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To the Graduate Council:

I am submitting herewith a dissertation written by Basel K. Al-Dabbagh entitled "Interrelationship among magnesium, potassium and platelets in hypomagnesemic ewes." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

James K. Miller, Major Professor

We have read this dissertation and recommend its acceptance:

John P. Hitchcock, James B. McLaren, Monty J. Montgomery, Robert S. Dotson

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

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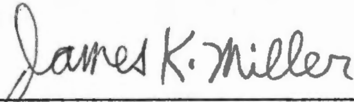

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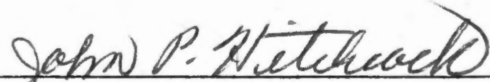

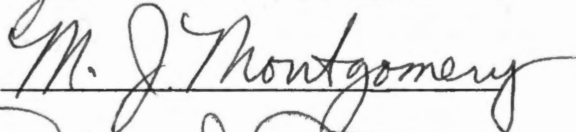

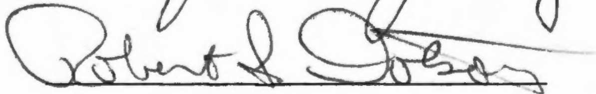

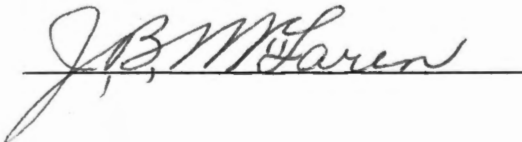

Accepted for the Council:

Vice Chancellor
Graduate Studies and Research

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INTERRELATIONSHIP AMONG MAGNESIUM, POTASSIUM AND PLATELETS IN HYPOMAGNESEMIC EWES

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Basel K. Al-Dabbagh

March, 1985

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ABSTRACT

Sixteen Finnish cross bred ewes were used as models of cows on pasture in two experiments to investigate relationships between dietary magnesium (0.04 and 0.24% Mg), previous Mg status, potassium (0.7 and 3.5% K) and platelet function during pregnancy and lactation. In the first experiment, 12 ewes were assigned to four diets which contained either deficient or adequate Mg supplemented with adequate (diet 1 and 2) or high K (diet 3 and 4). In the second experiment eight ewes from the first experiment and eight ewe lambs born to ewes from the first experiment were fed either deficient (diet 1) or adequate Mg (diet 2) to investigate the effect of previous Mg status, either in utero or as an adult ewes, on later performance.

Plasma Mg was higher ($P<.05$) in ewes fed diet 2 than in those fed diet 1. Supplementing diets with high K (diet 3 and 4) increased ($P<.05$) plasma K in ewes over those fed adequate K (diet 1 and 2) and lowered ($P<.05$) plasma Mg in ewes fed Mg-adequate (diet 4) but not Mg-deficient diets (diet 3). Ewes fed adequate Mg plus adequate K (diet 2) had lower ($P<.05$) systolic and diastolic blood pressure than those fed deficient Mg plus adequate K (diet 1). When K was high systolic, diastolic and mean arterial pressures were lower ($P<.05$) in ewes fed a deficient Mg diet (diet 3) than in those fed the adequate Mg diet (diet 4). In vitro platelet delay time was longer and % aggregation velocity was higher ($P<.05$) in ewes during the preliminary than the gestation periods. In vitro platelet reactivity

measurements were not affected by Mg or K treatments in ewes after parturition or during lactation. Thromboxane B_2 concentration in plasma of ewes was higher ($P<.05$) during gestation than during the preliminary period but 6-keto-prostaglandin $F_{1\alpha}$ concentration was not affected.

Previous Mg deficiency lowered ($P<.05$) plasma K and increased ($P<.01$) packed cell volume but had no effect on plasma Mg and Calcium (Ca). There was no effect of previous Mg deficiency in ewes on blood pressures or heart rate. In the second experiment feeding adequate Mg diets resulted in similar effects which were observed in the first experiment. Plasma K and blood glucose in ewes fed experimental diets were lower ($P<.01$) during the second than in the first experiment and the ewes developed abnormal health problems before parturtion in the second experiment. The problem was recognized as copper (Cu) toxicity due to high Cu content of corn gluten meal and possibly low molybdenum (Mo) in the diets.

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CHAPTER I

INTRODUCTION

Magnesium is one of the major minerals recognized as essential for all animals. The amount required varies among species and between classes of animals within species. Clinical hypomagnesemia can become a major problem in ruminants during pregnancy or lactation when they are maintained primarily on early spring pasture with limited supplemental feed. In the United States this disturbance usually occurs in beef cows in early stages of lactation, but also occurs in ewes and dairy cows. Although the problem has been investigated extensively, many question without satisfactory answers remain. Hormonal changes known to accompany decreased concentrations of plasma magnesium in clinical or laboratory animal studies can be induced by some of the conditions present when hypomagnesemic tetany is a threat. Tetanic convulsion often ending in death is the most clinically obvious sign in hypomagnesemic ruminants but the specific cause of the final seizure has not been identified. Hypomagnesemia in ruminants has been investigated by two approaches. In one, cows in late pregnancy or early lactation have been maintained on pasture considered to be "tetanigenic." In another, confined smaller ruminants (usually wethers or calves) have been fed diets of known composition. In the former approach, lack of control over weather and thus over forage composition has made results unpredictable and grazing cattle are not readily available for detailed physiological measurements.

Although feed composition can be controlled in the latter approach, conditions prevailing when hypomagnesemic tetany is a threat can not be simulated closely because stresses imposed by pregnancy, parturition, and lactation are missing. Desirable features of both approaches could be combined by feeding controlled rations to pregnant ewes in confinement.

The interrelationships between plasma electrolytes and platelet function in prevention of cardiovascular diseases and strokes in humans have not been emphasized until recently. Hypomagnesemia, for example, could contribute to abnormal platelet function in several ways. Magnesium is necessary for maintenance of the platelet disc shape and is required for platelet disaggregation, while Ca can induce the aggregation (Ardlie et al., 1970). Magnesium is also important in maintaining mitochondrial integrity, retaining myocardial K, and may control the Na and Ca pump which is essential in maintenance of normal coronary vascular tone (Seelig and Heggtveit, 1974, Altura and Altura, 1982). Excess dietary K can reduce dietary availability and utilization of Mg (Fontenot et al., 1973). Magnesium deficiency can contribute to elevated cholesterol, lesions of the vascular wall and hyperactivity of platelets (Seelig and Heggtveit, 1974).

Since the discovery of prostacyclin and thromboxane A_2, much effort has been expended in studying the mechanisms of action of these compounds. Prostacyclin produced by the endothelial cell lining of the vascular system disaggregates platelet clumps, prevents

platelet aggregation, and causes vasodilation. Thromboxane A_2 synthesized in platelets constricts arterial smooth muscle, promotes platelet aggregation, and induces the platelet release reaction (Moncada and Vane, 1977, 1979; Samuelsson et al., 1978). Because of the opposing effects of prostacyclin and thromboxane A_2 synthesis, there is the potential for the delicate control of hemostasis and arterial thrombosis in vivo. That is, thromboxane A_2 promotes platelet aggregation and is a vasoconstrictor, while prostacyclin prevents platelet aggregation and is a vasodilator.

Due to the numerous factors involved in grass tetany and their effect on platelet function, the research presented in this dissertation was proposed to investigate the interrelationship among Mg, K, and platelets in hypomagnesemic ewes. The hypothesis to be tested is that vascular endothelial damage in Mg deficient animals could reduce formation of prostacyclin while thromboxane A_2 formation by hyperactive platelets is unaffected or even increased. This would tip the balance in favor of thromboxane, thus favoring thrombus formation and vasospasm which could be significant mortality risk factors in Mg deficient animals.

Objectives

A) To determine effects of diets low in Mg, high in K, or both on concentrations of Mg, K and Ca in plasma and erythrocytes of pregnant or lactating ewes.

B) To measure effects of hypomagnesemia, hyperkalemia, or both on platelet reactivity in vitro, concentrations of vasoactive substances (thromboxane A_2 and prostacyclin) in blood plasma, and blood pressure.

C) To determine effects of previous Mg deficiency, either in utero or as adults, on response of pregnant or lactating ewes to low Mg intake a year later.

D) To evaluate results of the above measurements in light of clinical hypomagnesemia in ruminants.

CHAPTER II

REVIEW OF LITERATURE

Hypomagnesemia in Ruminants

Clinical hypomagnesemia can become a major problem when pregnant or lactating ruminants are maintained on lush, rapidly growing pasture with limited supplemental feed in late winter or early spring. This abnormal condition, first documented by Sjollema in 1932, has more commonly been called grass tetany, wheat pasture poisoning, winter tetany, lactation tetany, or grass staggers. Milk tetany is associated with calves fed only milk. Grass tetany is characterized by a set of classic physical signs. These usually include restlessness, incoordination, profuse salivation, progressive intensity in excitement, muscle spasms, collapse, convulsive struggles, abnormal neurological reflexes, nystagmus, pounding heart beats and finally extreme respiratory distress produced by pulmonary insufflation and loss of ability to exhale trapped air from the lungs (Wilkinson, 1980). Post-mortem findings in cattle which died in hypomagnesemic tetany included extensive hemorrhages in the pleural cavity, on the epicardium and endocardium, throughout the cardiovascular system, the thymus, lung, and muscle, fragmentation of the internal elastic membrane of arteries, thrombus formation, and ecchymotic hemorrhages in aorta, pulmonary artery, heart and kidney (Rook and Storry, 1962; Haggard et al., 1978; Ohshima et al., 1973).

The concentration of Mg in plasma or serum for normal pigs, cattle, sheep, and goats rangesfrom about 1.2 to 3.8 mg/dl of Mg. The lower normal limit in cattle is 1.7 mg/dl, and values below that can properly be referred to as hypomagnesemia (Rook and Storry, 1962). Wilson (1964) suggested that the majority of healthy animals will have plasma or serum Mg concentrations between 1.7 and 3.0 mg/dl and the symptoms of nervous origin are likely to occur if the concentration falls below 1.0 to 1.2 mg/dl. In clinical cases serum Mg level is usually below 0.7 mg/dl (Rogers, 1979). However, some animals show no symptoms at very low concentrations while others show symptoms above 1.2 mg/dl. Serum Mg values of .5 mg/dl or less were not invariably associated with clinical signs and in general, mature animals appeared to be less sensitive to low values than calves (Rook and Storry, 1962).

Not only are low plasma Mg levels associated with this disorder but many times plasma Ca levels are also reduced especially during long periods of chronic low Mg concentration. Parr (1957) indicated that serum Ca levels of cattle usually lie within a range of 8.5 to 11.5 mg/dl with an average value of about 10 mg/dl. In the cases of hypomagnesemia, however, a rapid fall to values below 8.0 mg/dl often occurs (Rogers, 1979).

