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I am submitting herewith a thesis written by Nancy Ramsey entitled "Effects of Dietary Imbalance of Magnesium, Calcium and Potassium on Plasma Insulin in Sheep and Cattle." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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EFFECTS OF DIETARY IMBALANCE OF MAGNESIUM,
CALCIUM AND POTASSIUM ON PLASMA INSULIN
IN SHEEP AND CATTLE

A Thesis
Presented for the
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ABSTRACT

Different levels of magnesium, calcium and potassium were fed to 12 mature wethers and 30 Angus cows to investigate the effects of these cations on plasma concentrations on plasma immunoreactive insulin. Experiment I consisted of two phases each repeated in two trials. In the first phase, four groups of three wethers each were assigned to two dietary levels of magnesium (0.04 and 0.24%) and calcium (0.06 and 0.30%) in a 2X2 factorial arrangement of rations.

Dietary variables in the second phase were magnesium (0.04 and 0.27%) and potassium (2.1 and 4.7%). In experiment II, three groups of 10 cows each were assigned either to normal hay, tetany prone hay or tetany prone pasture as the only feed.

Plasma magnesium concentrations were reduced by low dietary magnesium in wethers. Cows on tetany prone hay and pasture heavily fertilized with nitrogen and potassium had lower plasma magnesium concentrations than cows on normal hay. Plasma calcium was not affected by ration in wethers but was significantly higher ($P < .05$) in cows fed normal hay than in those fed tetany prone hay. Plasma potassium of wethers was not affected by dietary magnesium and calcium levels when dietary potassium was held constant, but was increased ($P < .05$) when the wethers were fed the higher level of potassium regardless of magnesium intake. Plasma potassium of the cows was not influenced ($P < .05$) by ration. Plasma immunoreactive insulin of both sheep and cows was lower ($P < .05$) than levels reported as normal and was not affected by experimental ration.

Previous reports have shown that hyperkalemia can stimulate insulin secretion and that insulin can reach higher levels in hypomagnesemic animals. However, these changes were not detected in wethers fed semipurified diets containing different levels of magnesium or in cows fed hay or pasture which induced hypomagnesemia.

TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION	1
II. REVIEW OF LITERATURE	3
History of Hypomagnesemia	3
Magnesium Absorption	5
Effect of Insulin on Magnesium	6
Insulin and Glucagon	6
Factors Affecting Insulin Secretion	8
III. EXPERIMENTAL PROCEDURE	16
General Design of Experiment	16
Management and Procedure	16
Chemical Analysis	25
IV. RESULTS AND DISCUSSION	28
Feed Consumption	28
Balance Data/Phase I	30
Plasma Data	33
V. SUMMARY	53
LITERATURE CITED	55
VITA	63

LIST OF TABLES

TABLE	PAGE
I. Experimental Design for Sheep: Experiment I	17
II. Factors Considered in Allotment of Sheep to Rations	20
III. Ingredients in Basal Sheep Rations	21
IV. Analysis of Rations for Sheep: Experiment I	22
V. Forage Analyses: Experiment II	24
VI. Feed Consumption of Sheep: Experiment I (Grams $\pm$ S.E.M.)	29
VII. Daily Magnesium Balance of Wethers During Collection Periods: Experiment I Phase I (Grams $\pm$ S.E.M.)	31
VIII. Daily Calcium Balance of Wethers During Collection Periods: Experiment I Phase I (Grams $\pm$ S.E.M.)	32
IX. Plasma Magnesium, Calcium, Potassium and Immunoreactive Insulin: Experiment I	34
X. Plasma Magnesium, Calcium, Potassium and Immunoreactive Insulin: Experiment II	38
XI. Correlation Coefficient for Sheep Plasma Magnesium, Calcium, Potassium and Immunoreactive Insulin: Experiment I Phase I	48

LIST OF FIGURES

FIGURE	PAGE
1. Effect of Dietary Magnesium on Plasma Magnesium Experiment I, Phase I	35
2. Effect of Dietary Magnesium on Plasma Magnesium Experiment I, Phase II	36
3. Average Plasma Magnesium Experiment II	39
4. Average Plasma Calcium Experiment I, Phase I	40
5. Average Plasma Calcium Experiment I, Phase II	41
6. Average Plasma Calcium Experiment II	43
7. Average Plasma Potassium Experiment I, Phase II	44
8. Effects of Dietary Potassium on Plasma Potassium Experiment I, Phase II	46
9. Average Plasma Potassium Experiment II	47
10. Effect of Magnesium and Calcium Deficiency on Immunoreactive Insulin Experiment I, Phase I	49
11. Effect of Dietary Potassium on Plasma Immunoreactive Insulin Experiment I, Phase II	50

CHAPTER I

INTRODUCTION

The conditioning resulting in the occurrence of hypomagnesemic tetany or "grass tetany" exist worldwide. Thousands of animals are lost yearly because of this nutritional disease. It is difficult to obtain accurate figures for either the incidence of hypomagnesemia or death losses as a result of grass tetany. Nationwide, the losses cost cattle owners many millions of dollars annually plus the loss of productivity in hypomagnesemia without death.

Grass tetany appears to be more of a problem on farms with high productivity and above average management practices. It strikes cows grazing early vegetative grasses which are low in total readily available carbohydrates (sucrose, fructose, glucose and other hexoses) and magnesium, but high in potassium (Metson et al., 1966; Lentz et al., 1976). Symptoms of hypomagnesemic tetany are incoordination, loss of appetite, staggering and falling, muscle twitching, grinding of the teeth and labored breathing. If the animal is not treated, convulsions resulting in death may occur.

The metabolism and regulation of magnesium is not clearly understood. Walser (1967) stated that there does not seem to be any endocrine mechanism whose primary function is to regulate plasma magnesium. The administration of insulin and glucose tend to lower plasma magnesium (Whang et al., 1966).

The purpose of this study was to determine the effect of different levels of magnesium, calcium and potassium on plasma immunoreactive insulin in mature wethers and Angus cows. Lentz et al. (1976) have shown that an increase in potassium in blood can stimulate insulin release. Elevated insulin can result in lower blood potassium by transferring potassium from extracellular fluids to intracellular spaces (Hiatt et al., 1974). Elevated insulin can also reduce plasma magnesium concentrations (Persson and Luthman, 1974), by increasing its tissue uptake (Aikawa, 1960).

CHAPTER II

REVIEW OF LITERATURE

I. History of Hypomagnesemia

Hypomagnesemia and hypomagnesemic tetany have been studied for years. Researchers have found that the 3rd and 4th lactations are the most critical periods. An average of 22% of all cows with calves under 8 weeks of age, grazing early spring pasture is affected by hypomagnesemia or hypomagnesemic tetany.

In mature dairy cows, lactation can cause a decrease in magnesium 3 to 4 weeks after parturition and an increase later in lactation (Grace, 1972). To prevent hypomagnesemia, a dietary source of magnesium should be provided every day to maintain serum magnesium at 2.0 mg/100 ml during early, mid and late lactation (O'Kelley and Fontenot, 1969).

Adult ruminants have very low reserves of mobilizable magnesium within the body (Rogers et al., 1971). Because of this, ruminants are dependent on an almost continuous net absorption of magnesium from the alimentary tract in amounts adequate to replace losses in the milk and for tissue requirements. Any marked decrease in the net absorption of magnesium from the alimentary tract will result in a rapid fall in the blood magnesium level (Rogers et al., 1971). Unless magnesium absorption is increased, any sudden demand by the mammary gland or other tissues for large amounts of magnesium will cause hypomagnesemia which may then result in tetany.

Magnesium deficiency occurs in adult ruminants grazing on fresh spring and fall pastures in certain areas of the world (McAleese et al., 1961). A variety of pasture mixtures prone to precipitating grass tetany has been reported to be deficient in both magnesium (Sjollem, 1932; Kemp, 1960) and in readily fermentable carbohydrates (Metson et al., 1966). A reduction in magnesium availability to ruminants grazing early vegetative pasture high in potassium is thought to be a contributing factor in the development of hypomagnesemia (Madsen et al., 1976).

When dietary potassium levels are excessively high, it interferes with the ability of livestock to utilize dietary magnesium (Gross and Jung, 1978). Grass that is heavily fertilized with potassium in the early part of the season has lower levels of calcium and phosphorus. This depresses the availability of calcium to ruminants grazing the grass. The high dietary potassium gives rise to high excretion of potassium in the urine which is related to a high excretion of magnesium (Mudd, 1970).

Large doses of potassium given to sheep can reduce apparent digestibility of magnesium, decrease magnesium in urine, temporarily decrease blood magnesium and increase magnesium in feces (Stehling, 1971).

Studies by House and Van Campen (1971) and Newton et al. (1972) with radioactive ^{28}Mg have shown that the primary effect of an increased intake of potassium is to reduce the absorption of magnesium from the alimentary tract rather than drastically increasing its excretion into the intestine.

Tomas and Potter (1976) have shown that a high potassium load in sheep may reduce the retention of absorbed magnesium. Their results

indicated that most of the response to potassium was due to its effect within the rumen and part of the response was due to a more generalized influence of the potassium load on the whole animal. This could have arisen from changes in the volume of fluid flowing to and from the rumen and excreted via the kidney as a result of an increased water intake (Suttle and Field, 1967). Duarte (1974) has shown that rats fed a high potassium diet had a low plasma concentration of magnesium and excreted reduced amounts of magnesium in the urine.

Prevention and Treatment of Hypomagnesemic Tetany

Supplemental magnesium should be added to the diet of cattle to prevent hypomagnesemic tetany and maintain plasma magnesium at 2.0 mg/100 ml during early, mid and late lactation (O'Kelley and Fontenot, 1969; McLaren et al., 1975).

An emergency treatment researched by Bell et al. (1977) has been successful in preventing death from hypomagnesemic tetany. This treatment is an enema consisting of 60% magnesium chloride in water.

II. Magnesium Absorption

There is a conflict among researchers on the absorption site of magnesium in ruminants. Care and Van't Klooster (1965) reported that the intestine was the major site of magnesium absorption in ruminants. But according to recent studies by Axford et al. (1975), Ben-Gehedalia et al. (1975) and Tomas and Potter (1976) the main site of the net uptake of magnesium is from the reticulo-rumen and colon.

III. Effect of Insulin on Magnesium

Effects of insulin on plasma magnesium have been studied by various workers. Rayssiguier and Larvor (1972) reported no changes in magnesium levels in rats after insulin shock, but plasma magnesium was reported to decrease in man after intravenous administration of insulin and glucose by Whang et al. (1966). Aikawa (1960) reported that tissue magnesium content increased in rabbits after treatment with insulin and glucose. Insulin injected intravenously in young sheep at a dose of 0.5 i.u./kg caused a decrease in plasma magnesium and calcium, while infusion of glucose had no significant influence on plasma magnesium (Persson and Luthman, 1974).

The decrease in plasma magnesium sometimes observed after the administration of insulin may be due to increased uptake of magnesium by tissue (Aikawa, 1960).

IV. Insulin and Glucagon

The pancreas is the site of production of the hormones insulin and glucagon. Insulin is produced by the beta cells and glucagon by the alpha cells of the pancreas as well as the salivary glands and the jejunum (Smith, 1979).

Insulin is made up of two polypeptide chains called A and B. Chain A has a disulfide bond between the 66th and 71st position. There is also two disulfide bonds that hold chains A and B together. These bonds are important to the activity of insulin. Insulin is inactivated by an enzyme called insulinase which uses reduced glutathione which provides hydrogen to break the disulfide bonds. The islets in the pancreas of

man can only store a five-day supply of insulin. Insulin is released within one-half minute following glucose elevation in man but, the half-life of insulin is only 3 to 4 minutes in man (Grodsky and Forsham, 1966) and 13 to 15 minutes in ruminants.

Glucagon is made of one long chain of 29 amino acid residues. It has no sulfur groups as does insulin. The pancreatic secretion of glucagon is stimulated by activity, stress, fasting, starvation and injections of amino acids especially by the gluconeogenic amino acid (alanine). Food entering the stomach will cause an increase in glucagon which in turn stimulates release of insulin.

