorus rations. Also, fecal phosphorus excretion was decreased ($P < .01$) by decreasing the phosphorus level in high-calcium diets. When fecal phosphorus was expressed as a percentage of intake, the higher-calcium or higher-magnesium diets caused a greater ($P < .01$) excretion than lower-calcium or lower-magnesium diets.

The higher levels of magnesium and the lower level of dietary phosphorus caused greater ($P < .01$) excretions of urinary phosphorus than the opposite levels of either of these elements. Also, the calcium x phosphorus interaction was significant; it was caused by an increased urinary phosphorus excretion, when ($P < .01$) the level of calcium was decreased in high-phosphorus diets and by a reduction ($P < .01$) when the phosphorus level was reduced in the low-calcium diets.

Phosphorus retention, when expressed in grams, was higher ($P < .01$) in high-phosphorus diets and was subject to a magnesium x phosphorus interaction ($P < .05$): the reduction of phosphorus level either in high- or low-magnesium diets decreased ($P < .01$) retention of phosphorus; however, the high-magnesium diets had a greater ($P < .01$) effect than did the low-magnesium level. The higher level of phosphorus promoted greater ($P < .01$) percentage retention than the lower level. Percentage retention of phosphorus was affected by a calcium x magnesium interaction ($P < .05$), which resulted from reduced ($P < .05$) retention when the calcium level was reduced in high-magnesium ration but increased ($P < .05$) when the calcium level was reduced in low-magnesium rations.

When phosphorus retention was expressed as a percentage of the absorbed, low-calcium diets promoted greater ($P<.01$) retention than did the high-calcium rations. Low-magnesium diets also promoted greater retention ($P<.05$) than the high-magnesium diets, while the high-phosphorus diets promoted greater ($P<.05$) retention than did the lower levels. Two interactions were significant (calcium x magnesium and magnesium x phosphorus), but both are readily explained by a consideration of the fecal and urinary excretions.

Forbes (1963) found that both calcium and phosphorus retentions were increased by the addition of phosphorus without respect to the supply of magnesium. The results of the present experiment in general agree with those results with the apparent exception that the higher magnesium level reduced phosphorus ($P<.01$) retention in low-magnesium rations and had no effect ($P<.05$) when the level of phosphorus was high. As the highest level of magnesium in the present experiment corresponded to the lowest level in Forbes' experiment, these results in actuality confirm and extend his results.

Hematological results are shown in Table VII and in no case did treatments cause differences ($P>.05$).

Table VIII exhibits blood plasma levels of calcium, magnesium and phosphorus. Animals consuming the high-calcium diets had higher ($P<.05$) calcium levels than those consuming the low-calcium diets. Plasma magnesium levels were lower ($P<.01$) in animals fed rations containing the higher level of calcium; however, the levels were affected by a calcium x magnesium x phosphorus interaction: the higher level of calcium decreased ($P<.01$) plasma magnesium level only if dietary levels of both magnesium and phosphorus were low. Also, the higher level of magnesium

TABLE VII

EFFECTS OF TWO LEVELS EACH OF CALCIUM, MAGNESIUM AND PHOSPHORUS ON BLOOD CONSTITUENTS

	Diets								Std. Error
	1 +++[a]	2 ++-	3 +-+	4 +--	5 -++	6 -+-	7 --+	8 ---	
RBC x 10^6/mm^3	6.36	6.53	6.22	6.36	6.38	6.34	7.13	6.49	0.45
Hb., gm./100 mg.	12.34	13.54	12.68	12.58	12.52	12.40	12.48	12.82	0.59
Packed cells, %	43.4	42.7	44.3	43.5	44.3	42.6	42.7	44.8	0.85
Mean corp. Hb., µµg.	19.6	21.4	21.0	19.8	19.8	19.7	18.0	20.7	1.81
Volume index	0.79	0.76	0.85	0.79	0.81	0.78	0.71	0.84	0.05
Mean corp. vol. μ^3	68.9	66.1	73.6	68.4	70.0	67.7	61.7	71.7	4.51
Mean corp. Hb. concentration, %	28.4	31.9	28.6	28.9	28.3	29.1	29.2	28.6	1.40

[a]Calcium, magnesium and phosphorus, respectively. The plus (+) sign refers to the higher level and the minus (-) to the lower level of each element.

TABLE VIII

EFFECTS OF TWO LEVELS EACH OF CALCIUM, MAGNESIUM AND PHOSPHORUS ON THE BLOOD PLASMA MINERAL CONTENTS

	Diets								
	1 +++[a]	2 ++-	3 +-+	4 +--	5 -++	6 -+-	7 --+	8 ---	Std. Error
Calcium, mg./100 ml.[b]	8.37	8.54	8.86	8.36	8.06	8.26	8.03	8.06	0.25
Magnesium, mg./100 ml.[c]	1.99	2.18	2.09	1.51	2.13	1.88	1.97	1.97	0.14
Phosphorus, mg./100 ml.[d]	4.2	4.6	5.4	5.3	3.9	5.0	4.0	5.6	0.43

[a]Calcium, magnesium and phosphorus, respectively. The plus (+) sign refers to the higher level and the minus (-) to the lower level of each element.