Absorption of Mg by the gastrointestinal tract as well as the availability of Mg have been of major concern in better understanding the etiology of hypomagnesemia. Factors which influence the availability range from binding of Ca and Mg by the digesta to mineral

interrelationships. The earliest studies on Mg absorption suggested that Mg was absorbed from the middle third of the small intestine. Other areas of the digestive tract of the ruminant were not considered to be important sites of Mg absorption under normal dietary conditions. Stewart and Moodie (1956) dosed sheep with Mg salts and collected venous blood from different sites of the alimentary tract. They demonstrated that after dosing with 122 g of $MgSO_4$, the principle site of Mg absorption is probably the duodenum and small intestine. Phillipson and Storry (1965) found similar results and demonstrated that the rumen epithelium appeared to be relatively impermeable to both Ca and Mg. Research by Care and Van't Klooster (1965) indicated that Mg was absorbed from the upper ileum in sheep and appeared to reach a limiting value with increasing intraluminal Mg concentration. It was postulated that the rumen does not appear to be an important site of net absorption when Mg concentration is within normal limits; however, after supplementation with 37.6 g of Mg acetate Mg absorption increased. Similar finding were reached by Smith (1957, 1959a,b) using calves as experimental models.

More recent work has demonstrated that the fore stomachs are the major sites of absorption with the small intestine being more involved in net secretion of Mg back into the tract (Rogers and Van't Klooster 1969; Grace et al., 1974; Ben-Ghedalia et al., 1975). Net Mg absorption was lowered only when K was infused to the rumen, and the reduction was almost entirely due to reduced absorption of Mg from the stomach (Tomas and Potter, 1976). When two levels of Mg (.1 and .2%) and three levels of K (.6, 2.4, and 4.8% dry basis) were fed to

cannulated wethers (Greene et al., 1983b) it was observed that Mg was absorbed prior to entry to the small intestine and that feeding 2.4 and 4.8% K decreased Mg absorption by 24.4 and 61.2%, respectively. A net secretion of Mg into the small intestine was also found. The large intestine can absorb small amounts of Mg from dietary sources (Rogers and Van't Klooster, 1969, Kemp et al., 1973; Grace et al., 1974; Stevenson and Unsworth, 1978). The rectum may absorb large amounts from enemas of $MgCl_2$ and this has proven to be a very effective method in increasing the plasma Mg in cattle (Bell et al., 1977). Bell et al. (1977) indicated that using 60 g of $MgCl_2 6H_2O$ in 200 ml of water as a rectal infusion would increase the plasma Mg level significantly within 20 minutes.

The mechanism by which Mg absorption takes place is a process that involves some active transport (Brown et al., 1978). The amount of Mg absorbed depends on intake and availability (Rogers, 1979; McKim, 1983). The Mg content in most diets for cows ranges from 0.10 - 0.30% of the D.M.; in spring, levels are often lower, 0.14 - 0.18% of forage D.M. Spring grass and winter diets usually have lower levels of available Mg (Rogers, 1979). High levels of K, and the stresses of changing weather, parturition and initiation of lactation depress Mg availability. Fontenot (1979) found that feeding a high K level (4.5% dry basis) decreased Mg absorption and increased fecal Mg to the same extent. The relationship of low blood Mg concentration to low dietary Mg content and increased fecal Mg loss accompanying elevated K intake is well documented

(Fontenot et al., 1960; Newton et al., 1972; Miller et al., 1980, McKim; 1983; Suttle and Field, 1967, 1969).

Mineral and Dietary Interrelationships

The effect of other minerals on the absorption of Mg are a major contributing factor during hypomagnesemia with K having the most extensive effect. Other major mineral interactions are Ca and P or the combination of both is more effective in accentuating Mg deficiency (Jacobson et al., 1972). A significant interaction between level of Mg and form of P in diet was found by Dutton and Fontenot (1967) with inorganic P causing a greater decrease in plasma Mg than organic form. Chicco et al. (1973) fed dietary levels of Ca, P, and Mg varying with Ca content of 0.13 to 0.78%, P 0.12 to 0.36%, and Mg 500 to 7,750 ppm to wethers to study the interrelationship of these minerals. High dietary Ca decreased Mg in the bone and plasma, partly due to increased fecal excretion of Mg. Feeding increased levels of Mg did not influence fecal excretion of Ca or bone concentrations; however, excess Mg reduced both urinary and plasma Ca. Results indicated that increased Ca decreased Mg utilization while Mg had the same effect on Ca. These effects could be explained by the indication that Ca and Mg may compete for the same absorptive mechanism involving a limited carrier system for both minerals (Care and Van't Klooster, 1965).

Cows may become severely hypomagnesemic less than a week after an abrupt change to early spring pasture. Ramsey et al. (1982)

attributed this to reduced Mg intake, lowered Mg availability due to high K content of forage, or a combination of these factors. Ramsey et al. (1982) found that the addition of excess K to a low Mg diet fed to wether lambs increased the rate of plasma Mg decline over that due to low Mg alone. Numerous other researchers have concluded similar results with the addition of a high K intake by the ruminant (Kunkel et al., 1953; Fontenot et al., 1960; Bohman et al., 1969; Yano et al., 1982).

House and Van Campen (1971) attributed the decline in plasma Mg partially to decreased Mg absorption, but mainly to a direct depressing effect of K on the circulating Mg. The absorption and retention of K increases in response to increased intake of this cation and it has been postulated that cells take up and retain more Mg as the level of K in the cell increases. House and Bird (1975) postulated a similar theory about the effect of high K lowering urinary Mg. Lentz et al. (1976) demonstrated that intravenous infusion of K will increase insulin and glucose in Mg deficient calves. They suggested circulating Mg was transferred into tissues, thus lowering serum Mg. Therefore, high K could exert an effect by altering intermediary metabolism in some way.

Excessive amounts of K fertilizers have been a contributing factor to this increased K intake by animals on pasture. Blaxter et al. (1960) indicated two possible ways that this might affect the incidence of hypomagnesemia and tetany. It is known that an excess of K will reduce the Mg concentration within the plants and secondly, excess soil K increases the K content of the plants; therefore, increasing animal intake of this mineral.

It is apparent that the mechanism by which K reduces Mg in plasma involves an interference with the absorption of Mg. Greene et al. (1983b) fed cannulated wethers two levels of dietary Mg (0.1 and 0.2%) and three levels of K (0.6, 2.4, and 4.8%, dry basis) to determine how K affects Mg absorption. The data verified that Mg is absorbed prior to the small intestine and that feeding 2.4 and 4.8% K decreased Mg absorption by 24.4 and 61.8%.

The effect of excess K on Mg absorption has in turn had a pronounced effect on the excretion patterns of Mg. Excess K intake is accompanied by a reduction in urinary excretion of Mg that appears to result from this lowered absorption (Fontenot et al., 1973; Frye et al., 1975). According to Suttle and Field (1967) adding K to the diet of sheep decreased urinary output of Mg by 33%. In another study by McKim (1983), calves fed Mg deficient or Mg adequate plus supplemental K diets excreted less Mg in the urine than did calves fed an adequate Mg diet. Similar results were found by Blaxter and Rook (1954a,b) which indicated that the amount of Mg excreted in the urine was always small relative to that excreted in the feces. They found that when the dietary Mg concentration was high, as much as 17 mg out of a total of 448 mg consumed was excreted in the urine, but low intakes were invariably associated with the excretion of only a few mg daily. Rook and Storry (1962) reported that urinary excretion of Mg reflects the nutritional adequacy of the diet, and the transfer of an animal to a diet deficient in Mg was followed by a progressive fall of serum Mg and reduction of urinary Mg excretion until at a threshold value, excretion in the urine ceased.

Most recently, aluminum (Al) has been postulated to chelate Mg thereby reducing its availability. Allen and Robinson (1980) supported this hypothesis by showing that rumen contents of animals which died of tetany contained 2,373 ppm Al, while non-tetany animals rumen contents averaged 405 ppm Al.

Dietary constituents of a forage or diet could also affect absorption and availability of Mg. There seems to be a negative correlation between the crude protein content of a forage or diet and the Mg availability. Kemp (1960) indicated that as dietary protein content fed to dairy cattle increased, the available Mg decreased. Toothill (1963) concluded that an increase in the dietary protein increased the percent absorption of Mg from 71.7 to 73.8 in rats. However, Moore et al. (1972) found no changes in Mg absorption by feeding two levels of protein (10 and 30%) to sheep and a similar finding was reported by Grace and Macrae (1972).

Some early spring pastures have been noted to contain not only low amounts of Mg but also low quantities of readily fermentable carbohydrates. Madsen et al. (1976) fed sheep with or without supplemental glucose to study this as a contributing factor in hypomagnesemia. Results indicated that supplementing the diet with glucose during high periods of risk may maintain plasma Mg levels thereby reducing tetany. In a related article Miller et al. (1976) concluded that absorption of Mg was increased by glucose supplementation of a hay diet but urinary excretion of Mg was also increased, therefore, retention remained unchanged. However, this increased

plasma Mg associated with supplementation may be due to an alteration in intermediary carbohydrate metabolism.

Lactose is another energy source implicated in hypomagnesemia. Forbes (1961) concluded that the addition of 0.25% lactose at the expense of glucose was accompanied by decreased weight gain, increased absorption, urinary excretion, and balance of Ca with increased urinary excretion and decreased balance of Mg.

Platelet Function in Hypomagnesemia

Platelets are minute round or oval discs only about 2 microns in diameter. They are fragments of megakaryocytes extremely large cells of the hemopoietic series found in the bone marrow, which release the platelets into the blood. The normal concentration of platelets in the blood is between 200-400 thousand per cubic mm (Johnson, 1971).

Two forms of platelet granules have been identified: Alpha-granules of moderate density contain enzymes that are characteristically associated with lysosomes. The second type of granule is associated with storage sites for ADP, ATP, serotonin, platelet factor 3, platelet factor 4, and calcium (Simmons, 1980).

Under normal conditions, platelets circulate for about 10 days as disc-shaped cells. Adhesion of platelets to collagen or basement membrane exposed by damage to the vascular endothelium is considered to be a primary step in hemostasis (Jamieson, 1974; Simmons, 1980). After this adhesion the platelets expel the alpha and dense granules,

resulting in the release of specific platelet constituents (e.g. ADP, arachidonic acid, epinephrine, norepinephrine, histamine, and 5-hydroxytryptamine). These constituents possess properties that cause aggregation of platelets (Cooper et al., 1976; Simmons, 1980). When plasma clots in the presence of platelets, the clot retracts. This retraction is proportional to the number of platelets present and depends on a reaction between ATP and a platelet contractile thrombosthenin (Simmons, 1980).