Function of Insulin and Glucagon

Insulin is considered to be the hormone which promotes the storage of metabolic fuels within cells. It clears the blood of glucose and stores it as glycogen in the tissues until needed. Insulin also increases the movement of glucose into many peripheral tissues (Bowen, 1964; West and Passey, 1967) including both muscle and fat. It has the ability to lower the K_m constant of the transport systems of muscle tissue. The K_m or Michaelis is defined as the amount of substrate needed to stimulate an enzyme at half maximum velocity. Insulin acts on glucokinase and hexokinase to lower their K_m constant so that the liver is able to take up more glucose. Glucose can not enter the adipose tissue without insulin. Response of ruminants to insulin is slower than in nonruminants (McDonald, 1977).

The function of glucagon is opposite to that of insulin. When blood glucose levels are lowered, glucagon causes the breakdown of liver glycogen to glucose which enters the blood to elevate blood glucose. In

a study by Madsen et al. (1976) two bull calves weighing approximately 100 kg were given 1 mg of glucagon intravenously which resulted in an increase in insulin and glucose.

It is important to keep a good glucagon-insulin ratio to maintain proper glucose levels. The ruminant is probably not as dependent upon this insulin-glucagon scheme as the nonruminant (McDonald, 1977). This has been explained by the fact that the ruminant is less dependent upon blood glucose and more dependent upon blood levels of volatile fatty acids as a source of energy (McDonald, 1977).

V. Factors Affecting Insulin Secretion

Insulin basically regulates carbohydrate and fat metabolism. It enhances the permeability of glucose through the cell membrane. Factors other than blood glucose which may increase insulin release include glucagon, amino acids, medium and short chain fatty acids and gastrointestinal hormones. Insulin release may be inhibited by low feed intake or starvation and low temperature.

Glucose and Fatty Acids

Glucose metabolism in adult ruminants differs in many respects from that occurring in other mammals. In simple stomached animals, dietary carbohydrates are digested to glucose and other simple sugars, absorbed into the portal blood and utilized by the body. Where as in ruminants only small amounts of glucose are absorbed and glucose must be synthesized from nonhexose sources (Lenz, 1970).

In roughage fed ruminants carbohydrates are fermented in the rumen to volatile fatty acids (VFA) (Bergman, 1973). The ruminant dependent's

less upon blood glucose and more upon blood levels of volatile fatty acids as a source of energy. The most important of the VFA's are acetic, propionic and butyric acid which are produced in a ratio of 70:20:10. This ratio can vary depending upon the type of diet and rumen microorganisms; but these three VFA's furnish 70% of the ruminants total caloric requirements.

The release of insulin in ruminants does not depend primarily on plasma glucose concentration (Bassett, 1974; Manns and Boda, 1967; Horino et al., 1968). The effects of volatile fatty acids on insulin secretion has been investigated by an increase in propionate, butyrate and valerate levels in blood plasma (Bergman, 1973). Manns and Boda (1967) observed that propionate and butyrate stimulate insulin secretion and hyperglycemia in sheep. There was an increase in plasma insulin in wethers (less than one year of age) when injected with propionate, butyrate and glucose (Trenkle, 1970a). No effect was observed with injections of acetate. Horino et al. (1968) reported that propionate and butyrate stimulated insulin secretion in sheep as well as cattle while acetate had no effect. Manns et al. (1967) presented evidence that butyrate and propionate had a direct influence on the pancreas. Trenkle (1970a) and Stern et al. (1970) observed a marked increase in plasma insulin in ruminants with butyrate injection and a small increase with the injection of glucose. As sheep increase in age there appears to be a reduction of insulin release due to stimulation by glucose or butyrate (Manns and Boda, 1965).

Amino Acids

Infusions of arginine, lysine, leucine, phenylalanine and amino acid mixtures have been associated with elevated plasma insulin in cattle and sheep (Davis, 1972; Hertelendy et al., 1970; McAtee and Trenkle, 1971). In early studies the role of amino acids in the regulation of plasma insulin was questionable because of the high doses required for stimulation and because there was not a large increase in concentrations of plasma amino acids in ruminants after feeding (Theurer et al., 1966). In recent work with sheep, Bassett et al. (1971), showed a correlation between plasma insulin levels and plasma concentrations of branched chain amino acids.

Gastrointestinal Hormones

Other factors which may regulate the release of insulin are the gastrointestinal hormones. Intestinal hormones have been found to stimulate and regulate insulin secretion in nonruminants after meals (Unger and Eisentraut, 1969). Baile et al. (1969) reported that pancreozymin would stimulate insulin secretion but secretin had no effect in feed goats. In another study intravenous injections of pancreozymin and secretin in fasted sheep resulted in the stimulation of insulin secretion (Trenkle, 1972).

From these studies it appears that intestinal hormones may cause a rise in insulin during the first hour after feeding. Combinations of these hormones along with glucose, amino acids and short chain fatty acids probably maintain insulin secretion for several hours after fasting.

Type of Feed, Anorexia and Starvation

Nutritional factors are involved in the regulation of pancreatic endocrine secretions. Nutrients absorbed following feeding directly or indirectly stimulate insulin secretion which is necessary for the storage of excess nutrients in the form of glycogen, fat and protein.

In experiments conducted by Kamalu (1970), young milk fed calves and mature lambs which received a hay and concentrate ration had elevated plasma insulin levels. The nature of insulin response was different in calves and mature lambs—insulin release being greater in the milk fed calves (Kamalu, 1970). In calves, the rise in insulin levels paralleled the rise in plasma glucose concentration. However, in lambs there was no relationship between plasma glucose levels and plasma insulin levels. Instead the insulin secretion paralleled rumen volatile fatty acid concentrations. This indicated that glucose is more important in regulating insulin secretion in the milk-fed but not in the adult ruminant (Kamalu, 1970).

Diet affects plasma insulin in sheep by altering the short chain fatty acid pattern in the rumen. Sheep fed grain as opposed to those fed hay had an increase in propionate and butyrate in the rumen (Donefer et al. 1963; Luther, 1964; Bassett, 1974). The increase in propionate and butyrate produced in the rumen of ruminants fed a high grain diet results in an increase in plasma insulin (Trenkle, 1970a; Donefer et al., 1963; Luther, 1964; Bassett, 1974).

Animals that were full fed had higher insulin levels than animals that were fasted (Romsos and Leveille, 1974; Trenkle, 1970a; Bassett, 1974; Nestal et al., 1970; Unger and Eisentraut, 1969; Vance et al.,

1968; Anguilar-Parada et al., 1969; Heding and Anderson, 1973). The insulin level in blood plasma declines in starvation. Horino et al. (1968) reported a decrease in plasma insulin levels; from 35 ± 5 uU/ml to 12 ± 2 uU/ml after one day of fasting to a minimum value of 4 to 5 uU/ml after three days of fasting (Misbin et al., 1970). McAtee and Trenkle (1971) reported that plasma insulin was not related to plasma glucose concentration after feeding or after 46 to 58 hours of fasting in heifers. Other workers have shown similar results as McAtee and Trenkle in sheep and lactating dairy cows (Horino et al., 1968; Athanasion and Phillips, 1978).

Prolonged starvation does not result in the establishment of a steady basal level of plasma insulin. A diurnal variation in plasma insulin in young men was observed during starvation by Freinkel et al. (1968). Insulin was found to be higher in the morning than in the evenings.

Temperature

Lowering the temperature can decrease insulin secretion in the isolated perfused rat pancreas (Loubatieres-Mariani et al., 1978) and intact rats (Reach et al., 1978). Sykes et al. (1969) reported that exposure to $+8^{\circ}\text{C}$ caused a 12% reduction in plasma magnesium levels of sheep but had no effect on calcium, sodium or potassium levels.

Magnesium and Calcium

The calcium/magnesium ratio is a primary regulator of the insulin secretory process (Curry et al., 1977). An alteration of the physiologic ratio of calcium to magnesium results in a marked alteration of total insulin secretion.

An adequate amount of calcium in extracellular fluids is required for insulin secretion in response to glucose or other secretagogues for insulin (Witzel and Littledike, 1973).

The ability of glucose to stimulate insulin secretion by the isolated perfused pancreas of the rat (Grodsky and Bennett, 1966) or rabbit (Milner and Hales, 1967) is inhibited by omission of calcium from the medium. These studies suggest that the calcium ion may play an important role in the mechanism of insulin release from the beta cells.

Insulin secretion is reduced as a result of pronounced hypocalcaemia following parturition (Kolb, 1977). Athanasion and Phillips (1978) observed in dairy cows that plasma insulin concentrations remained constant in the prepartum period. Following parturition the mean plasma insulin concentration decreased for 5 days to values below 10 uU/ml. By the 8th day after parturition the plasma insulin concentration had returned to prepartum concentrations, which was about 10 uU/ml.

Infusion of glucose in a cow with severe hypocalcemia fails to increase blood levels of insulin; however, if calcium is administered prior to the injection of glucose there is a rapid and substantial increased insulin secretion (Littledike et al., 1969).

Insulin secretion by isolated pancreas is inhibited when magnesium is omitted from the medium, but elevated magnesium can also inhibit insulin secretion (Bennett et al., 1969). Lentz et al. (1976) found potassium stimulated insulin secretion was higher in magnesium deficient calves than in normal calves. Curry et al. (1977) reported that magnesium plays an important role in the regulation of insulin secretion by altering the sensitivity of the beta cells of the Islets of Langerhans to glucose.

Calcium and magnesium may compete for the same active site at the cell membrane or at the granule membrane and when sites are bound by magnesium ions, calcium ions cannot exert permissive or facilitating effects on secretion.

Potassium Relationship to Insulin Secretion

Many membrane systems require potassium and calcium ions for excitation and transport (Weiss and Bianchi, 1965). The potassium-calcium effect on insulin secretion is thought to occur at the membrane level.

In obese subjects fasted for two weeks, potassium deficiency appeared to be a contributory factor causing hypoinsulinemia. Fasting obese subjects given a potassium supplement were found to have significantly better glucose tolerance and insulin secretion than control subjects given no potassium (Anderson et al., 1969). Grodsky and Forsham (1966) concluded that in men the potassium ion can independently promote insulin release.

Comparison of Biological and Radioimmunoassays of Insulin

Plasma insulin in ruminants as determined by biological assays is not directly comparable to measurements by radioimmunoassay. The difference between the two assays may be caused by factors in plasma that increase uptake and metabolism of glucose but does not react with antibodies to insulin. Berson and Yalow (1964) concluded that insulin measured by radioimmunoassay represents the active circulating form of the hormone.

Plasma Insulin in Ruminants

Plasma insulin levels of 10 to 50 uU/ml for sheep (Herelendy et al., 1970; Horino et al., 1968; Manns and Boda, 1967; Trenkle, 1970b; Trenkle, 1971), 15 to 50 uU/ml for cattle (Hertelendy et al., 1970; Irvin and Trenkle, 1971; McAtee and Trenkle, 1971; Trenkle, 1970b; Young et al., 1970) have been reported. The level of plasma insulin usually increases after feeding and declines during starvation.

CHAPTER III

EXPERIMENTAL PROCEDURE

I. General Design of Experiment

Two experiments were conducted to study the effect of different dietary levels of magnesium, calcium and potassium on the level of plasma insulin in 12 mature wethers and 30 Angus brood cows.

Experiment I consisted of two phases with two trials within each phase and two collection periods within each trial. Each phase had four rations with different dietary levels of magnesium, calcium and potassium. Three sheep were assigned to each of the four rations (Table I).

In experiment II, 10 Angus cows were assigned to each of three rations consisting of control hay, tetany prone hay and tetany prone pasture. The control hay was predominantly orchard grass considered to contain normal levels of magnesium and potassium. The tetany prone forages was fescue grown on land fertilized heavily with nitrogen and potassium and were considered likely to induce hypomagnesemia in cattle consuming them.

II. Management and Procedure

Animals

Fifteen mature wethers were selected from Blount farm at The University of Tennessee according to weight and uniformity of appearance to be used in the first experiment. The wethers were sheared, weighed

TABLE I. EXPERIMENTAL DESIGN FOR SHEEP: EXPERIMENT I

Phase I				
Trial	Rations			
	1	2	3	4
	(Basal)	(0.32%Ca)	(0.25%Mg)	(0.24%Mg, 0.28%Ca)
1	3*	3	3	3
2	3	3	3	3
Phase II				
Trial	Rations			
	1	2	3	4
	(Basal)	(4.70%K)	(0.29%Mg)	(0.26%Mg, 4.70%K)
1	3	3	3	3
2	3	3	3	3

* Number of wethers.

and then placed in dual-type metabolism stalls similar to those described by Briggs and Gallup (1949) so that fecal and urine collections and individual feed consumption could be recorded. All wethers were made as comfortable as possible and appropriate animal care was practiced.