[b]High calcium (8.53) > low calcium (8.10)($P < .05$).

[c]1. Low calcium (1.99) > high calcium (1.94)($P < .01$).
2. Calcium x magnesium x phosphorus interaction ($P \leq .05$).

[d]1. Low magnesium (5.075) > high magnesium (4.425)($P < .05$).
2. Low phosphorus (5.125) > high phosphorus (4.375)($P < .05$).

were effective in increasing ($P < .01$) plasma magnesium level only when adequate calcium and phosphorus were present.

Plasma phosphorus level was depressed ($P < .05$) by the higher dietary magnesium level. It was also found that the higher dietary phosphorus level caused lower ($P < .05$) levels than the lower dietary level. No explanation is offered for this observation except to point out that a calcium x phosphorus interaction which approached significance ($P < .05$) was present.

Histological examination of the organs of the slaughtered animals did not in general show very evident calcifications. Livers of two rats from diet number 6, one from 8 and one fed the number 5 diet showed only slight calcifications. Three out of the five animals in diet 3 showed some calcifications in the kidneys. This organ was also affected in one animal in diets 1, 2, 5 and 8. These results indicate no clear cause-effect relationship attributable to the diets. Forbes (1963) found that his results did not allow him to predict the appearance of kidney calcification, beyond the criterium of the mere content of calcium, magnesium and phosphorus in the urines.

Even in a study carried on by Carrillo et al. (1961), in which two levels of dietary magnesium (24 and 240 ppm) are compared against two levels each of potassium and protein, the effect of magnesium in the production of kidney tissue alternations by calcium deposition did not appear to be clear cut.

In the present study, the animals fed the lowest level of dietary magnesium developed deficiency symptoms; the symptons were loss of hair, reddish ears and emaciation.

The results presented herein indicate that the calcifications found in enteque seco are caused by conditions more complex than an imbalance of calcium, magnesium and phosphorus. Studies conducted by Carrillo *et al.* (personal communication) indicate that dietary factors such as protein, potassium, zinc and copper may also be involved. Further study of the complex problem is indicated.

SUMMARY

There were evidences indicating that a disease affecting cattle in Argentina, characterized by extreme emaciation, stiffness of the legs and calcifications in joints and soft tissues, was due to an imbalance in the dietary ratios of certain mineral elements. In an attempt to reproduce such calcifications, two levels of calcium, magnesium and phosphorus were fed to 40 male albino rats, in a 2 x 2 x 2 factorial arrangement of treatments. Collection of feces and urine was made during the 28-day growth trial. Weight gain, feed consumption, feed efficiency, blood analyses and histological changes were also response criteria.

Treatments did not affect weight gain, feed consumption or feed efficiency in the rats.

Calcium balance was affected by significant calcium x magnesium and magnesium x phosphorus interactions: low dietary levels of magnesium improved calcium retention only in rations containing high levels of calcium or phosphorus, thus the highest retention of calcium was found in rats consuming the diet containing a high level of calcium and phosphorus and a low dietary level of magnesium.

Magnesium balance was greater when a high level of this element was in the diet. A high dietary phosphorus level caused less negative balance than the lower level of this element.

Phosphorus retention was higher when the high phosphorus diets were fed. High dietary magnesium level caused a significant reduction of phosphorus retention in low phosphorus diets.

Magnesium deficiency symptoms in rats fed the low magnesium diets were apparent by the fifteenth day after the experiment was initiated.

Animals consuming the high-calcium diets had higher plasma calcium levels than those consuming the low calcium diets. Plasma magnesium levels were lower in animals fed rations containing the higher level of calcium; however, plasma magnesium level was affected by a calcium x magnesium x phosphorus interaction: the higher level of dietary calcium decreased plasma magnesium level only if dietary levels of both magnesium and phosphorus were low. Also, the higher level of dietary magnesium was affective in increasing plasma magnesium level only when adequate calcium and phosphorus were present.

Kidney calcifications appeared in only a few of the animals, thus no clear cut cause-effect relationship was established.

LITERATURE CITED

Arnold R. M. and G. Bras. 1956. Observations on the morbid anatomy and histology of manchester wasting disease of cattle in Jamaica, and related conditions in other countries of the Americas. Am. Jour. Vet. Res. 17:630.

Carrillo, B. J. and O. P. Gaggino. 1961. Unpublished report.

Carrillo, B. J., W. G. Pond, L. Krook, F. E. Lovelace and J. K. Loosli. 1961. Response of growing rats to diets varying in magnesium, potassium and protein content. Proc. Soc. Exp. Biol. and Med. 107:793.