Gross lesion in cattle which had died in hypomagnesemic tetany included fragmentation of the internal elastic membrane of arteries, thrombus formation and ecchymotic hemorrhages in the aorta, pulmonary artery, heart and kidney (Ohshima et al., 1973; Haggard et al. 1978). Hughes and Tonks (1956, 1959, 1962) have described similar lesions in hearts and lungs of rabbits in which intravascular platelet aggregation was induced by entirely different experimental methods. Two observationscommon to all experiments were lung hemorrhages resulting from rupture of pulmonary arterioles occluded by platelet aggregates and thromboemboli generated in the pulmonary capillary bed and which also lodged in the coronary arteries. Similar platelet aggregates in the pulmonary microvasculature were the only significant post-mortem finding in human victims of unexpected sudden death (Pirkle and Carstens, 1974).

Plasma electrolyte imbalances could contribute to abnormal platelet function in several ways. Magnesium is necessary for maintenance of the disc shape and is required for platelet disaggregation, while

Ca which can induce the initial shape change in platelets and is necessary for platelet aggregation (Ardlie et al. 1970), can intensify Mg deficiency (O'Dell et al., 1960). Zinc can inhibit aggregation of platelets (Chvapil, 1973; 1977). Excess dietary K can reduce dietary availability and utilization of Mg (Fontenot et al. 1973). Magnesium deficiency can contribute to elevated cholesterol, lesions of the vascular wall and hyperactivity of platelets (Seelig and Heggtveit, 1974). In vitro addition of Mg to platelet rich plasma delayed clumping of platelets (Hughes and Tonks, 1965).

The role of Mg in prevention of cardiovascular diseases and strokes, and requirements for normal muscle function have been recognized by many researchers (Seelig and Heggtveit, 1974; Seelig, 1983; Iseri et al., 1983; Singh et al., 1983; Brautbar, 1984). Muscle weakness, fibrillation, spasticity and an electromyogram characteristic of myopathy commonly occur with Mg deficiency. Seelig and Heggtveit (1974) reviewed the interrelationship of Mg in ischemic heart disease. They reported that Mg was important in maintaining mitochondrial integrity, normal contraction with the formation of Mg-ATP complex, and retaining myocardial K. Their hypothesis was that with the depletion of cardiac Mg, mitochondrial swelling progresses to disorganization and disruption. Another study by Altura and Altura (1982) indicated that extracellular Mg was important in control of coronary arterial tone via regulation of vascular membrane Mg-Ca exchange sites. A reduction in extracellular Mg may produce coronary vasospasm and potentiation of vasoconstrictor agents by

allowing excess entry of extracellular Ca. A lowering or an elevation in extracellular Na in the face of Mg deficit would exacerbate the coronary vasospasm noted with reduction in only extracellular Mg. Their data suggested that Mg may control Na-Ca pump which is essential for maintenance of normal coronary vascular tone. Brautbar (1984) fed low Mg diets to rats and examined mitochondrial bioenergetics in heart and skeletal muscle. The results of his study showed that low Mg was associated with: 1) Impaired myocardial energy production, but with no alteration in coupling of the mitochondria; 2) altered myocardial cell membrane biochemical integrity. He concluded that impaired energy provision for the function of the various enzymes, and abnormal cell membrane permeability, may mediate the myocardial and skeletal muscle cellular abnormalities in Mg depletion. Altura and Altura (1982) reported that Mg deficiency can lower the extra-cellular concentration of Mg ions, causing greater cerebral arterial contraction thus producing cerebrovasospasm. Recent study with rats by Fisher and Giroux (1984) suggested that Mg deficiency alters Na transport. This may alter the membrane potential of the heart and could account for the cardiac arrhythmia associated with Mg deficiency.

Magnesium in the form of $MgSO_4 \cdot 7H_2O$ has been successfully used to inhibit premature labor, control and/or prevent eclampsia, and control pregnancy hypertension in women (Gant and Worley, 1980; Elliott and Colonel, 1983). Three hundred fifty-five patients with diagnoses of premature labor were treated with $MgSO_4$ as a tocolytic agent in the study conducted by Elliott and Colonel (1983). After

a 4 gm intravenous bolus of $MgSO_4$, a constant infusion was started at 2 gm/hr, and decisions to increase or decrease the rate were based on the patient clinical response. Their data demonstrated that delivery was successfully delayed in the majority of patients, and the incidence of unexplained failure of tocolysis was only 2%. Side effects occurred at 7% and necessitated stopping the drug at only 2% in patients. They reported that $MgSO_4$ may compete with Ca in the uterine smooth muscle, leading to a breakdown in ATP-driven actin and myosin interactions, thus causing a relaxing effect on the myometrial layer of placenta and inhibit premature labor. They also concluded that $MgSO_4$ should be considered as the drug for tocolysis in patients with premature labor.

Pre-eclampsia and eclampsia are disorders associated with pregnancy in women. Clinically, these disorders are characterized by hypertension, edema and proteinuria, with a possibility of renal failure and consumptive coagulopathy (Bonnar et al., 1977). In severe cases, they cause morbidity and death of the pregnant patient and her fetus. Gant and Worley (1980) reviewed, classified, and presented a plan of management for these disorders based upon readily obtained laboratory and clinical data. They recommended the use of $MgSO_4 \cdot 7H_2O$ in hypertensive pregnant women, and reported the reasons for using it. They stated that this agent is recommended because (1) it definitely controls and/or prevents eclampsia; (2) the patient is alert and awake and not heavily sedated; (3) the already compromised, frequently distressed fetus is not further jeopardized by the

anticonvulsant, and (4) magnesium sulfate therapy is easily managed and imposes a minimal burden on nursing and physician time.

A transient, mild reduction in blood pressure often occurs after $MgSO_4 \cdot 7H_2O$ is administered as an intravenous bolus. Therapeutic anticonvulsant blood levels are achieved when the concentration of Mg reaches 4 to 4.5 m Eq/liter. However, signs of Mg toxicity are reached at approximately 10 m Eq/liter, at which point the patellar reflex is lost. Therefore, any order for the administration of $MgSO_4 \cdot 7H_2O$ must specify that the drug is not to be given in the absence of patellar reflexes (Gant and Worley, 1980).

Prostaglandin and Thromboxane Interrelationships to Hypomagnesemia

The origin of prostaglandin and thromboxane, and their role in platelet aggreation have been reported by Hamberg et al. (1974a). Linoleic acid is an essential fatty acid which is metabolized to arachidonic acid. Arachidonic acid, another essential fatty acid and an important component of membrane phospholipids, is the starting compound of the metabolic cascade. Upon demand, arachidonic acid may be released from the cell wall by the enzyme phospholipase. Free arachidonic acid is rapidly coverted to a set of unstable metabolites including prostaglandin endoperoxides, PGG_2 and PGH_2. The substance PGH_2 is an important compound since it is metabolized by three pathways: 1) In the presence of thromboxane synthetase enzyme PGH_2 is converted to thromboxane A_2, which is an unstable compound and is rapidly hydrolyzed to a stable end product

thromboxane B_2; 2) In the presence of prostacyclin synthetase, PGH_2 is hydrolyzed to prostacyclin, and then to a stable end product 6-keto-prostaglandin $F_{1\alpha}$; 3) In the absence of either thromboxane or prostacyclin synthetase emzymes, PGH_2 could be converted to different stable metabolites, PGE_2, PGF_2, or PGD_2, enzymatically or spontaneously. PGG_2 and PGH_2 play an important role in the metabolism of arachidonic acid for they are precursors of thromboxane and prostacyclin, which have opposing biological properties. An imbalance between formation of these compounds could lead to dramatic pathologic consequences.

Thromboxane synthetase enzyme is known to be present in the microsomal fraction of platelets in large amounts (Samuelsson et al., 1978). Prostacyclin synthetase enzyme was found to be present in the vascular wall of different species including man by Moncada and Vane (1979). Hamberg and Samuelsson (1973) postulated that when thromboxane A_2 is released, it causes constriction of arterial smooth muscle, promotes platelet aggregation, and induces the platelet release reaction. They also found it to be unstable in aqueous solution with a half-life of about 0.5 minutes at 37°C. On the other hand prostacyclin was found to be a powerful vasodilator, an inhibitor of platelet aggregation, and unstable in aqueous solution of neutral pH with a half-life of about 2 to 3 minutes at 37°C (Moncada and Vane, 1977). Since both these substances are unstable and have short half-lives, researchers usually measure levels of their stable hydrolysis products (Thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$) by radioimmunoassay (Granstrom et al., 1976;

Remuzzi et al., 1981; Olson et al., 1982; Watson et al., 1984). The levels of those products should reflect the levels of their precursors thromboxane A_2 and prostacyclin, respectively.

Under normal conditions platelets interact with the vessel wall and initiate the production of endoperoxides which serve as substrates for prostacyclin synthetase in the endothelium (Moncada and Vane, 1977). They postulated that these series of events could be the mechanism by which the vessel wall protects itself against platelet deposition, since prostacyclin release would inhibit platelets and cause vasodilation, vascular damage could lead to platelet deposition in the endothelial wall and the development of thrombosis. Thrombus growth and initiation and development of thrombosis depend mainly on the degree of injury (Poole et al., 1958). Stemerman (1974) reported that severe damage or even physical detachment of the endothelial layer has to occur before the formation of thrombosis. Minor damage leads to platelet thrombi which are rapidly washed out by the circulation.

Moncada and Vane (1977) stated that beyond certain limits, when the endothelium is destroyed or physically detached, then the platelets will start adhering to the subendothelial layers which produce less prostacyclin and contain pro-aggregating materials like collagen. Interaction of platelets with these layers will redirect the biosynthetic pathway from prostacyclin to thromboxane A_2 and favor platelet aggregation with the consequent formation of a mural thrombus. As the thrombus grows, its size may be limited as the depositing platelets begin to come in contact with undamaged,

prostacyclin producing cells, and this could lead to detachment of part or all of thrombus. Moncada and Vane (1977) supported this hypothesis by demonstrating that cutting rabbit mesenteric and coeliac rings induced an increase in the release of prostacyclin which then diminished after several washings of the tissue.

Addition of 10-30 ng/ml of endoperoxides, prepared from vesicular glands of sheep, to suspensions of washed human platelets resulted in rapid aggregation (Hamberg et al., 1974b). They noted that the half-life of the prostaglandin endoperoxides in aqueous medium was significantly longer than that of "rabbit aorta-contracting substance" released from guinea pig lung, which indicates that the prostaglandin endoperoxides differ in their biological potency. Schneider (1977a,b) isolated a collagen species from aortas of aged burros which was an extremely potent inducer of platelet aggregation. This collagen was found to effectively activate both ovine and bovine platelets in vitro. Intravenous infusion of the collagen into tranquilized/heparinized guinea pigs caused almost immediate disappearance of platelets from circulating blood (Schneider et al., 1979). This was followed rapidly by left venticular hypertension which persisted for approximately 5 minute before receding toward baseline pressure. This reaction was associated with a series of abnormal ventricular electrical conduction events closely mimicking acute ischemic heart disease or myocardial infarction in humans (Schneider and Kelman, 1979).