The wethers were given an adjustment period of 6 to 8 days. Because of low hematocrit readings and high fecal egg counts, all wethers were dewormed with Tramisol as prescribed. During the adjustment periods of both phases, all wethers were started on hay and slowly transferred to the basal diet with 0.24% magnesium and 0.28% calcium. All rations were refrigerated at 4°C to prevent spoiling. The wethers were maintained on ration 4 until all wethers were consuming an average of 1200 g of the diet per day. On the 8th day of the adjustment period, 12 wethers were selected from the 15 and put on the four rations. Wethers were fed and watered with distilled water once a day.

Each phase of experiment I was conducted for 12 weeks with 6 weeks in each trial. The first phase started in August and the second in April. During phase I, electrodiagnostic evaluations were performed on all wethers including nerve conduction velocities (NCV) and electromyographic (EMG) evaluation of selected muscles. These data will not be presented in this thesis. At the end of each trial and each phase, wethers were released from their metabolism stalls, weighed and put in a pen to exercise.

Thirty Angus brood cows were selected at Holston farm of The University of Tennessee, according to an age range of 3 to 15, uniformity of appearance and expected calving data to be used in the

second experiment. This experiment began in October and ran through the first of April.

The cows were divided into three groups with 10 cows in each group. Two groups of cows were placed in stanchion stalls in an old dairy barn at Holston and started on their hay rations and the third group was turned out on the experimental pasture.

Rations

Three wethers were assigned according to weight and hematocrit reading (Table II) to each of four dietary rations in the two phases of the first experiment. The composition of the basal diets and mineral mix is shown in Tables III and IV. The dietary rations for phase I, trial one and two were: (1) basal (magnesium and calcium deficient); (2) basal with 0.32% calcium; (3) basal with 0.25% magnesium and (4) basal with 0.24% magnesium and 0.28% calcium. The rations in phase II, trial one and two were: (1) basal (magnesium deficient) with 1.98% potassium; (2) basal with 4.7% potassium; (3) basal with 0.29% magnesium and 2.16% potassium; and (4) basal with 0.26% magnesium and 4.66% potassium. These diets were balanced according to NRC recommendations for all nutrients except for those with imbalances of magnesium, calcium and potassium. The semipurified sheep diets were made by adding the premixes of minerals and vitamins with the major ingredients in a Marion horizontal mixer. Mineral compounds were finely ground when necessary and thoroughly mixed in a Hobart mixer. The complete diets were sampled as soon as mixed and a composite of daily samples was taken during the collection period.

TABLE II. FACTORS CONSIDERED IN ALLOTMENT OF SHEEP TO RATIIONS

Experiment I, Phase I		Mature Wethers			
		Ration			
Factor		1	2	3	4
Average hematocrit (%)		37	43	31	38
Average body weight (kg)		84	86	85	84

Experiment I, Phase II		Mature Wethers			
		Ration			
Factor		1	2	3	4
Average hematocrit (%)		29	30	30	31
Average body weight (kg)		87	87	88	81

TABLE III. INGREDIENTS IN BASAL SHEEP RATIONS

Ingredients	%
Corn Cobs	60
Casein	4
Alpha Protein	4
Dextrose	15
Starch	7.2
Oil	2
Urea	2
Mineral Mix	5.6
Vitamins	.2
100 %	
Mineral Mix	Amount/110 kg Rations
$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	781.000 g
NaCl	550.000 g
KI	.014 g
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	2.400 g
$\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$	.055 g
ZnCl_2	11.470 g
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	18.000 g
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	26.000 g
Se premix	22.000 g
1410.939 g	

3% added K as KCl to all diets in Phase I.

1% added K as KCl to diets 1 and 3 in Phase II.

4% added K as KCl to diets 2 and 4 in Phase II.

0.4% added Ca as CaCO_3 to diets 2 and 4 in Phase I.

0.2% added Mg as MgO to diets 3 and 4 in Phase I and II.

TABLE IV. ANALYSIS OF RATIONS FOR SHEEP: EXPERIMENT I

Phase I				
Nutrient	Rations			
	1	2	3	4
Magnesium	.035	.035	.249	.236
Calcium	.062	.317	.057	.283
Potassium	2.973	3.183	2.859	2.753
Phase II				
Nutrient	Rations			
	1	2	3	4
Magnesium	.041	.037	.285	.261
Calcium	.281	.252	.266	.228
Potassium	1.980	4.711	2.166	4.661

In experiment II, 10 cows were randomly assigned to each of the following treatments: (1) normal hay as control; (2) hay from pasture which had produced grass tetany in grazing cows; and (3) fescue pasture heavily fertilized with nitrogen and potassium. The mineral analysis of the dietary treatments of experiment II is shown in Table V.

Data Collected

Blood samples were taken via jugular veinipuncture using heparinized syringes and tubes on Monday morning of each week from each wether in experiment I. Phase I blood samples were kept at room temperature after being drawn from the sheep, during phase II the blood samples were placed in ice immediately. In experiment II, blood samples were taken from each cow using the same fashion once a month at the beginning of the experiment day 0 and twice a month beginning with day 59 until the end of the experiment.

Daily feed consumption of each wether was recorded. Two weeks after the wethers had been assigned to a ration they were put on a collection period for 7 days. Daily urine and fecal excretions were measured and approximately 10% of the total daily samples of urine and feces were collected. Five ml of concentrated HCl was put in the urine jars to acidify the urine and reduce mineral precipitation. Thymol was added to the fecal samples as they were collected to prevent molding. Urine and fecal samples collected were kept refrigerated at 2-8°C. At the end of the 7-day period, a composite sample was taken from the collection samples of urine and feces and analyzed for magnesium, calcium and potassium by using an Atomic Absorption Spectrophotometer.

TABLE V. FORAGE ANALYSES: EXPERIMENT II

Nutrient ^a	Rations		
	Normal Hay	Tetany Prone Hay	Pasture
Magnesium	.167	.240	.200
Calcium	.515	.430	.407
Potassium	1.679	2.536	1.885

^aPercentage.

Milk, urine and fecal samples were taken from the cows while on a 7-day collection period to be analyzed for mineral content. These data will not be presented in this paper. Feed samples from each experiment were taken during the collection period to be analyzed for the minerals mentioned earlier.

Statistical Analysis

Correlation, Proc GLM and Duncan's Multiple Range test were run on the sheep and cow data to determine the effect of treatments on plasma immunoreactive insulin (Barr et al., 1976).

III. Chemical Analysis

Plasma

Mineral analysis. Plasma samples were diluted to a known volume with 2 ml of Lanthanum Trioxide (0.5%) added to prevent interference from phosphate. A Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer was used to determine levels of magnesium, calcium and potassium for all samples collected during both experiments.

Immunoreactive insulin analysis. Plasma immunoreactive insulin (IRI) was assayed using a commercial kit.¹ The principle of radioimmunoassay of hormones is the binding of radioactive labeled hormone to a specific antibody to form a labeled antigen-antibody complex. This procedure takes advantage of the ability of the unlabeled hormone to compete with the labeled hormone for binding to the antibody. In

¹Phadebas Insulin Kit, Pharmoeia Laboratories, Inc., Piscataway, New Jersey.

comparison with other immunological procedures radioimmunoassay has the greatest applicability to study plasma hormones in animals (Berson and Yalow, 1964).

It is important that the hormone being measured in plasma exhibits the same immunological properties of the hormone used as the standard. This was not established on the sheep plasma but Lentz et al. (1976) compared the standard insulin solution provided with the Phadebas Insulin Test Kit with similar dilutions of bovine insulin and obtained identical standard curves.

Feces

A 100 g sample of the feces was dried for 48 hr at 60°C. The samples were then ground and a well-mixed 2 g sample was ashed in a crucible for 3 hr at 600°C. After ashing the samples were diluted with 3 ml of 6 N HCl and brought to a volume of 25 ml with glass distilled water. A known volume was taken from the 25 ml's and analyzed for magnesium, calcium and potassium.

Urine

Five ml of each urine sample was put in a 100°C oven overnight. After the moisture had been completely removed the residue was treated like the fecal samples and analyzed for the same minerals.

Feed

While the animals were on collection, small samples of their refusals were taken each day for analyses to determine intake of feed nutrients. A 2.0 g sample of each diet was ashed for 3 hr at 600°C, diluted with 3 ml of 6 N HCl and brought to 25 ml with glass distilled

water. This volume of the 25 ml was diluted further and mineral content was determined using a Perkin-Elmer 303 Atomic Absorption Spectrophotometer. Lanthanum Trioxide was added to all magnesium and calcium dilutions to prevent phosphate interference.

CHAPTER IV

RESULTS AND DISCUSSION

I. Feed Consumption

The mean feed consumptions for animals on phases I and II, experiment I, are shown in Table VI. No differences due to treatment was seen in feed consumption of sheep in phase I of experiment I. The average feed consumption of wethers in phase I was 1114 g per day. However, in phase II wethers on rations high in potassium had lower ($P < .05$) feed intake than those on rations with low potassium (Table VI). A slight difference was noted in feed consumption of cows between treatments in experiment II with the cows on pasture having the lowest dry matter intake during the collection period.

Discussion

A general low feed intake was observed in both phases of experiment I. In phase I of experiment I, there was no difference between rations in feed consumption but the feed consumption was low in all groups. A deficiency of magnesium in diets can lower the feed intake of sheep according to work conducted by Chicco et al. (1973). The low feed consumption could be due to the fine texture of the feed or environmental factors.

Wethers in phase II of experiment I on rations 2 and 4 had lower ($P < .05$) feed consumption than those on rations 1 and 3 probably because of the high potassium (4.7%) in their rations as compared to the rations with 2.1% potassium. This agrees with Newton et al. (1972) and Kunkel

TABLE VI. FEED CONSUMPTION OF SHEEP: EXPERIMENT I (GRAMS $\pm$ S.E.M.)

Phase I				
Period	Rations			
	1	2	3	4
1	1194 $\pm$ 314	1269 $\pm$ 117	1291 $\pm$ 56	1364 $\pm$ 61
2	1222 $\pm$ 295	1333 $\pm$ 116	1276 $\pm$ 143	1019 $\pm$ 645
3	693 $\pm$ 489	1070 $\pm$ 320	1024 $\pm$ 338	933 $\pm$ 486
4	1125 $\pm$ 388	930 $\pm$ 416	975 $\pm$ 537	1095 $\pm$ 524
Means	1059 ^a	1151 ^a	1142 ^a	1103 ^a
Phase II				
Period	Rations			
	1	2	3	4
1	1485 $\pm$ 176	902 $\pm$ 223	1435 $\pm$ 229	1029 $\pm$ 379
2	1479 $\pm$ 168	987 $\pm$ 224	1476 $\pm$ 131	1091 $\pm$ 44
3	1142 $\pm$ 104	1069 $\pm$ 193	1200 $\pm$ 0	1001 $\pm$ 243
4	1169 $\pm$ 144	1061 $\pm$ 265	1200 $\pm$ 0	1021 $\pm$ 233
Means	1319 ^a	1005 ^a	1328 ^a	1035 ^b

Means in a line with different superscripts are significantly different ($P < .05$).

et al. (1953) who observed a lower feed intake in lambs on a high potassium ration.

II. Balance Data/Phase I

The magnesium balance for each collection period in phase I is presented in Table VII. During the first collection period, all wethers had a negative magnesium balance. In the second collection period, only wethers on diets 2 and 3 had negative magnesium balance. A negative magnesium balance was found in wethers consuming diets 1 and 4 in the third collection. A positive magnesium balance was observed in wethers on all 4 diets during the fourth collection. The main excretory route of magnesium was the feces.

During the first and second collections all wethers had a negative calcium balance although diets 3 and 4 had 0.3% calcium (Table VIII). Wethers on diets 1, 3 and 4 had negative calcium balances in the third and fourth collections. The largest amount of calcium was excreted in the feces (Table VIII).