Colby, R. W. and C. M. Frye. 1951. Effect of feeding high levels of protein and calcium in rat rations in magnesium deficiency syndrome. Am. J. Phys. 166:408.

Duncan, C. W., C. F. Huffman and C. S. Robinson. 1935. Magnesium study in calves. I. Tetany produced by a ration of milk or milk with various supplements. J. Bio. Chem. 108:35.

Fiske and SubbaRow. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375.

Forbes, R. M. 1963. Mineral utilization in the rat. I. Effects of varying dietary ratios of calcium, magnesium and phosphorus. J. Nutr. 80:321.

Forbes, R. M. 1964a. Mineral utilization in the rat. II. Restoration of normal tissue levels of magnesium and calcium following magnesium deficiency. J. Nutr. 83:44.

Forbes, R. M. 1964b. Mineral Utilization in the rat. III. Effects of calcium, phosphorus, lactose and sources of protein in zinc-deficient and in zinc-adequate diets. J. Nutr. 83:225.

Hellerstein, E. E., J. J. Vitale, P. L. White, D. M. Hegsted, N. Zamchek and M. Nakamara. 1957. Influence of dietary magnesium on cardiac and renal lesions of young rats fed an atherogenic diet. J. Exp. Med. 106:767.

Henderson, J. M. 1942. Rev. Vet. of Hawaii.

Lignieres, Jose. 1912. Arteriosclerosis epidemic in sheep. Am. Vet. Rev. 42:284.

Lueker, C. E. and G. P. Lofgreen. 1961. Effects of intake and calcium to phosphorus ratio on absorption of these elements by sheep. J. Nutr. 74:233.

Mallette, M. F., P. M. Althouse and C. O. Clagett. 1960. Biochemistry of Plants and Animals. John Wiley & Sons, Inc. New York, New York.

Maynard L. A. and J. K. Loosli. 1962. Animal Nutrition (5th Edition). MacGraw-Hill Book Company, Inc. New York, New York.

O'Dell, B. L. and J. E. Savage. 1960. Effect of phytic acid on zinc availability. Proc. Soc. Exptl. Biol. and Med. 103:304.

O'Dell, B. L., J. M. Johe and J. E. Savage. 1964. Zinc availability in the chick as affected by phytate, calcium and ethylene-diamine-tetraacetate. Poultry Sci. 43:415.

Orent, E. R., H. D. Kruse and E. V. MacCollum. 1934. Studies on magnesium deficiency in animals. 4. Chemical changes in the bone with associated blood changes, resulting from magnesium deprivation. J. Biol. Chem. 106:573.

Pardi, M. C. and S. A. Dos Santos. 1947. Ossificacao pulmonar e calcificacao vascular en bovines do partanal matogrsssense. Veterinaria 1:3.

Perkin-Elmer. 1964. Analytical Methods for Atomic Absorption Spectrophotometry. Manual by Perkin-Elmer, Norwalk, Connecticut.

Pires, R. E. 1942. Ossificacao en pulmao de un bovino. Rev. Facul. Med. Vet. Sao Paulo 2:77.

Rook, J. A. F. and J. E. Storry. 1962. Magnesium in the nutrition of farm animals. Nutr. Abstr. and Rev. 32:1055.

Schutte, Karl H. 1964. The Biology of the Trace Elements (1st Edition). J. B. Lippincott Co. Philadelphia, Pennsylvania.

Steel R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc. New York, New York.

Stillings, B. R., J. W. Bratzler, L. F. Marriott and R. C. Miller. 1964. Utilization of magnesium and other minerals by ruminants consuming low and high nitrogen-containing forages and vitamin D. J. Animal Sci. 23:1148.

Tibirica, P de Q. T. 1927. Arteriosclerose bovine. An. Facul. Med. (Sao Paulo) 2:311.

Tufts, E. V. and D. M. Greenberg. 1937. The biochemistry of magnesium deficiency. J. Bio. Chem. 122:715.

Vasconcelos, A. 1916. Ossificacao do pulmao de un bovino. Rev. Vet. Zool. 4:390.

Vitale, J. J., P. L. White, M. Nakamura, D. M. Hogsted, N. Zamchek and E. E. Hellerstein. 1957. Interrelationships between experimental hypercholesteremia, magnesium requirement and experimental atherosclerosis. J. Exp. Med. 106:757.

Waldroup, P. W., C. B. Ammerman and R. H. Harms. 1965. Availability of phytic acid phosphorus for chicks. Poultry Sci. 44:880.

Wintrobe, M. M. 1961. Clinical Hematology (5th Edition). Lae and Febiger. Philadelphia, Pennsylvania.

Wise, M. B., A. L. Ordoveza and E. R. Barrick. 1963. Influence of variations in dietary calcium:phosphorus ratio on performance and blood constituents of calves. J. Nutr. 79:79.

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