Prostacyclin production was significantly depressed in fetal and placental vascular tissue from patients with severe pre-eclampsia

in comparison to vascular tissues from women with uncomplicated pregnancy (Remuzzi et al., 1980). Severe pre-eclampsia in this study was defined on the basis of a rise in diastolic blood pressure (>100 mm Hg) during the third trimester, heavy proteinuria (>2 g/24h) and edema. Their results indicated that prostacyclin concentrations decreased from 191.1 ± 29.1 ng/mg tissue in women with uncomplicated pregnancy to 65.4 ± 12.6 ng/mg tissue in patients with severe pre-eclampsia. In a later study plasmatic regulation of vascular prostracyclin was compared in non-pregnant women, women in early (<13 weeks) normal pregnancy, in late (>34 weeks) normal pregnancy, and in late (>29 weeks) pregnancy complicated by severe pre-eclampsia (Remuzzi et al., 1981). Plasmatic activity of prostacyclin stimulating factor during early pregnancy was comparable with that in non-pregnant women, whereas in late pregnancy the activity was significantly depressed. Patients with severe pre-eclampsia showed activities within the range of those in the control, non-pregnant women but higher than those in comparable women with normal pregnancies. In this study plasma samples for measurement of activity of prostacyclin stimulating factor were prepared from citrated venous blood and incubated with rat aortic rings. Prostacyclin was measured as both platelet aggregation inhibitory potency and the amount of the stable prostacyclin derivative 6-keto-prostaglandin $F_{1\alpha}$. Blood pressure, urate, and proteinuria were signifcantly higher in patients with severe pre-eclampsia than the control group. However, platelet counts declined in plasma of patients with pre-eclampsia compared

with women in early or late normal pregnancy but the decline was not significant. Watson et al., (1984) studied $MgSO_4$ effect on prostacyclin release by cultured human umbilical vein endothelial cells. They noted that exposing endothelial cells to $MgSO_4$ (0 to 5 mM) and Na-arachidonate amplified cellular release of 6-keto-prostaglandin $F_{1\alpha}$ in a dose-dependent manner from 8.2 ng/dish at 0 $MgSO_4$ to 15.6 ng/dish for 5mM of $MgSO_4$. Prostacyclin production by endothelial cells was 2.3 fold greater when cells were incubated with plasma obtained from pre-eclamptic patients on $MgSO_4$ therapy compared to pretherapy plasma. They concluded that $MgSO_4$ mediates enhanced production of prostacyclin by endothelial cells with a maximal effect in a range achieved during therapy of pre-eclampsia. These results were in agreement with previous conclusions drawn by other researchers with respect of using $MgSO_4$ as a tocolytic agent to inhibit premature labor and control pre-eclampsia in women (Gant and Worley, 1980; Elliott and Colonel, 1983).

Although $MgSO_4$ treatment was successful in women with complicated pregnancy, the etiology of hypertension that occurs during pregnancy remains unknown (Gant and Worley, 1980). Magnesium intake, availability and/or absorption may be inadequate during pregnancy. Chronic Mg deficiency may cause damage or lesions of the endothelial vascular walls. This may reduce prostacyclin production and lead to elevation in thromboxane A_2: prostacyclin ratio. Elevated thromboxane A_2: prostacyclin ratio may predispose hypertension and disorders during pregnancy in women.

CHAPTER III

EXPERIMENTAL PROCEDURE

Animals and Treatments

First Experiment

Twelve Finnish cross ewes in the second month of pregnancy were kept on a progressively deteriorating pasture in the fall and early winter of 1982, and adjusted gradually to a concentrate mixture (diet 2) designed to provide adequate amounts of known nutrients (NRC, 1975). The ingredients included and mineral mixture composition of the experimental diets are shown in Table 1 and 2.

After two months, ewes were moved to a barn and three ewes were randomly assigned to each of four pens and fed concentrate free choice plus 2 Kg per pen daily of wheat straw containing 0.08% Mg. After two weeks the concentrate was modified by omission of MgO and/or addition of KCl and $KHCO_3$ to contain 0.04% (Diet 1 and 3) or 0.24% (Diet 2 and 4) Mg and 0.7% (Diet 1 and 2) or 3.5% (Diet 3 and 4) K (Table 2) . Calcium content of the concentrate was increased from 0.28% during gestation to 0.46% after parturition (Table 3) to meet recommended allowances for lactation (NRC, 1975). New born lambs had access to their mother's feed in addition to suckling.

TABLE 1. COMPOSITION OF DIETS FED TO PREGNANT OR LACTATING EWES (FIRST EXPERIMENT)

Ingredient	I.F.N.[a]	Adequate K		High K	
		Low Mg	Adequate Mg	Low Mg	Adequate Mg
		(kg/100 kg)			
Corn gluten meal	5-02-900	12	12	12	12
Soybean protein	5-08-038	6	6	6	6
Coarse corn cobs	1-02-783	10	10	10	10
Fine corn cobs	1-02-783	31.4	31	26.5	26.1
Corn starch	4-02-889	18	18	19.2	19.2
Dextrose	4-02-125	15	15	15	15
Corn oil	4-02-889	1	1	1	1
Mineral mix[b]		6.3	6.7	10	10.4
Vitamin mix[c]		.3	.3	.3	.3

[a]International Feed Number.

[b]Table 2.

[c]One kilogram supplies the following: Vitamin A, 900,000 IU; Vitamin D, 106,250 IU; Vitamin E, 5,500 IU; Ascorbic acid, 45.0 g; Inositol, 5.0 g; Choline chloride, 75.0 g; Menadione, 2.25 g; P-Aminobenzoic acid, 5.0 g; Niacin, 4.25 g; Riboflavin, 1.0 g; Pyridoxine hydrochloride, 1.0 g; Thiamine hydrochloride, 1.0 g; Calcium pantothenate 3.0 g; Biotin, 2.0 mg; Folic acid, 9.0 mg; Vitamin B-12, 0.1 mg.

TABLE 2. COMPOSITION OF MINERAL MIXTURE FOR EWE DIET (FIRST EXPERIMENT)

Ingredient	Adequate K		High K	
	Low Mg	Adequate Mg	Low Mg	Adequate Mg
	(g/100 kg)			
$CaCO_3$[a]	264	264	264	264
$CaH_4(PO_4)_2 \cdot H_2O$[b]	672	672	672	672
KCl	860	860	3880	3880
NaCl	1000	1000	1000	1000
$NaHCO_3$	3360	3360	----	----
$KHCO_3$	----	----	4000	4000
$FeSO_4 \cdot H_2O$	127	127	127	127
$CuSO_4 \cdot 5H_2O$	2.1	2.1	2.1	2.1
$CoSO_4 \cdot 7H_2O$	.1	.1	.1	.1
$ZnCl_2$	9.6	9.6	9.6	9.6
$MnSO_4 \cdot H_2O$	16.6	16.6	16.6	16.6
MgO	----	367	----	367
Se premix[c]	20	20	20	20

[a]Increased to 600 g in lactation ration.

[b]Increased to 1150 g in lactation ration.

[c]Contain 500 μg/gm of selenium.

TABLE 3. MINERAL ANALYSIS OF DIETS FED TO PREGNANT OR LACTATING EWES (FIRST EXPERIMENT)

Element and period	Adequate K		High K	
	Low Mg	Adequate Mg	Low Mg	Adequate Mg
	(%)			
Magnesium				
Preliminary	.24	.24	.24	.24
Gestation	.03	.26	.04	.25
Lactation	.03	.22	.04	.28
Calcium				
Preliminary	.28	.28	.28	.28
Gestation	.28	.27	.29	.29
Lactation	.46	.45	.48	.48
Potassium				
Preliminary	.70	.70	.70	.70
Gestation	.73	.69	3.55	3.23
Lactation	.64	.60	3.48	3.57
Phosphorus				
Preliminary	.34	.34	.34	.34
Gestation	.31	.34	.28	.28
Lactation	.40	.42	.48	.48

Second Experiment

Eight mature ewes and eight ewe lambs from the first experiment were used. Four of the ewes which had been fed a Mg-deficient diet in the first experiment and four ewe lambs born to Mg-deficient ewes, were compared with the remaining ewes and ewe lambs which had received adequate Mg.

Ewes in both age classifications were kept with a ram while on pasture and then moved to the barn. In the barn all animals were fed a control diet with a slight modification of diet 2 in the first experiment (Table 4 and 5). The control diet was supplemented with MgO to contain 0.25% Mg. A Mg-deficient diet, containing less than 0.05% Mg (Table 6) was made by omitting MgO from the control diet. After a one month preliminary period, where the ewes were fed the control diet, they were randomly assigned to Mg-deficient (diet 1) or Mg-adequate (diet 2) according to age, class and previous Mg status (Table 7).

Physiological Measurements and Collection of Data

In both experiments, samples were obtained weekly during the preliminary and experimental periods. Weekly blood samples were obtained by jugular puncture with 18 gauge disposable needles and plastic syringes. Blood with heparin as anticoagulant was analyzed for packed cell volume, glucose in whole blood, and Mg, K, and Ca in plasma. Additional blood samples were obtained once during the preliminary period, during gestation, and after parturition. Blood was placed

TABLE 4. COMPOSITION OF DIETS FED TO PREGNANT EWES (SECOND EXPERIMENT)

Ingredient	I.F.N.[a]	Low Mg	Adequate Mg
		(kg/100 kg)	
Corn gluten meal	5-02-900	12	12
Soybean protein	5-08-038	6	6
Coarse corn cobs	1-02-783	10	10
Fine corn cobs	1-02-783	34.2	33.8
Corn starch	4-02-889	18	18
Dextrose	4-02-125	15	15
Corn oil	4-07-882	1	1
Mineral mix[b]		3.2	3.6
Vitamin mix[c]		.6	.6

[a]International Feed Number.

[b]Table 5.

[c]Same as table 1.

TABLE 5. COMPOSITION OF MINERAL MIXTURE FOR EWE DIET (SECOND EXPERIMENT)

Ingredient	Low Mg	Adequate Mg
	----------------(g/100 kg)----------------	
$CaHPO_4 \cdot 2H_2O$	916	916
NaCl	1000	1000
$KHCO_3$	1150	1150
$FeSO_4 \cdot H_2O$	127	127
$CuSO_4 \cdot 5H_2O$	2.1	2.1
$CoSO_4 \cdot 7H_2O$	.1	.1
$ZnCl_2$	9.6	9.6
$MnSO_4 \cdot H_2O$	16.6	16.6
MgO	----	367
Se premix[a]	20	20

[a]Contains 500 μg/gm of selenium.