Discussion

A balance study accounts for the intake of a nutrient in the food and for its excretion in feces and urine. It provides data for determining if there is a gain or loss of minerals by the body.

A large variation was observed in the magnesium balance. A negative calcium balance in all diets was observed in collections 1 and 2. In collections 3 and 4, only wethers on diet 2 had a positive balance (Table VIII). There appeared to be no relationship to a negative balance and the diets. The negative balance observed is typical of

TABLE VII. DAILY MAGNESIUM BALANCE OF WETHERS DURING COLLECTION PERIODS:
EXPERIMENT I PHASE I (GRAMS $\pm$ S.E.M.)

Period	Diet	Excretion				Balance
		Intake	Feces	Urine		
1	1	.39 $\pm$.10	.48 $\pm$.00	.02 $\pm$.01		-0.10 $\pm$.09
	2	.44 $\pm$.04	.42 $\pm$.05	.05 $\pm$.01		-0.04 $\pm$.05
	3	2.92 $\pm$.13	2.15 $\pm$.02	.68 $\pm$.08		-0.07 $\pm$.06
	4	3.18 $\pm$.14	2.32 $\pm$.28	1.00 $\pm$.10		-0.13 $\pm$.19
2	1	.39 $\pm$.09	.37 $\pm$.07	.02 $\pm$.01		0.01 $\pm$.03
	2	.44 $\pm$.03	.43 $\pm$.06	.03 $\pm$.01		-0.01 $\pm$.03
	3	2.63 $\pm$.30	2.39 $\pm$.34	.88 $\pm$.12		-0.39 $\pm$.16
	4	2.12 $\pm$ 1.34	1.62 $\pm$ 1.02	.68 $\pm$.44		0.07 $\pm$.23
3	1	.29 $\pm$.17	.33 $\pm$.14	.04 $\pm$.03		-0.07 $\pm$.11
	2	.43 $\pm$.11	.34 $\pm$.09	.03 $\pm$.01		0.06 $\pm$.02
	3	2.75 $\pm$.91	1.83 $\pm$.65	.83 $\pm$.41		0.09 $\pm$.15
	4	2.48 $\pm$ 1.29	1.68 $\pm$ 1.02	1.02 $\pm$.10		-0.22 $\pm$.32
4	1	.48 $\pm$.14	.44 $\pm$.11	.02 $\pm$.01		0.02 $\pm$.01
	2	.36 $\pm$.13	.33 $\pm$.11	.02 $\pm$.01		0.01 $\pm$.05
	3	2.60 $\pm$ 1.43	1.67 $\pm$.64	.79 $\pm$.51		0.17 $\pm$.29
	4	2.89 $\pm$ 1.36	1.75 $\pm$ 1.05	.76 $\pm$.35		0.38 $\pm$.11

TABLE VIII. DAILY CALCIUM BALANCE OF WETHERS DURING COLLECTION PERIODS:
EXPERIMENT I PHASE I (GRAMS $\pm$ S.E.M.)

Period	Diet	Excretion			
		Intake	Feces	Urine	Balance
1	1	.88 $\pm$.23	1.71 $\pm$.49	.26 $\pm$.19	-1.09 $\pm$.43
	2	4.04 $\pm$.34	4.55 $\pm$.23	.71 $\pm$.15	-1.22 $\pm$.33
	3	.90 $\pm$.04	1.44 $\pm$.05	.77 $\pm$.20	-1.30 $\pm$.16
	4	4.28 $\pm$.19	4.62 $\pm$.33	1.01 $\pm$.76	-1.34 $\pm$.74
2	1	.90 $\pm$.22	1.24 $\pm$.31	.42 $\pm$.35	-0.75 $\pm$.47
	2	4.37 $\pm$.00	4.00 $\pm$.53	.72 $\pm$.17	-0.50 $\pm$.43
	3	.89 $\pm$.10	1.23 $\pm$.28	1.23 $\pm$.38	-1.56 $\pm$.50
	4	3.20 $\pm$ 2.03	2.87 $\pm$ 1.33	.85 $\pm$.77	-0.52 $\pm$.48
3	1	.50 $\pm$.30	1.21 $\pm$.35	.22 $\pm$.16	-0.96 $\pm$.26
	2	3.64 $\pm$.97	2.65 $\pm$.65	.57 $\pm$.21	0.41 $\pm$.28
	3	.52 $\pm$.17	1.08 $\pm$.11	.70 $\pm$.20	-0.26 $\pm$.16
	4	2.78 $\pm$ 1.44	2.77 $\pm$ 1.44	.57 $\pm$.25	-0.56 $\pm$.24
4	1	.49 $\pm$.14	1.37 $\pm$.39	.39 $\pm$.22	-1.27 $\pm$.31
	2	3.00 $\pm$ 1.13	2.34 $\pm$.45	.42 $\pm$.23	0.23 $\pm$.53
	3	.36 $\pm$.19	1.08 $\pm$.05	.79 $\pm$.73	-1.50 $\pm$.59
	4	3.10 $\pm$ 1.45	2.56 $\pm$ 1.23	.66 $\pm$.30	-0.12 $\pm$.20

mature animals because they do not require these minerals for growth and development as do young animals. The variation in magnesium balance paralleled the work of Field (1962) and Chicco et al. (1973).

The largest magnesium and calcium loss was in the feces. The feces serves as a path of excretion of minerals that have been absorbed and metabolized as well as those that have escaped absorption. Calcium and magnesium can be excreted in the urine as well as the feces, but the distribution between the two varies with the species and can be influenced by dietary and age factors.

III. Plasma Data

Magnesium

A significant difference ($P < .05$) was found between plasma magnesium among rations in phase I (Table IX). Wethers on treatments 1 and 2 had lower plasma magnesium levels than wethers on treatments 3 and 4 (Figure 1). The average plasma magnesium for wethers on treatments 3 and 4 was 2.28 mg/100 ml as compared to 1.16 mg/100 ml for those fed rations 1 and 2. Addition of calcium to the diet further reduced plasma magnesium of wethers fed the magnesium deficient ration, but did not consistently affect plasma magnesium of wethers fed the adequate magnesium ration (Table IX and Figure 1).

In phase II, there was also a significant difference ($P < .05$) between plasma magnesium in wethers consuming rations 1 and 2 (low magnesium) as compared to wethers receiving treatments 3 and 4 (added magnesium) (Table IX). Wethers receiving treatments with 4.7% potassium averaged slightly lower plasma magnesium than those receiving treatments with 2.1% potassium (Figure 2).

TABLE IX. PLASMA MAGNESIUM, CALCIUM, POTASSIUM AND IMMUNOREACTIVE INSULIN: EXPERIMENT I

Phase I				
Minerals	Ration			
	1	2	3	4
Magnesium	1.23 ^a	1.08 ^a	2.27 ^b	2.29 ^b
Calcium	10.30 ^a	10.43 ^a	10.15 ^a	10.31 ^a
Potassium	17.47 ^a	16.00 ^b	16.58 ^{ab}	15.79 ^b
Insulin	12.25 ^a	12.95 ^a	13.47 ^a	13.90 ^a
Phase II				
Minerals	Ration			
	1	2	3	4
Magnesium	1.51 ^a	1.49 ^a	2.32 ^b	2.24 ^b
Calcium	9.55 ^{ab}	9.31 ^a	9.56 ^{ab}	9.68 ^a
Potassium	14.49 ^a	16.43 ^b	14.89 ^a	16.77 ^b
Insulin	10.92 ^a	9.50 ^a	9.27 ^a	9.76 ^a

Means in the rows with different superscripts are different ($P < .05$).

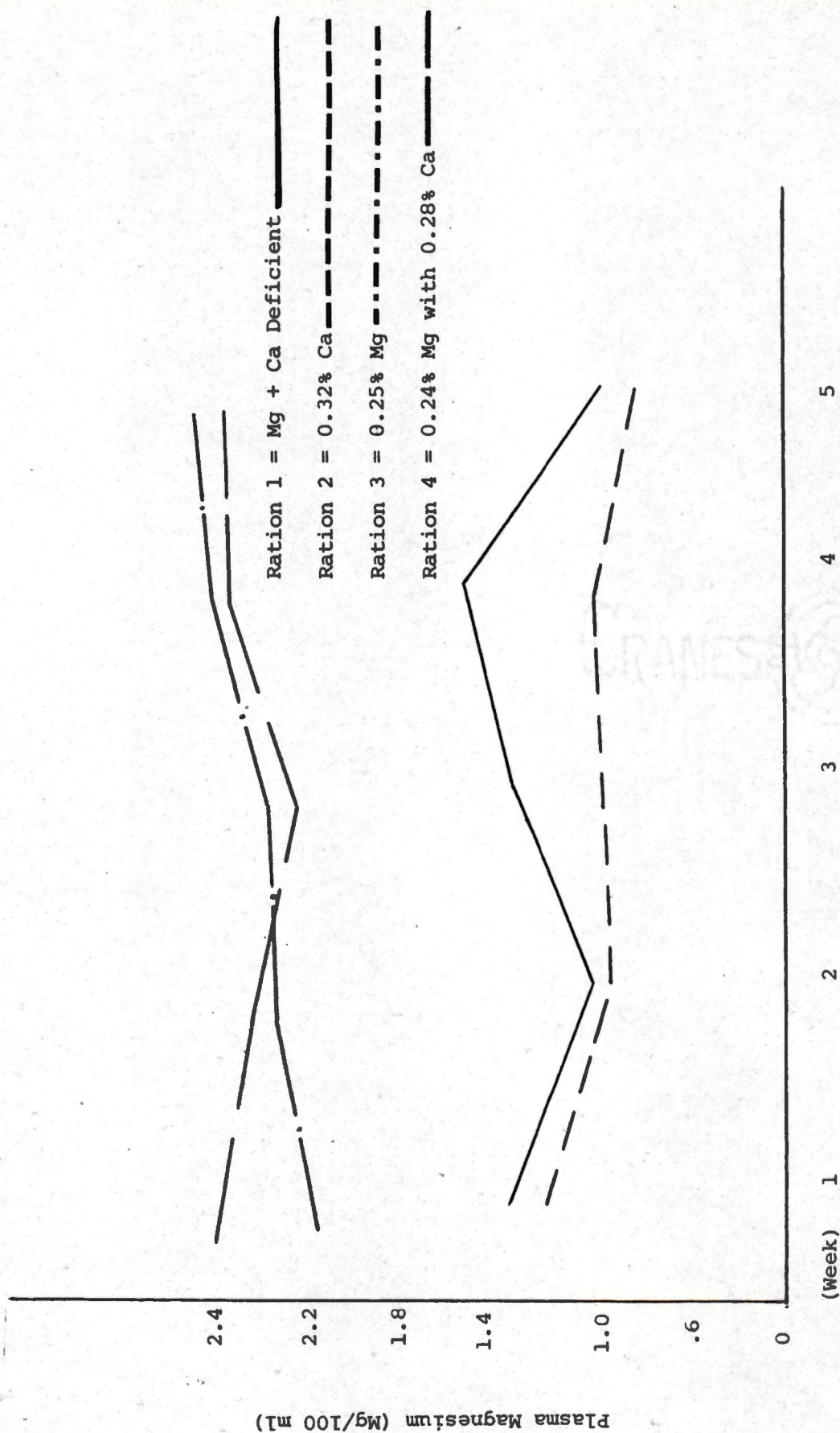


FIGURE 1. EFFECT OF DIETARY MAGNESIUM ON PLASMA MAGNESIUM EXPERIMENT I, PHASE I.

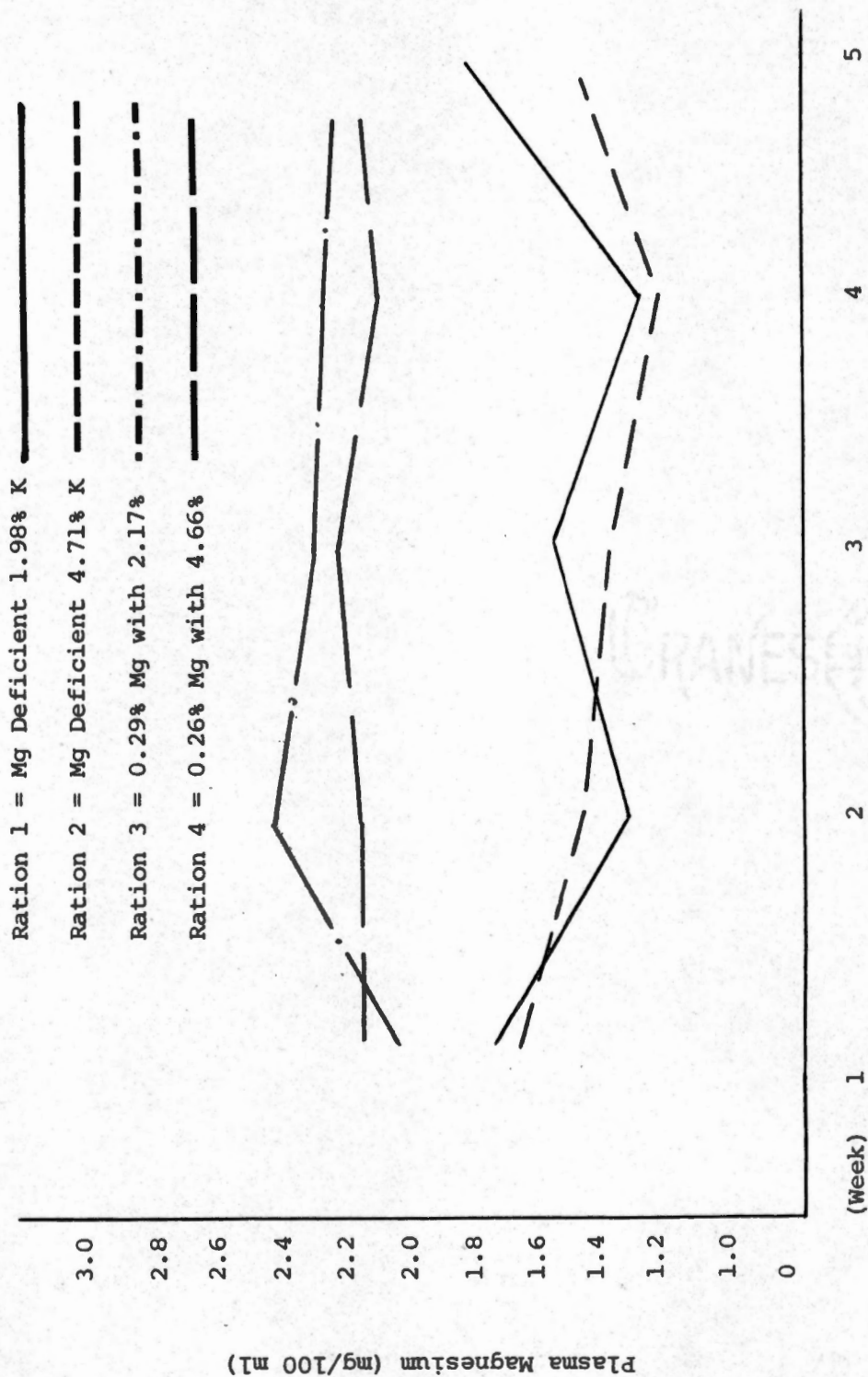


FIGURE 2. EFFECT OF DIETARY MAGNESIUM ON PLASMA MAGNESIUM EXPERIMENT I, PHASE II.

Cows on pasture and tetany prone hay had lower ($P < .05$) plasma magnesium levels than those on normal hay (Table X). Plasma magnesium in pasture cows showed a sudden drop by day 128 and a steady decrease until day 151 (Figure 3).

Discussion. Sheep and cows consuming magnesium deficient diets had lower plasma magnesium than animals on rations containing 0.26% magnesium. Lower plasma magnesium was observed in sheep consuming diets deficient in magnesium and containing 0.32% calcium as compared to plasma from sheep on a diet deficient in both magnesium and calcium. These results are in harmony with earlier reports which showed that calcium may be detrimental when added to low magnesium diets (Vitale et al., 1959; O'Dell et al., 1960).

Newton et al. (1972) and Duarte (1974) have observed a decrease in the absorption of magnesium when lambs and rats were receiving a high potassium diet. The same effect was observed in this study in sheep and cows on diets high in potassium. It appears that a high potassium intake that occurs when ruminants are first allowed access to lush pastures could have an effect on the absorption and utilization of magnesium.

Calcium

There were no significant differences between plasma calcium and treatments in either of the phases of experiment I (Figures 4 and 5), although there was a variation of calcium in ration of phase I. Rations 1 and 3 contained 0.06% calcium and rations 2 and 4 contained 0.30% calcium added as CaCO_3 .

TABLE X. PLASMA MAGNESIUM, CALCIUM, POTASSIUM AND IMMUNOREACTIVE INSULIN:
EXPERIMENT II

Minerals	Ration		
	Pasture	Tetany	Control
Magnesium	1.65 ^a	1.77 ^a	2.11 ^b
Calcium	9.88 ^{ab}	9.94 ^a	10.22 ^b
Potassium	14.82 ^a	15.52 ^a	15.22 ^a
Insulin	8.90 ^a	9.41 ^a	9.33 ^a

Means in rows with different superscripts are different ($P < .05$).

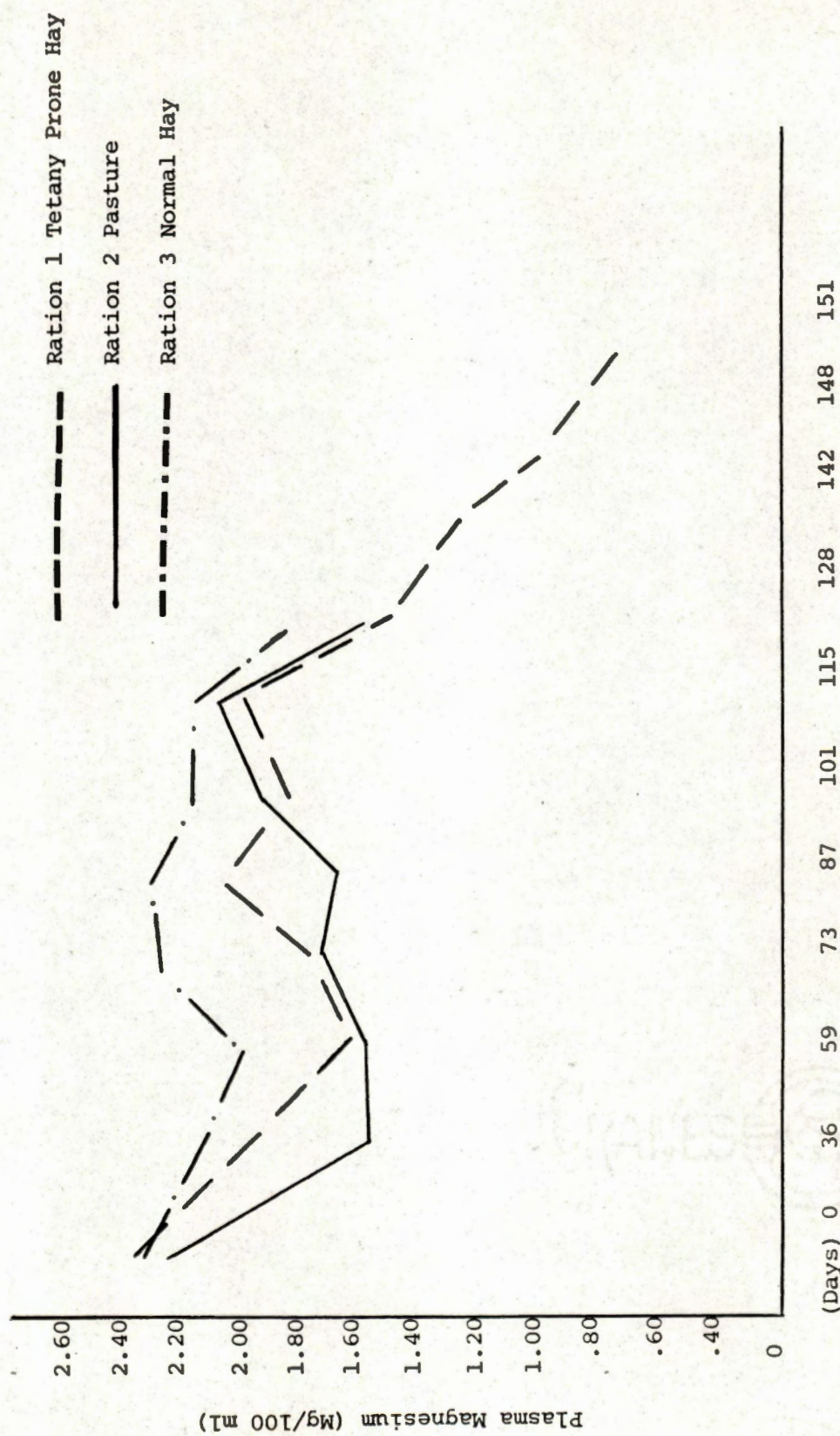


FIGURE 3. AVERAGE PLASMA MAGNESIUM EXPERIMENT II.

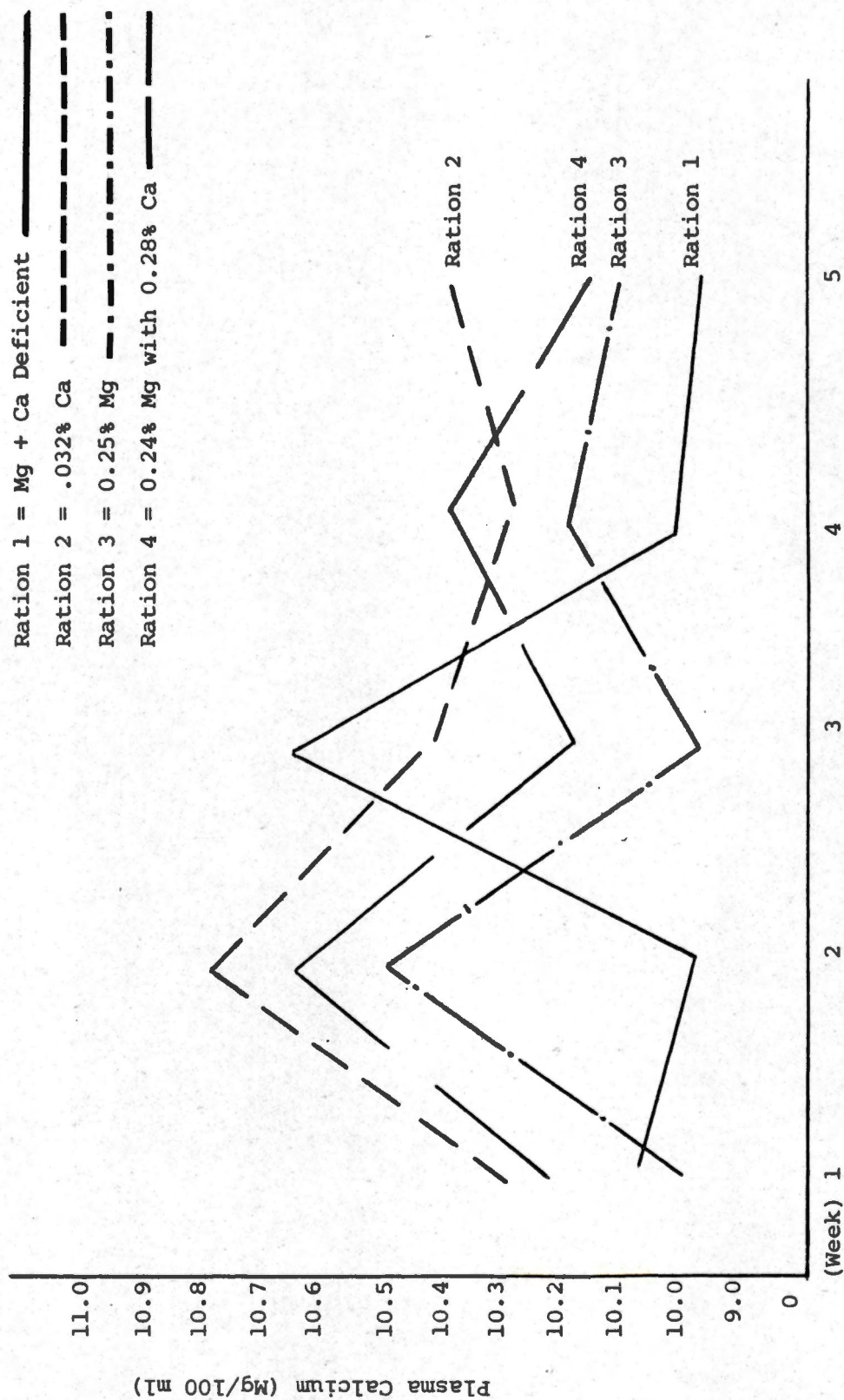


FIGURE 4. AVERAGE PLASMA CALCIUM EXPERIMENT I, PHASE I.