TABLE 6. MINERAL ANALYSIS OF DIETS FED TO PREGNANT EWES (SECOND EXPERIMENT)

Element and period	Low Mg	Adequate Mg
	(%)	
Magnesium		
Preliminary	.21	.21
Gestation	.02	.29
Calcium		
Preliminary	.30	.30
Gestation	.26	.24
Potassium		
Preliminary	.68	.68
Gestation	.94	.82
Phosphorus		
Preliminary	.32	.32
Gestation	.30	.29

TABLE 7. EXPERIMENTAL DESIGN FOR DETERMINING EFFECTS OF PREVIOUS MAGNESIUM DEFICIENCY ON RESPONSE OF PREGNANT EWES TO A MAGNESIUM-DEFICIENT DIET

Age class and previous Mg status	Dietary Mg level: Deficient	Dietary Mg level: Adequate
	(number of animals)	
Mature ewes		
Mg deficient	2	2
Mg adequate	2	2
Ewe lambs		
Mg deficient	2	2
Mg adequate	2	2

in plastic tubes containing one volume of anticoagulant solution (3.8% trisodium citrate and 0.5% dextrose in glass distilled water, pH 7, sterilized by filtration) for platelet counts and in vitro platelet reactivity assays and measurement of stable end products of plasma vasoactive substances (thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$).

Heart rate, mean arterial pressure, systolic, and diastolic blood pressure were measured weekly using an automatic sphygmomanometer (Dynamap research monitor, model 1255, Critikon, Inc.) with a pediatric cuff wrapped around the animals forelimb. Body weights of ewes were taken weekly.

Samples of each of the experimental diets were taken after mixing and kept in a refrigerator for mineral analyses. All disease conditions, treatments given and abnormal health observations were recorded daily.

Analytical Methods

Weekly blood samples obtained in both experiments were centrifuged at 5000 rpm for 20 minutesand plasma was withdrawn. Plasma was diluted to a known volume and analyzed for Mg, K, and Ca by atomic absorption spectrophotometry (Instrumentation Laboratories Model 551). Glucose in whole blood was measured weekly by using a commerical enzymatic colorimetric kit (Sigma Chemical Co., No. 510, Saint Louis, MO). In the second experiment, packed cell volume was estimated weekly by a micro-hematocrit method (Simmons, 1980).

Blood samples obtained for platelet count and aggregation test were centrifuged twice sequentially at 95 g for 30 minutes at 22°C. Each time, platelet-rich-plasma (PRP) was removed with a plastic pipet and pooled for each animal in plastic tubes. Total and aggregated platelets in PRP were counted by standard procedures.

In vitro reactivity of platelets to collagen addition was measured turbidimetrically with a self-calibrating photoelectric instrument and continuous chart recording (Platelet Aggregation Profiler, Model PAP-2A, Bio Data Corp.). In this test, 450 μl PRP were stirred at 37°C for 2 to 3 minute. Platelet activation was initiated by addition of 50 μl of appropriate diluent containing collagen in fibrillar form. Collagen in decreasing concentrations to extinction of platelet activation response were tested. Three variables of platelet sensitivity to activation with collagen were measured: 1) delay time in seconds between collagen addition to stirred PRP and onset of aggregation; 2) maximal percentage of aggregation accomplished in 7 minutes while stirring and incubating at 37°C; and 3) maximal aggregation velocity as percentage aggregation per minute calculated from the downward inclination of the turbidimetric recording during the most rapid phase of platelet clumping.

Thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ in plasma were measured using a commerical radioimmunoassay kits (New England Nuclear, a Du Pont Co., NEK-008, Boston, Ma.) with slight modification. Plasma sample size of 6-keto-prostaglandin was increased from 100 μl to 300 μl, since it was too low to detect.

Samples of experimental diets from both experiments were dried in a forced air oven at 100°C for 24 hours, cooled, weighed, then ashed at 600°C for three hours. These samples were dissolved in 3 ml of 6N HCL, quantitatively transferred to glass test tubes and diluted to a known volume with double distilled water. Diluted samples were analyzed for mineral composition. The mineral and chemical composition of the experimental diets used in both experiments are shown in Tables 3 (page 27), 6 (page 31) and 8.

Statistical Analysis

Data were analyzed statistically using SAS programs described by Barr et al. (1979). Analysis of variance procedures were used to evaluate data in the first and second experiment separately using the preliminary model:

$$Y_{ij} = U + d_i + e_{ij},$$

where:

Y_{ij} = dependent variables,

U = theoretical population means,

d_i = effect of diet i = 1-4 (First experiment),

i = 1-2 (Second experiment),

e_{ij} = random error.

where differences were determined, means were separated using the Student Neuman-Keuls test (Sokal and Rohlf, 1969) and Linear contrasts were used to separate the effect of dietary Mg, K, and their interactions.

TABLE 8. CHEMICAL COMPOSITION OF PRELIMINARY DIETS FED TO PREGNANT EWES DURING FIRST AND SECOND EXPERIMENT

Variable	First Experiment	Second Experiment
	---------------(DM Basis)---------------	
Dry Matter (%)	92.19	93.08
Crude Protein, (%)	13.75	15.73
Crude Fiber, (%)	19.87	26.45
Ash, (%)	5.05	3.87
Ether Extract, (%)	0.76	1.06
Nitrogen Free Extract, (%)	60.57	52.89

Data from both experiments were pooled and analyzed by analysis of variance using the final model:

$$Y_{ij} = U + d_i + A_j + e_{ij},$$

where:

Y_{ij} = dependent variables,

U = theoretical population means,

d_i = effect of diet, i = 1-6,

A_j = effect of animal, j = 12,

e_{ij} = random error

where differences were determined, orthogonal comparisons were used to separate the effect of Mg or first and second experiment effect. A probability level of 0.05 or less was used as the basis for determining whether a real difference existed among treatment means and experiments.

Data of the second experiment were evaluated in response to previous Mg status from the first experiment. The model used was:

$$Y_{ij} = U + d_i + A_j + e_{ij},$$

where:

Y_{ij} = dependent variables,

U = theoretical population means,

d_i = effect of diet, i = 1-4,

A_j = effect of animal, j = 4,

e_{ij} = random error

where differences were determined, orthogonal comparisons were used to separate the effect of Mg or previous and present Mg status. A probability level of 0.05 or less was used as the basis for determining a real difference existed among treatment means and Mg status.

CHAPTER IV

RESULTS

First Experiment

Plasma Minerals and Blood Glucose

Plasma Mg, K, and Ca, and glucose in whole blood were measured weekly during gestation and lactation periods. Table 9 shows the average values during the experimental period.

Plasma Mg was lower ($P<.05$) in ewes fed the Mg deficient than in those receiving Mg adequate diets when supplemented with adequate or high K (Table 9). However supplementing diets with high K caused a decrease ($P<.05$) in plasma Mg of ewes fed Mg adequate over those fed the Mg adequate and supplemented with adequate K. High dietary K did not influence plasma Mg levels of ewes that received the Mg deficient diet (Table 9).

By the fourth week of the experimental period, high dietary K started to lower plasma Mg in ewes receiving the adequate Mg diet compared to the adequate Mg adequate K group and averaged lower ($P<.05$) during the sixth, seventh and eighth week of experimental period (Figure 1).

Plasma K averaged higher ($P<.05$) in ewes fed high dietary K than in those receiving adequate K regardless of Mg treatments (Table 9). This increase became apparent during the seventh week and continued on until the twelfth week of experimental period (Figure 2). However there was no difference in plasma Ca or whole blood glucose

TABLE 9. COMPARISON OF 14 WEEKS MEANS FOR PREGNANT AND LACTATING EWES FED SEMIPURIFIED DIETS DURING FIRST EXPERIMENT

Variable	Adequate K		High K		
	Mg-deficient	Mg-adequate	Mg-deficient	Mg-adequate	SE[1]
Plasma Mg (m Eq/L)	1.58[c]	2.32[a]	1.55[c]	1.96[b]	0.04
Plasma Ca (m Eq/L)	4.21	4.23	3.78	4.00	0.08
Plasma K (m Eq/L)	4.44[b]	4.48[b]	4.73[a]	4.89[a]	0.04
Whole Blood Glucose (mg/dl)	54.18	51.55	53.56	54.57	0.94
Systolic (mm Hg)	134.06[a]	122.84[b]	115.00[c]	125.61[b]	1.49
Diastolic (mm Hg)	78.89[a]	68.90[b]	68.80[b]	76.98[a]	1.19
Mean Arterial Pressure (mm Hg)	95.62[a]	87.38[a,b]	85.48[b]	95.51[a]	1.33
Heart Rate Per Minute	134.26	122.47	128.10	133.17	2.23

[1]Standard error of mean.

[a,b,c]Differs ($P<.05$) from other means in same row and same K level not bearing the same superscript.