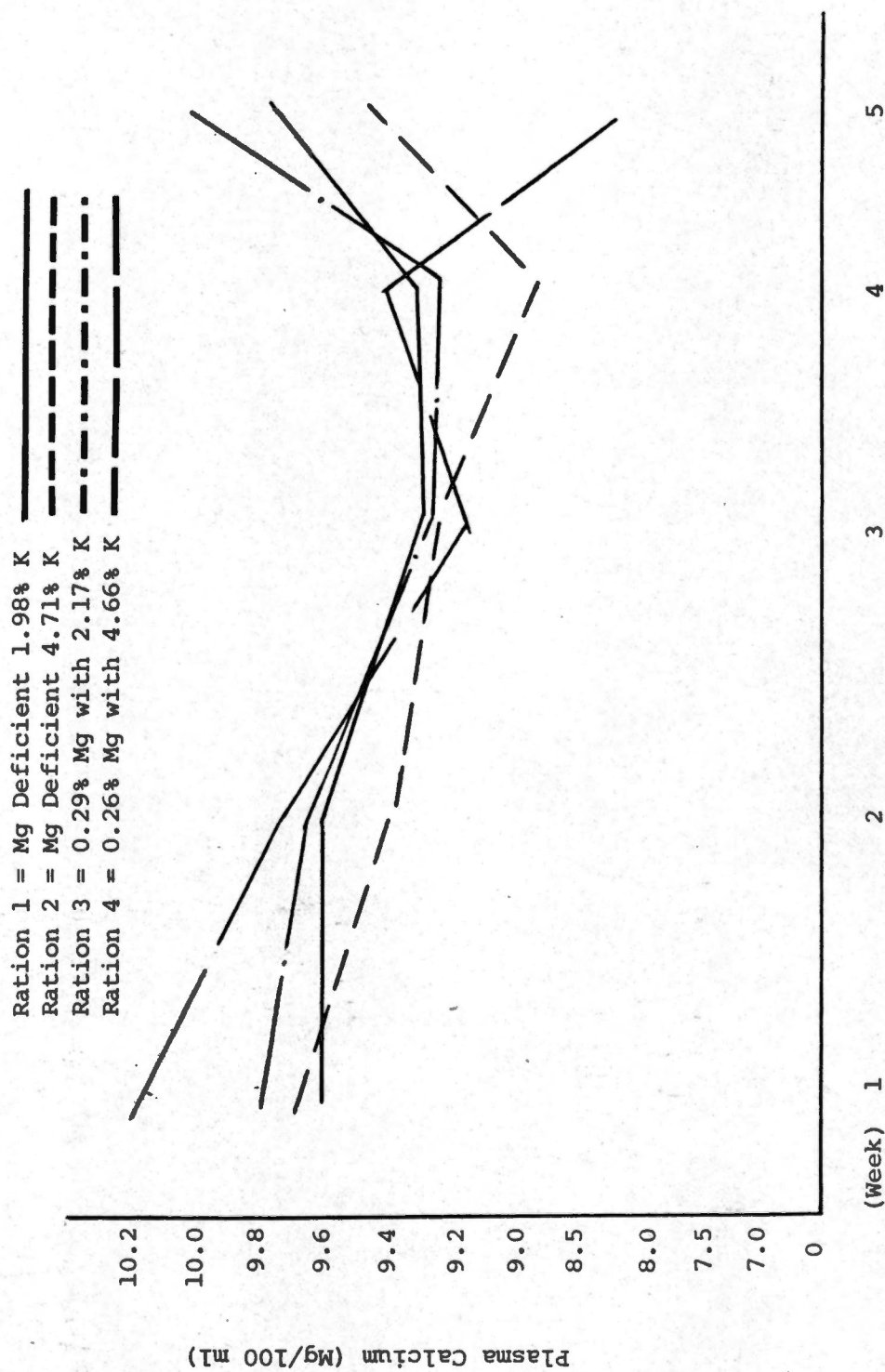


FIGURE 5. AVERAGE PLASMA CALCIUM EXPERIMENT I, PHASE II.

In experiment II, plasma calcium was lower in cows receiving tetany prone hay than those on control hay. There were no differences between plasma calcium in cows on pasture and those receiving tetany prone hay (Table X). The plasma calcium in cows showed a sudden drop by day 128, a slight increase by day 142 and then a steady decline until day 151 (Figure 6) which paralleled the decline in plasma magnesium

Discussion. Diet and bone serves as the two main sources supplying necessary calcium to the body for vital functions. The amount of calcium fed as well as the acidity or alkalinity of the ingesta influences calcium absorption (Storry, 1961). Dairy cattle, sheep and rats can adapt to their calcium intake (Hansard and Plumlee, 1954; Henry and Kon, 1953).

Researchers have shown an increase in serum calcium levels in lambs fed a high potassium ration or a high protein, high potassium ration (Kunkel et al., 1953; Fontenot et al., 1960). This was not observed in mature wethers on a 4.6% potassium diet in this study. Parallel declines in plasma calcium and magnesium in the cows on pasture (Figures 3 and 6) were not surprising since hypocalcemia often develops with magnesium deficiency despite adequate dietary calcium (Reddy et al., 1973). Highly significant positive correlations have been found between total serum calcium and magnesium in sheep (Belonje, 1976).

Potassium

The plasma potassium of the wethers were higher on those receiving ration 1 than those fed ration 2 or 4 during phase I, even though treatments 2, 3 and 4 did not differ (Figure 7). Plasma potassium was

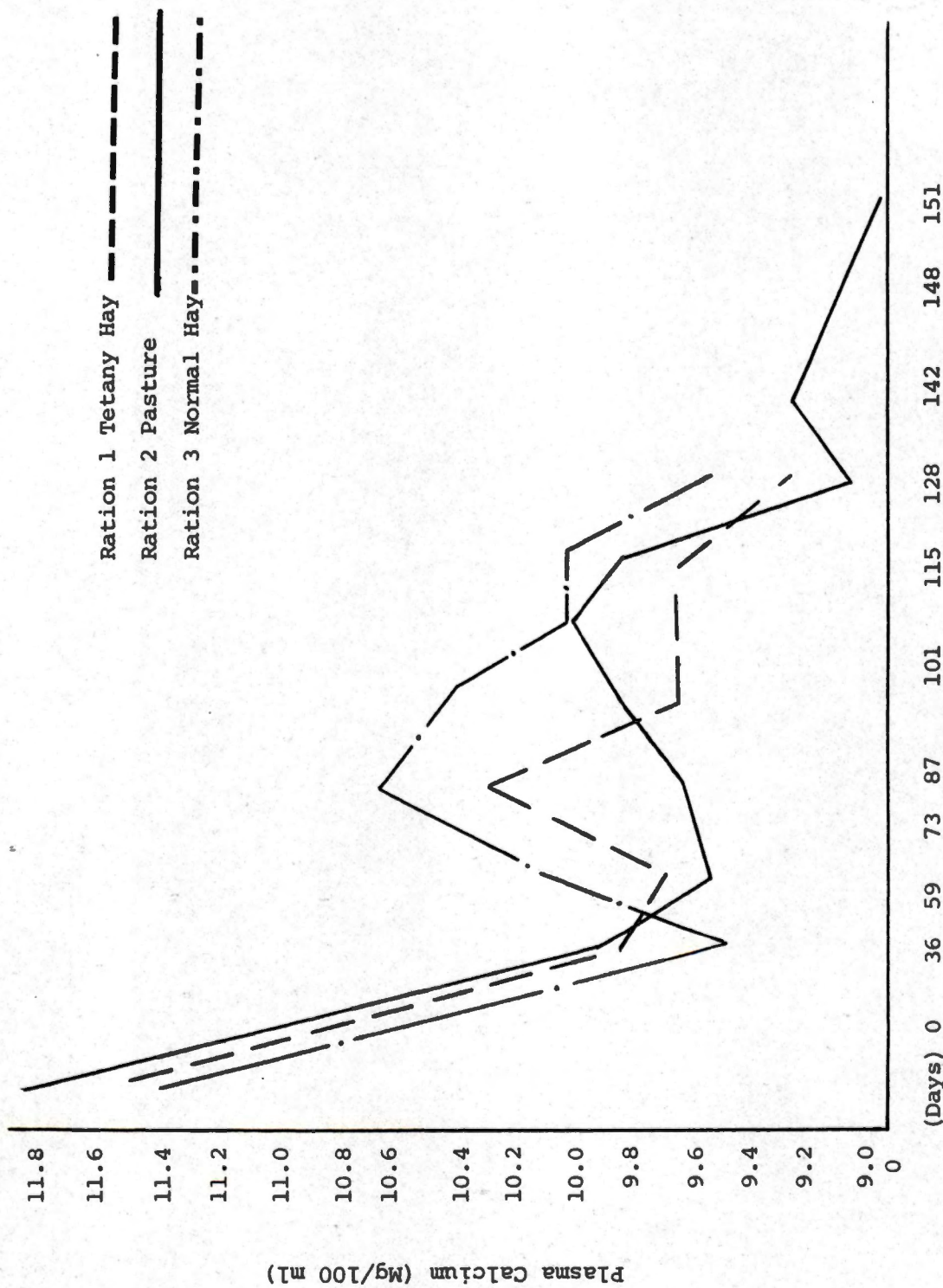


FIGURE 6. AVERAGE PLASMA CALCIUM EXPERIMENT II.

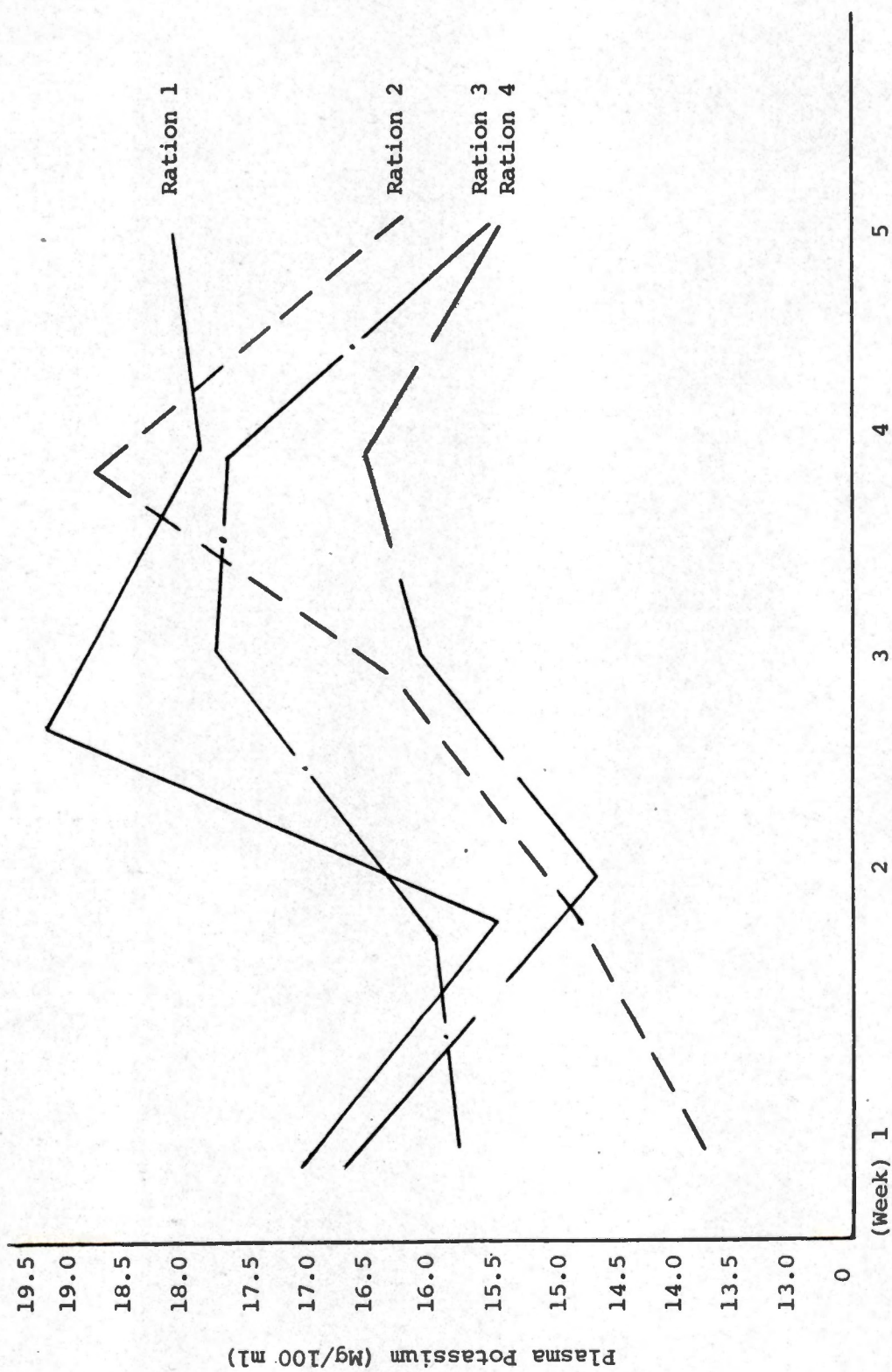


FIGURE 7. AVERAGE PLASMA POTASSIUM EXPERIMENT I, PHASE II.

significantly different ($P < .05$) in phase II (Table IX, page 34) and was directly related to amounts of potassium in the diets. Wethers fed the high potassium diet (4.6%) had higher potassium values ($P < .05$) than those on low potassium diets (2.1% potassium) (Figure 8). No significant difference was seen between plasma potassium for the cows on the three rations (Table X, page 38). However, on day 148, a sudden increase was seen in plasma potassium of cows on pasture (Figure 9) which was probably related to the high potassium content of the early spring pasture.

Discussion. The results obtained from this study are in agreement with work done with lambs by Newton et al. (1972). Plasma magnesium and calcium decreased in early spring while potassium increased. This supports the theory that less magnesium is available to ruminants grazing early spring pastures that are high in potassium.

Plasma Immunoreactive Insulin

Plasma immunoreactive insulin in sheep was positively correlated with both potassium ($P < .01$) and calcium ($P < .01$) in plasma but was not correlated with plasma magnesium (Table XI). No correlation was observed in phase II of experiment I. No significant differences were found between cow plasma insulin and treatments of experiment II (Table X, page 38).

A large variation of plasma immunoreactive insulin was seen in both phases of experiment I (Figures 10 and 11) and experiment II.

Discussion. The large variation in plasma immunoreactive insulin between animals on different treatments in both experiments could be due

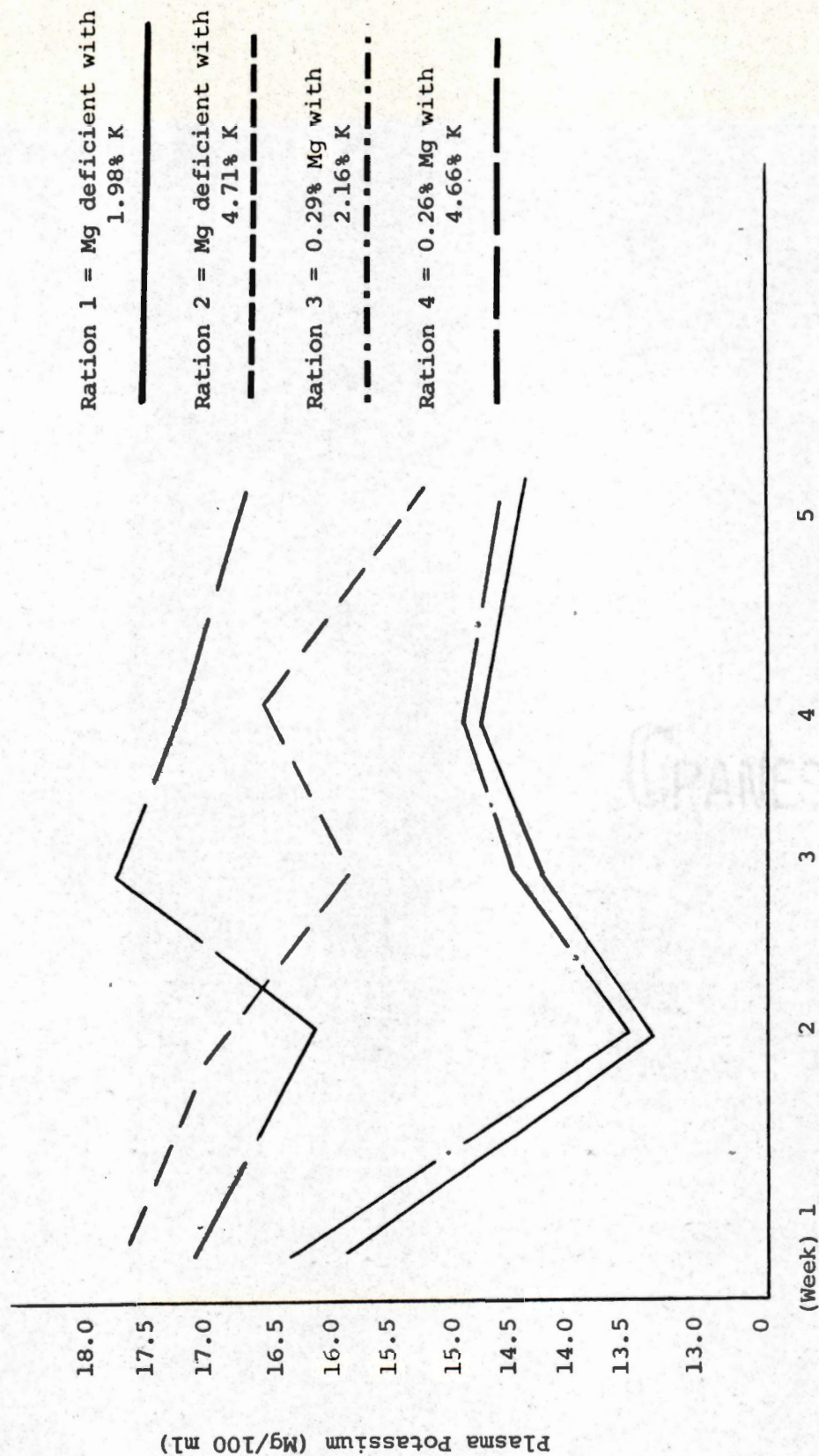


FIGURE 8. EFFECTS OF DIETARY POTASSIUM ON PLASMA POTASSIUM EXPERIMENT I, PHASE II.

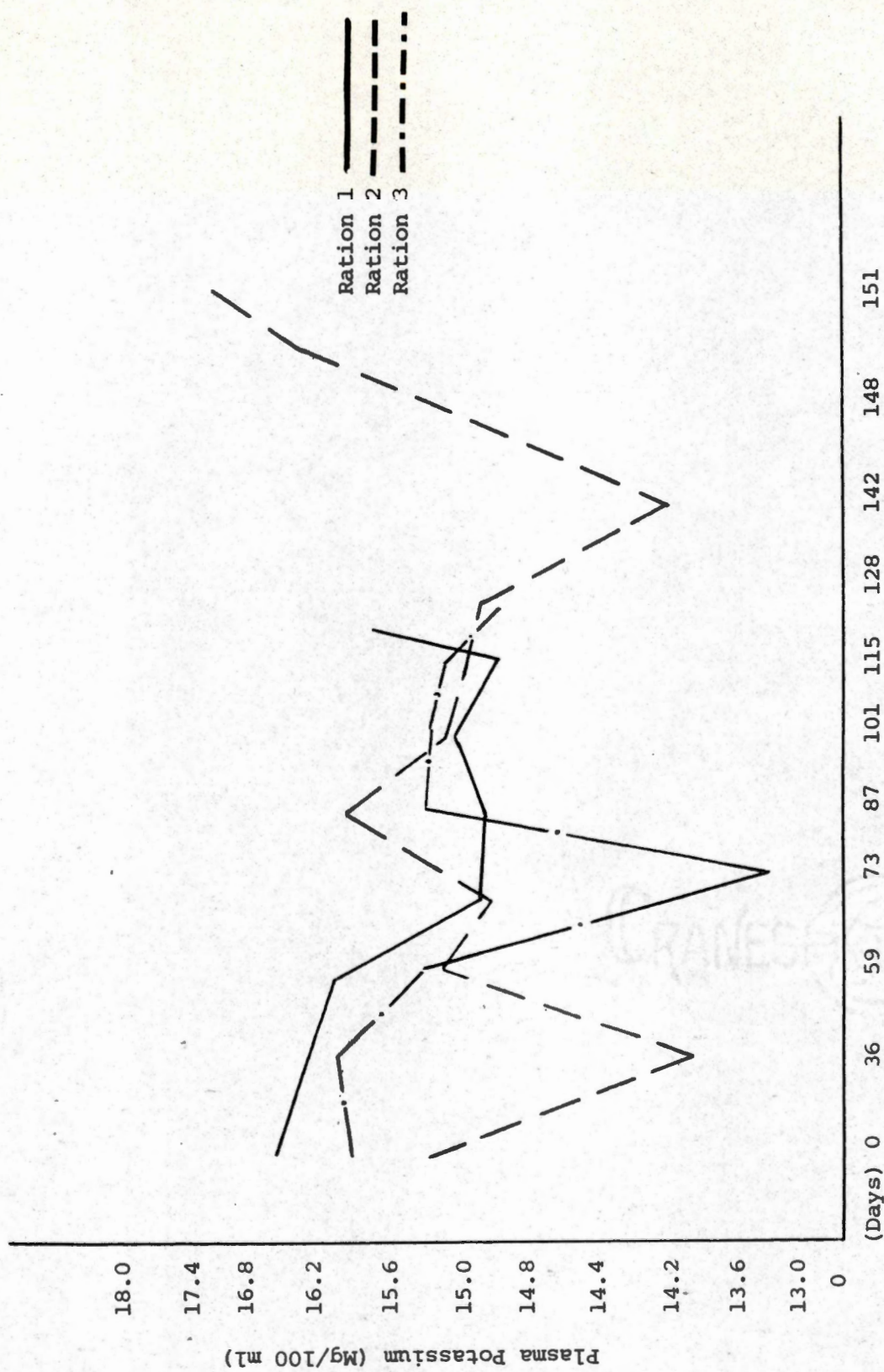


FIGURE 9. AVERAGE PLASMA POTASSIUM EXPERIMENT II.