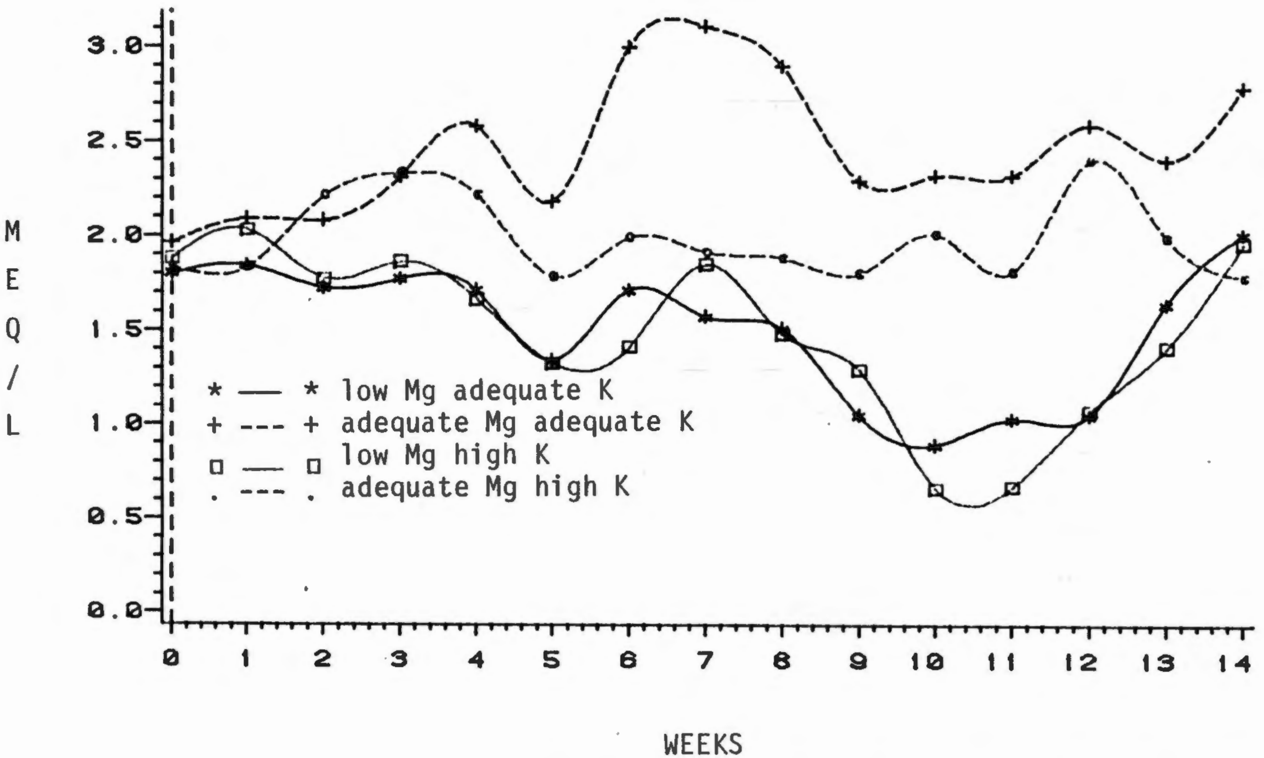

FIGURE 1. PLASMA MAGNESIUM CONCENTRATION IN PREGNANT OR LACTATING EWES FED SEMIPURIFIED DIETS DURING FIRST EXPERIMENT

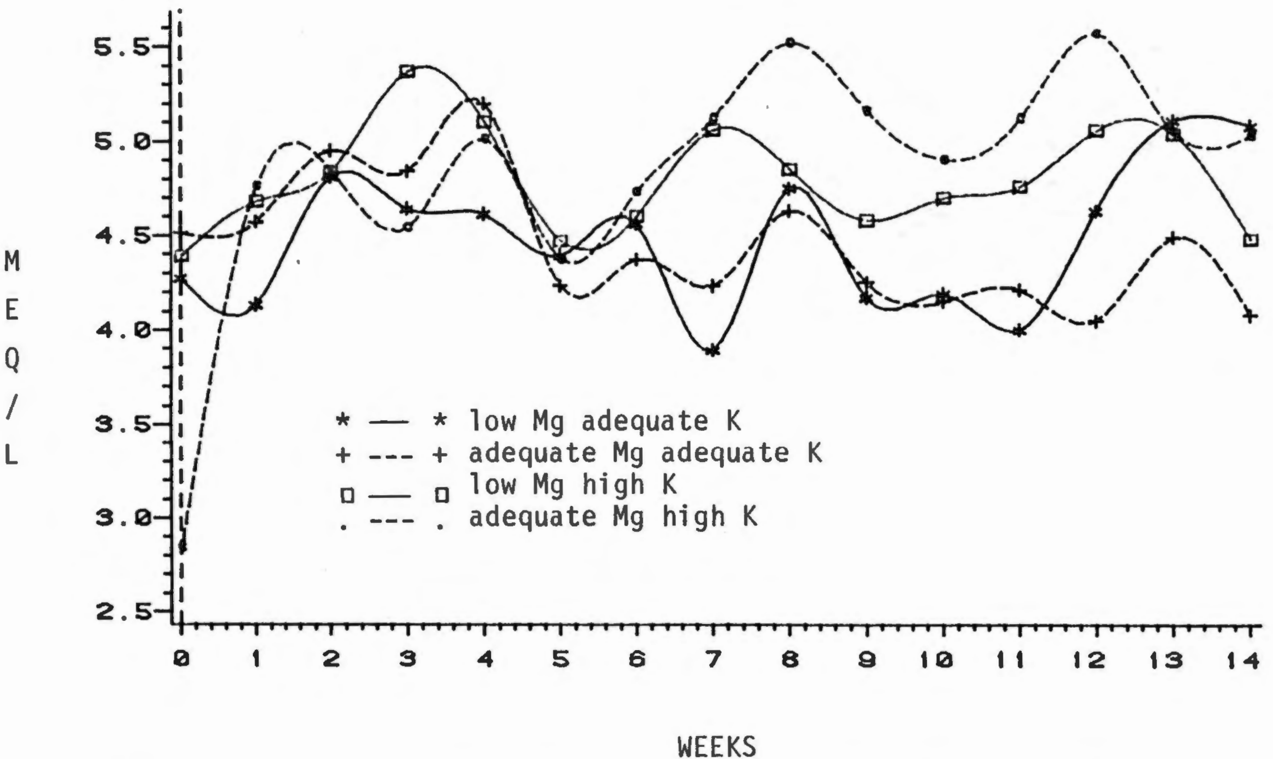

FIGURE 2. PLASMA POTASSIUM CONCENTRATION IN PREGNANT OR LACTATING EWES FED SEMIPURIFIED DIETS DURING FIRST EXPERIMENT.

of ewes due to Mg or K treatments (Table 9). Plasma Ca fluctuated through out the experimental period (Figure 3).

Blood Pressure and Heart Rate

Feeding ewes a Mg adequate diet supplemented with adequqte K caused a decrease ($P<.05$) in systolic and diastolic blood pressures over those receiving Mg deficient diet (Table 9). Also mean arterial pressure and heart rate averaged slightly lower but were not significant. However, the results were reversed when diets were supplemented with high K (Table 9). When dietary K was high, systolic, diastolic, and mean arterial pressure were lower ($P<.05$) in ewes fed Mg deficient diet than in those receiving Mg adequate diet. Heart rate averaged slightly lower but was not significant (Table 9).

In Vitro Platelet Test

In vitro platelet reactivity to burro collagen was measured in plasma of ewes during the preliminary and gestation periods (Table 10, Figure 4) after parturition (Figure 5), and during lactation (Figure 6).

Delay time to onset of aggregation lengthened with decreasing collagen dose during both preliminary and gestation periods (Figure 4). During the preliminary period, platelets in plasma tended to have slightly longer delay time than during the gestation period but differences were not significant except with addition of 2 μg ($P<.05$) of burro collagen (Table 10). However, maximal aggregation velocity % per minute progressively decreased with decreasing amounts (5,2,1 and 0.5μg) of burro collagen during both

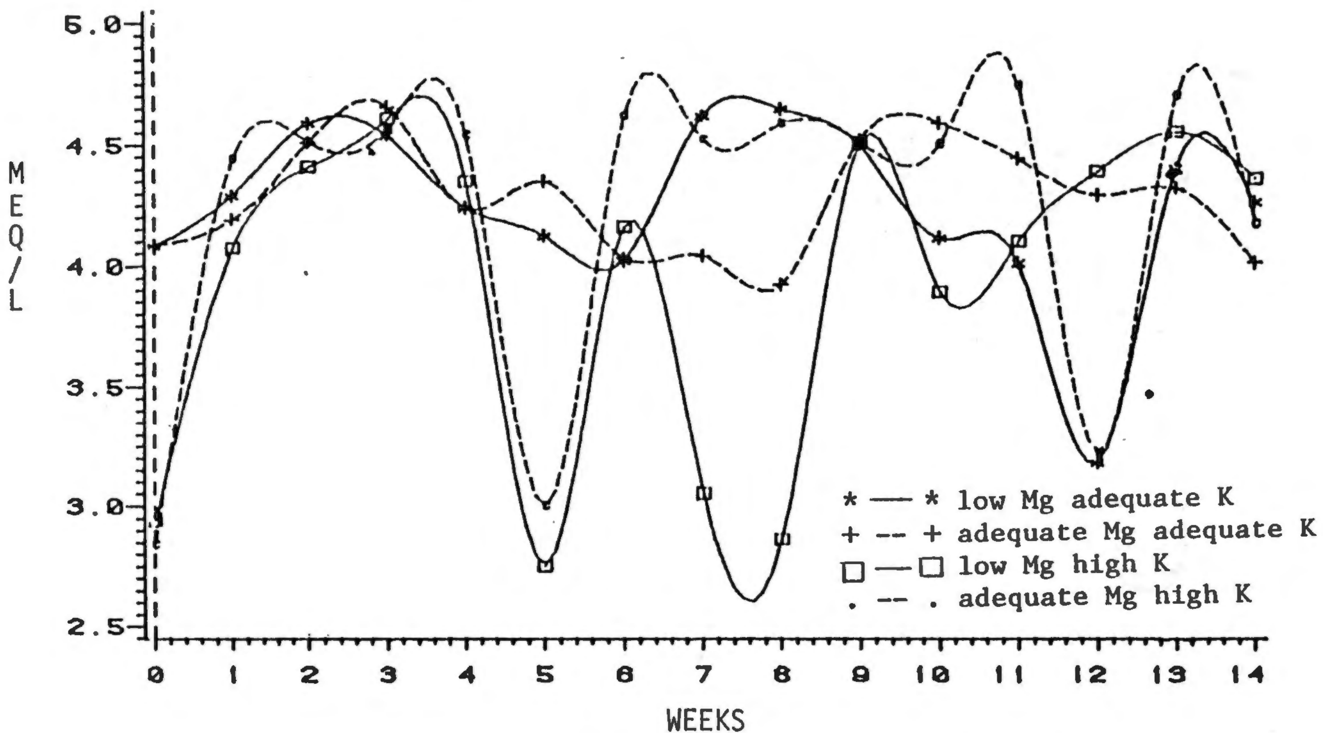

FIGURE 3. PLASMA CALCIUM CONCENTRATION IN PREGNANT OR LACTATING EWES FED SEMIPURIFIED DIETS DURING FIRST EXPERIMENT.

TABLE 10. IN VITRO PLATELET REACTIVITY TO BURROS COLLAGEN[1] FOR EWES DURING FIRST EXPERIMENT

Burros Collagen (Ug/ml)	Delay time[2] (SEC)			Velocity[3] (%/min)			Intensity[4] (%)		
	Preliminary	Gestation	SE[5]	Preliminary	Gestation	SE[5]	Preliminary	Gestation	SE[5]
10	8	7	0.46	80	70	2.69	88	87	1.74
5	10	8	0.44	87	73[a]	3.06	86	86	1.68
2	12	10[a]	0.52	80	66[a]	3.22	86	85	1.68
1	16	14	0.66	70	58[b]	2.41	84	84	2.11
0.5	21	19	0.91	59	52[a]	1.67	82	84	1.14

[1]Preliminary and gestation periods within measurement differ significantly (a, $P<.05$; b, $P<.01$).

[2]Delay time in seconds between collagen addition and onset of aggregation.

[3]Maximal aggregation velocity as percentage aggregation per minute during the most rapid phase of platelet clumping.

[4]Maximal percentage of aggregation accomplished in 7 minutes while stirring and incubating at $37^{0}C$.

[5]Standard error of mean.