TABLE XI. CORRELATION COEFFICIENT FOR SHEEP PLASMA MAGNESIUM, CALCIUM, POTASSIUM
AND IMMUNOREACTIVE INSULIN: EXPERIMENT I PHASE I ($P < .01$)

Variables	Magnesium	Calcium	Potassium	Insulin
Magnesium				
r	1.00000	-0.00464	0.02357	0.18807
probability	.0000	0.9574	0.7861	0.0559
Calcium				
r	-0.00464	1.00000	0.03636	0.25878
probability	0.9574	0.0000	0.6754	0.0080
Potassium				
r	0.02357	0.03636	1.00000	0.27493
probability	0.7861	0.6754	0.0000	0.0047
Insulin				
r	0.18807	0.25878	0.27493	1.00000
probability	0.0559	0.0080	0.0047	0.0000

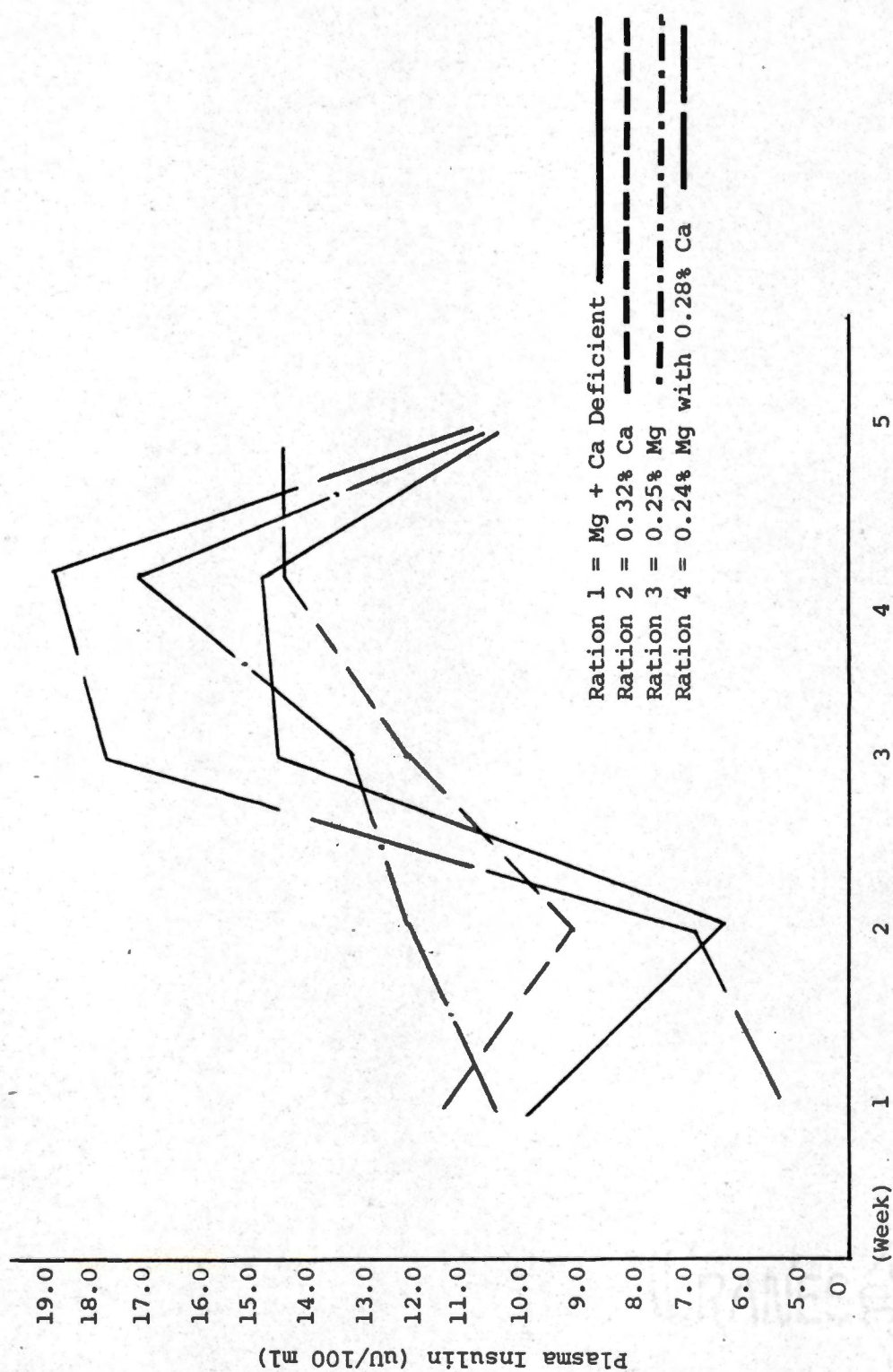


FIGURE 10. EFFECT OF MAGNESIUM AND CALCIUM DEFICIENCY ON IMMUNOREACTIVE INSULIN EXPERIMENT I, PHASE 1.

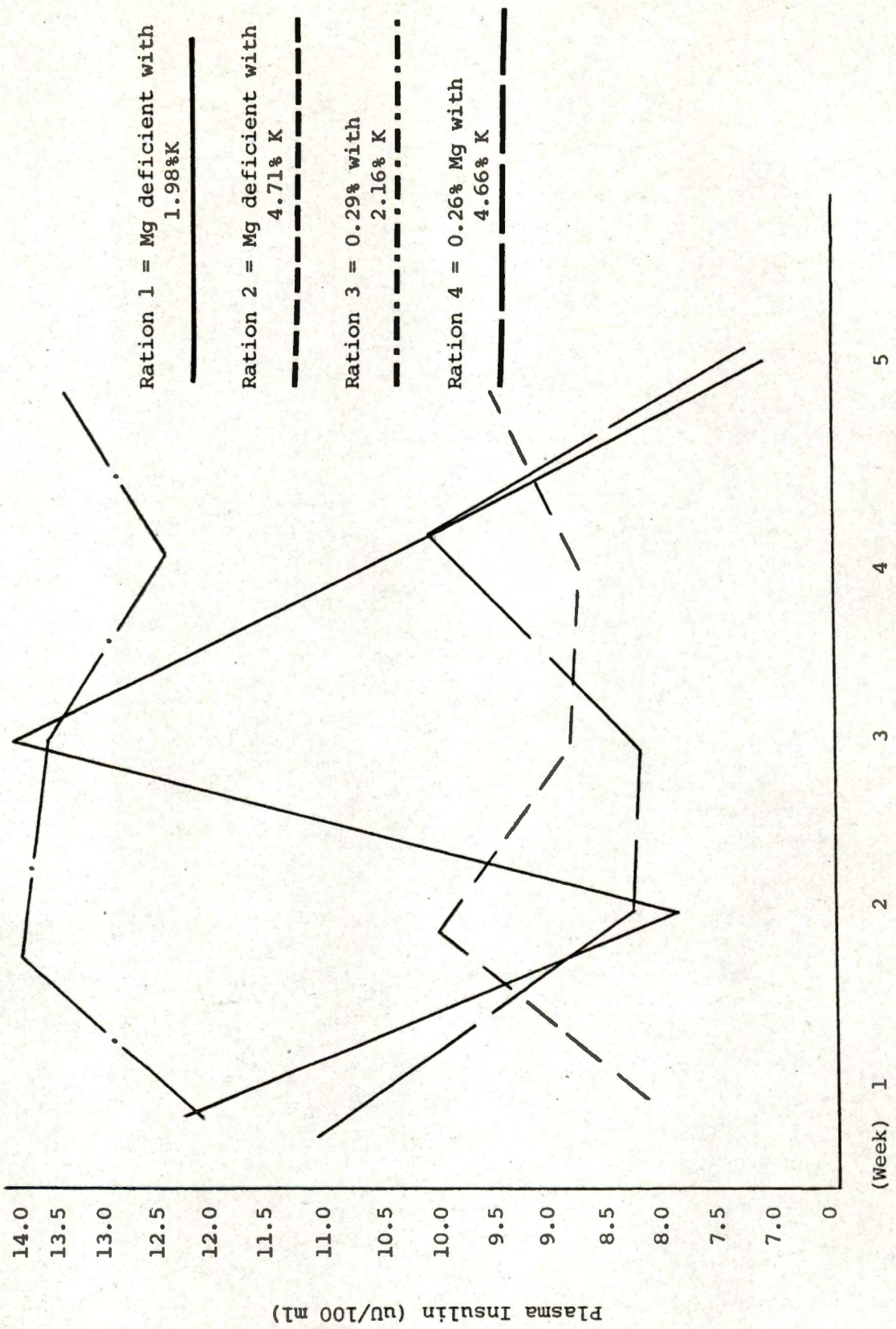


FIGURE 11. EFFECT OF DIETARY POTASSIUM ON PLASMA IMMUNOREACTIVE INSULIN
EXPERIMENT I, PHASE II.

to the circadian rhythm (McDonald, 1977) and the short half-life of insulin. The type of diet could also have affected the plasma insulin levels. It has been shown that animals fed grain have higher plasma insulin levels than animals fed hay (Trenkle, 1970a; Donefer et al., 1963; Luther, 1964; Bassett, 1974). No plasma insulin levels were found in the published literature for ruminants fed a semipurified diet. The feed intake of the wethers was slightly below normal and this could have contributed to the low plasma insulin values (Trenkle, 1972). Most of the studies on plasma insulin in sheep have been conducted with younger animals (less than one year old) (Trenkle, 1970a). The wethers in this experiment had an age range of 2-7 years. The effect of age on insulin secretion by ruminating animals needs further investigating.

The time of taking blood samples could have contributed some variation due to the circadian rhythm of insulin (McDonald, 1977). The once-a-week and monthly bleedings may not have given an accurate picture of insulin levels in the plasma of the wethers and cows because plasma insulin can change fairly quickly due to stress and excitement (Brockman, 1978; Brockman and Manns, 1976). The sheep were fed at four o'clock in the afternoon and blood samples taken at eight o'clock in the morning. This gives a 16 hr interval between feeding and blood sampling. Trenkle (1972) has shown that 12 hr after feeding there is more variation in plasma insulin. In a study conducted with heifers, plasma insulin values were: 11.2 uU/ml before feeding; 15.7 uU/ml 6 hr after feeding; and 4.5 uU/ml 46 to 60 hr after feeding. Hove (1974) has suggested that nocturnal sampling reduces variations in plasma insulin induced by feeding. The average mean values for the sheep on phase I was 14 uU/ml, phase II 8.94 uU/ml and cow plasma insulin average was 9.21 uU/ml.

Although potassium intake by cows grazing stockpiled forage should not have been excessive during the winter, their plasma insulin did not respond as expected to increasing potassium content of grass in the spring. Hove (1974) reported the mean plasma insulin level of dairy cows dropped by 50% during the first 45 days of lactation. Since the cows in the present study calved during the period on pasture, it is possible a potassium induced rise in plasma insulin was obscured by initiation of lactation. Also, hypomagnesemia in these cows apparently developed over a period of several weeks of relatively low magnesium intake. Severe weight losses indicated their feed intake were inadequate although available forage appeared to be adequate. Hypomagnesemia may have caused anorexia, one of the symptoms of magnesium deficiency in ruminants (McAleese et al., 1961). Plasma insulin levels in starved or ketotic cows have been found not to respond to glucose stimulation (Hove, 1978) and may not have responded to potassium.

Although hyperkalemia can stimulate elevated insulin levels in cattle (Lentz et al., 1976), the involvement of insulin in clinical grass tetany has not been established.

CHAPTER V

SUMMARY

Two experiments were conducted to determine effects of different dietary levels of magnesium, calcium and potassium on plasma concentrations of these cations on plasma immunoreactive insulin.

Four groups of three mature wethers each were fed semipurified diets varying in these minerals in two phases of experiment I. In the first phase, wethers fed magnesium deficient diets (0.04%) had lower plasma magnesium levels than those fed diets containing 0.2% magnesium regardless of dietary calcium (0.06 or 0.30%) when potassium averaged 2.9%. In the second phase, wethers receiving diets high in potassium (4.7%) had higher potassium and lower magnesium levels in plasma than wethers receiving a normal (2.1%) level of potassium. Plasma levels of calcium or immunoreactive insulin were not affected by dietary treatments in either phase of experiment I.

Dietary levels of these minerals were varied for three groups of 10 Angus brood cows by feeding them normal hay, tetany prone hay, or tetany prone pasture in experiment II. Plasma levels of both magnesium and calcium were lower in cows receiving hay or pasture likely to induce tetany than in cows fed normal control hay but plasma potassium and immunoreactive insulin were not affected by treatment.

These results indicate that different dietary combinations of magnesium, calcium and potassium had no effect on plasma immunoreactive insulin of sheep on semipurified diets or Angus cows on tetany prone

hay and pasture fertilized heavily with nitrogen and potassium. The potassium intake of the brood cows was low when they were grazing winter killed grass and increased markedly with new growth in the spring but this increase had no measurable effect on plasma immunoreactive insulin. This does not confirm results of work done by Lentz and associates (1976) who found that injected KCl increased immunoreactive insulin in bull calves and Holstein cows. Other factors, including plane of nutrition, time of blood sampling and stress may have also been involved. From the results of this experiment the involvement of elevated insulin in hypomagnesemic tetany in cattle overwintered on pasture is not clear.

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