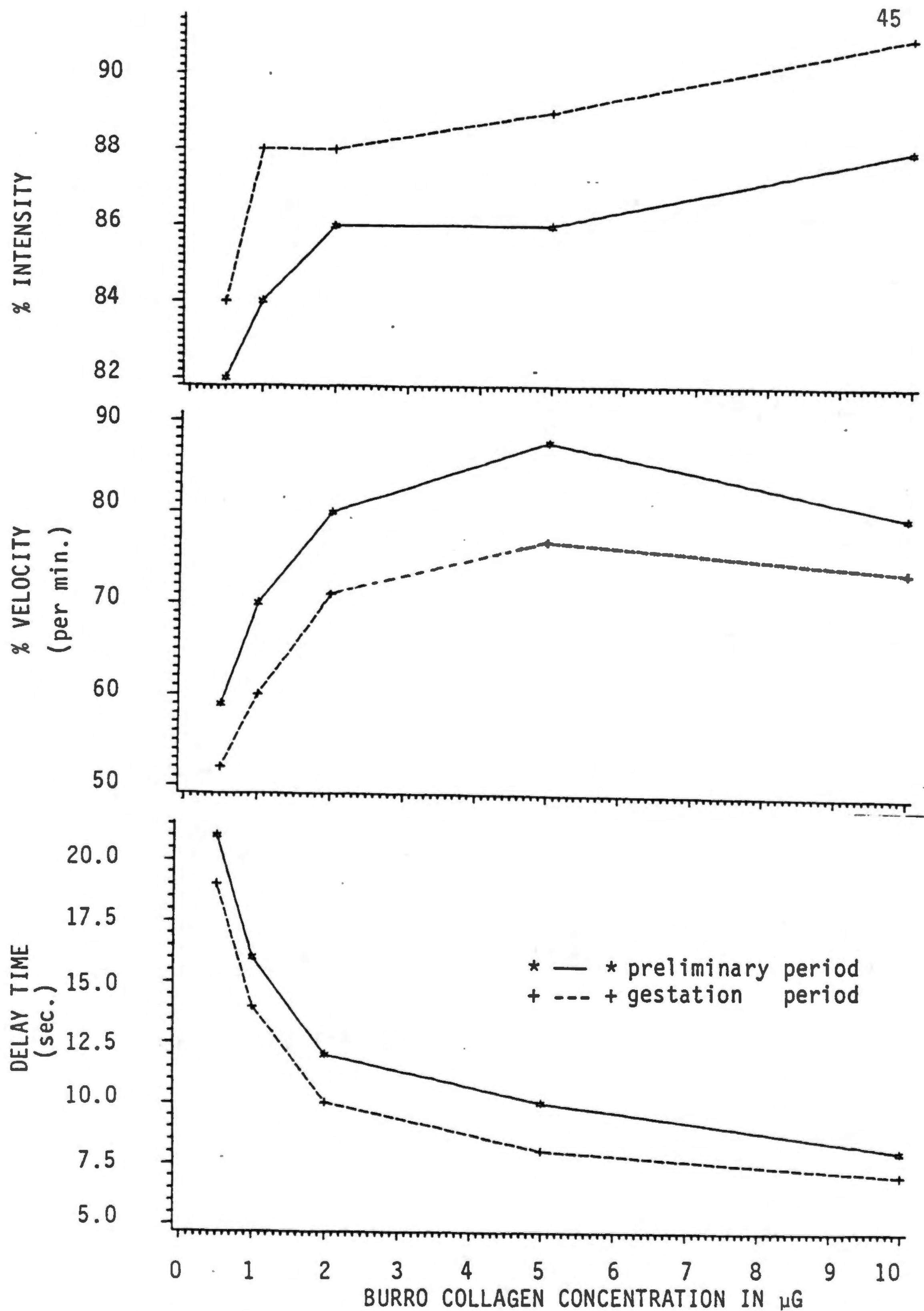

FIGURE 4. IN VITRO PLATELET REACTIVITY TO BURRO COLLAGEN IN EWES DURING PRELIMINARY AND GESTATION PERIODS (FIRST EXPERIMENT).

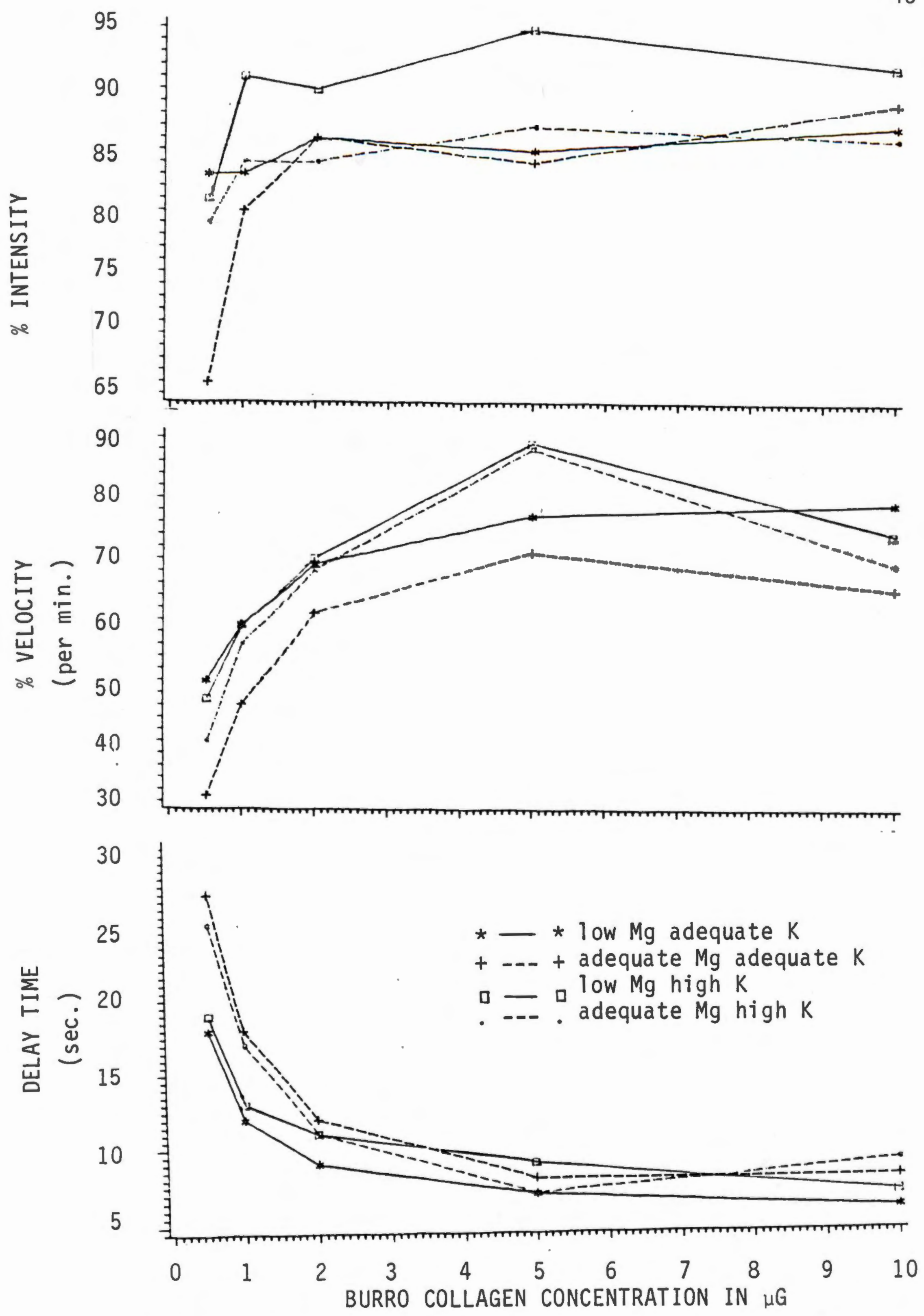

FIGURE 5. IN VITRO PLATELET REACTIVITY TO BURRO COLLAGEN IN EWES 24-48 HOURS AFTER PARTURITION (FIRST EXPERIMENT).

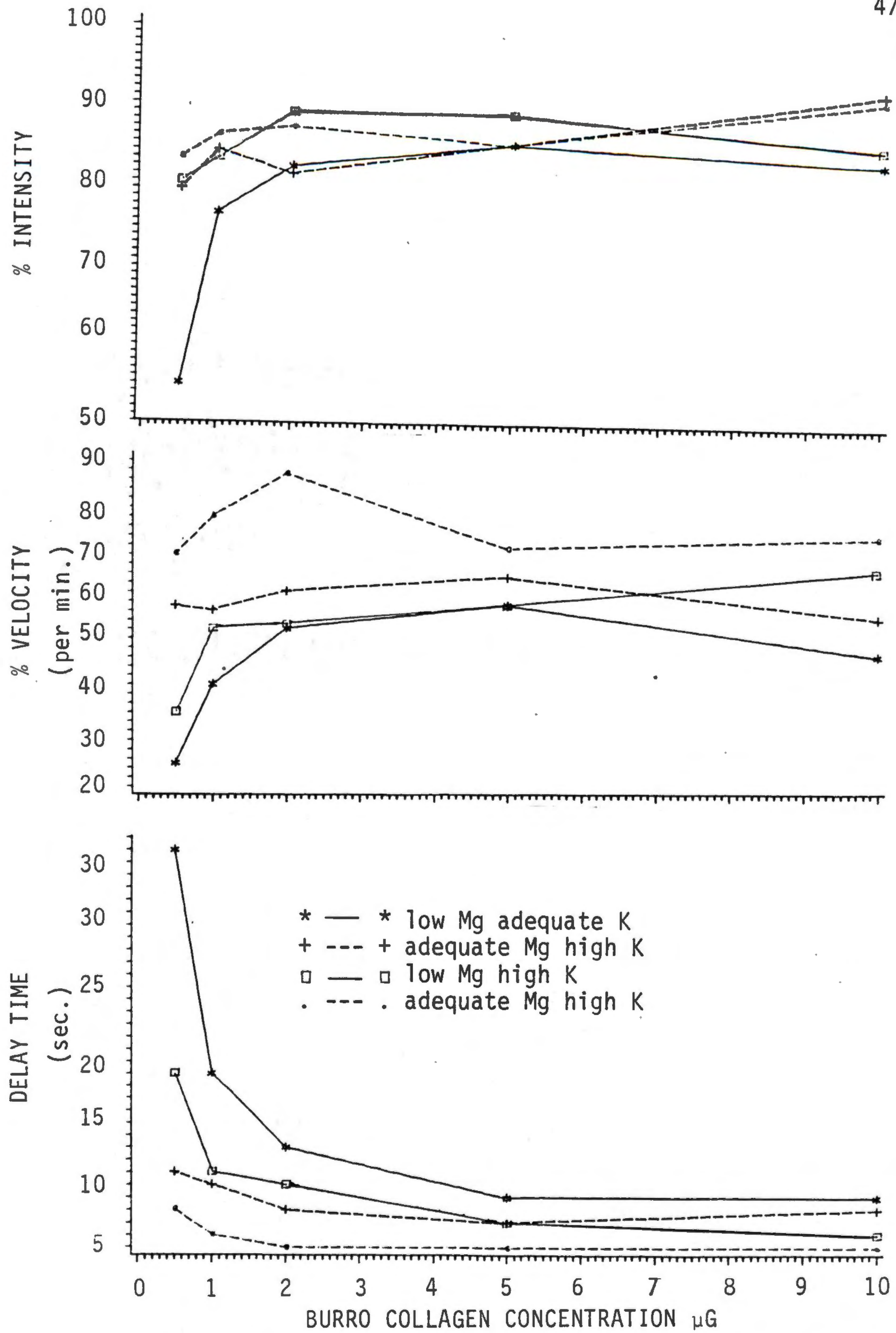

FIGURE 6. IN VITRO PLATELET REACTIVITY TO BURRO COLLAGEN IN EWES DURING LACTATION (FIRST EXPERIMENT).

periods and was higher ($P<.05$) during preliminary than during gestation periods (Table 10, Figure 4). There were no statistically significant differences between the two periods (Table 10) in maximal aggregation intensity accomplished in 7 minutes while stirring and incubating at $37^{o}C$ (Figure 4).

After parturition and during lactation in vitro platelet reactivity to burro collagen followed the same pattern as during preliminary or gestation periods. However, no differences were found in delay time, velocity, and intensity, due to Mg or K treatments after parturition (Figure 5) or during lactation (Figure 6).

Second Experiment

Results of the second experiment were evaluated through 8 weeks only since health of the ewes failed before parturition and they did not complete 14 weeks as in the first experiment. Least square means were used to evaluate data and orthogonal comparisons were made to separate effect of Mg or first and second experiment. The effect of dietary K treatment from first experiment and ewe lambs from second experiment were excluded from the comparison because these were not common to both experiments.

Plasma Minerals and Blood Glucose

Within 8 weeks of experimental period in the second experiment, plasma Mg ($P<0.01$) averaged lower but blood glucose ($P<.05$) was

higher in ewes fed the diet unsupplemented with Mg than in those receiving the Mg adequate diet (Table 11). There was no difference in plasma Ca among ewes fed the experimental diets.

Comparing results of the second to first experiment indicated that supplementing diets with Mg resulted in a similar effect (Figure 7) on plasma Mg but had no effect on plasma Ca (Figure 8) in both experiments (Table 11). However, plasma K and blood glucose were lower (P<.01) in the second than in the first experiment. The decline in plasma K during the second experiment was more noticeable during the earlier than later periods in comparison with the first experiment (Figure 9). Within 3 weeks, blood glucose in ewes began to decline (Figure 10) and continued on until the end of the second experimental period.

Blood Pressure and Heart Rate

Feeding an adequate Mg diet to ewes in the second experiment slightly lowered systolic, diastolic and mean arterial pressures, and heart rate compared to ewes receiving Mg deficient diet but differences were not significant (Table 11). However, when these results were compared to the first experiment, they indicated that systolic blood pressure and heart rate averaged lower (P<.05) but diastolic and mean arterial pressures did not differ between experiments.

Response to Previous Mg Status

Results of the second experiment were evaluated in response to previous Mg status from first experiment (Table 12). Present Mg

TABLE 11. LEAST SQUARE MEANS COMPARISON OF 8 WEEKS FOR PREGNANT EWES[1] FED SEMIPURIFIED DIETS DURING FIRST AND SECOND EXPERIMENT

Variable	First Experiment		Second Experiment		SE^2
	Mg-deficient	Mg-adequate	Mg-deficient	Mg-adequate	
Plasma Mg (m Eq/L)	1.84	2.13[a]	1.78	2.18[b]	0.04
Plasma Ca (m Eq/L)	4.21	4.41	4.50	4.33	0.06
Plasma K (m Eq/L)	4.42	4.55	3.58	3.66[d]	0.04
Whole Blood Glucose (mg/dl)	53.31	53.77	42.59	34.40[a,d]	0.86
Systolic (mm Hg)	126.46	125.63	120.29	118.54[c]	1.31
Diastolic (mm Hg)	72.84	70.66	73.50	70.50	0.94
Mean Arterial Pressure (mm Hg)	86.93	90.78	88.86	88.18	1.20
Heart Rate Per Minute	133.93	139.04	132.49	124.25[c]	1.98

[1]Ewe lambs from second experiment were not included in this comparison.

[2]Standard error of mean.

[a,b]Mg deficient differed significantly from Mg adequate (a, $P<.05$; b, $P<.01$).

[c,d]First experiment differed significantly from second experiment (c, $P<.05$; d, $P<.01$).

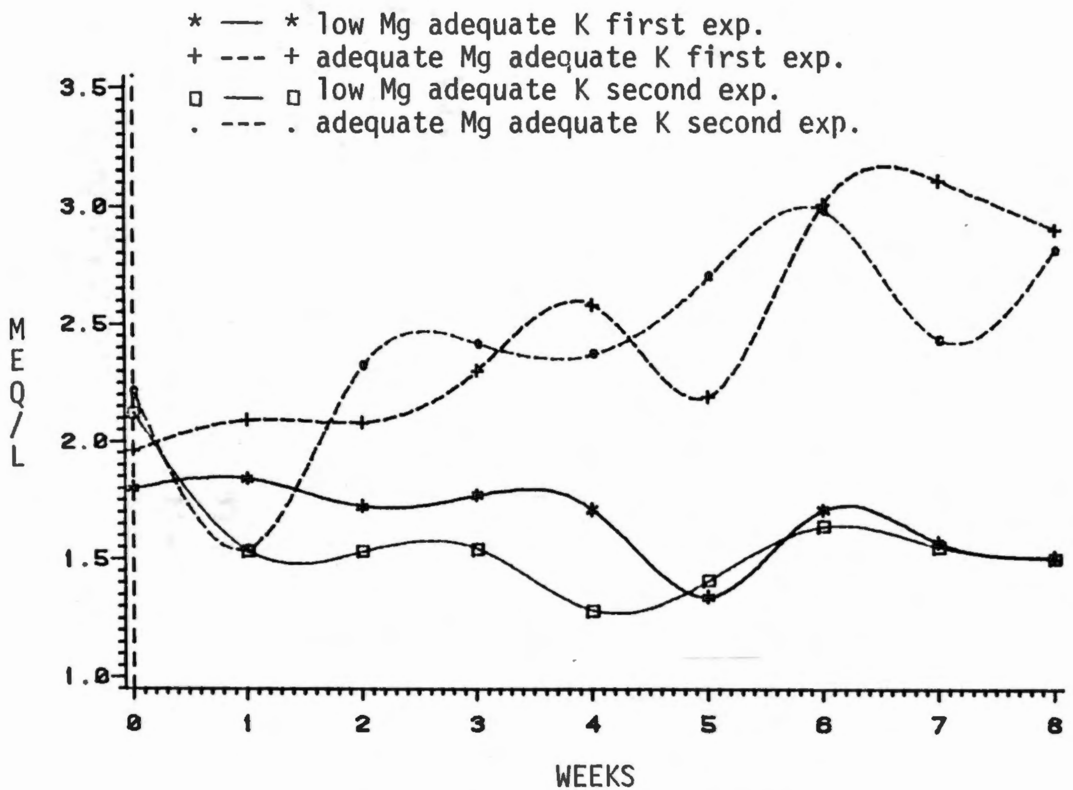

FIGURE 7. PLASMA MAGNESIUM CONCENTRATION IN PREGNANT EWES FED SEMIPURIFIED DIETS DURING FIRST AND SECOND EXPERIMENT.

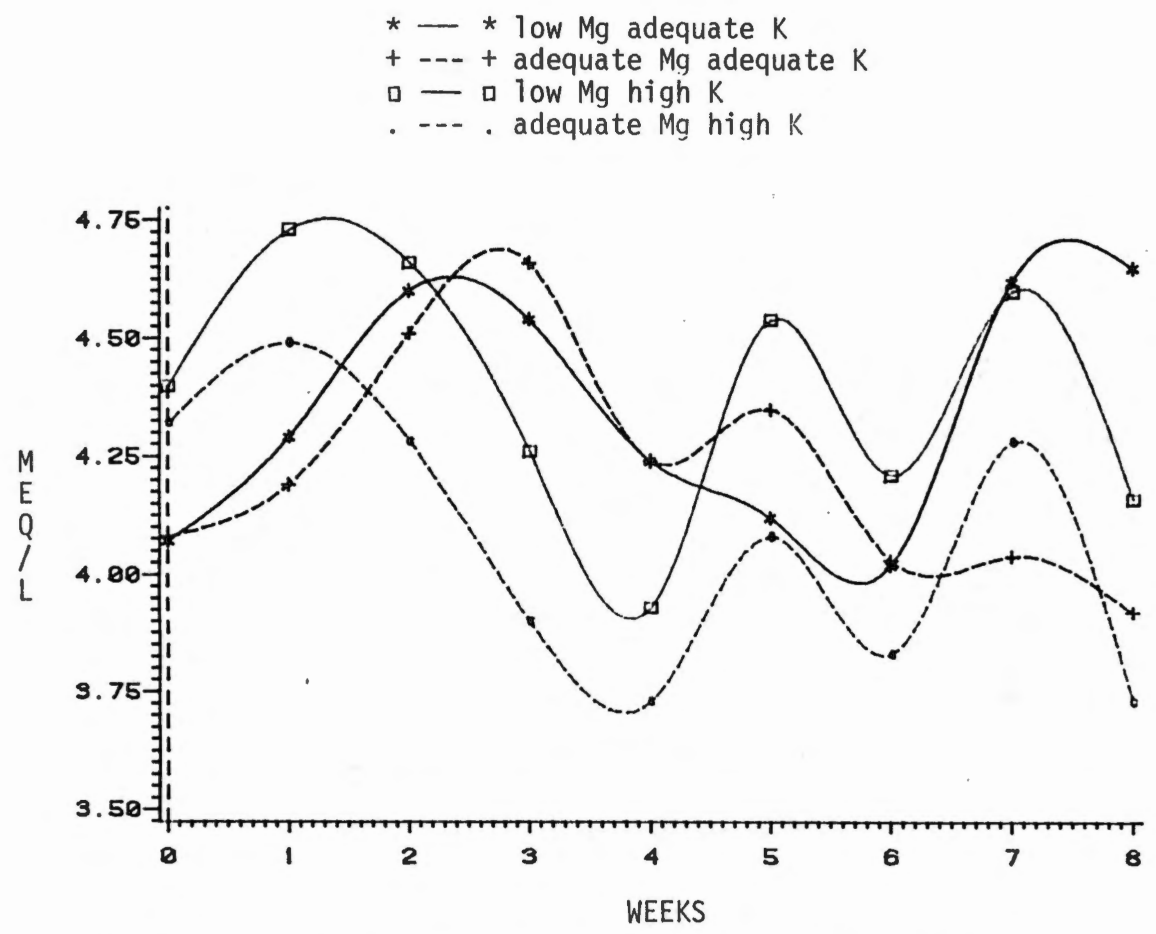

FIGURE 8. PLASMA CALCIUM CONCENTRATION IN PREGNANT EWES FED SEMIPURIFIED DIETS DURING FIRST AND SECOND EXPERIMENT.

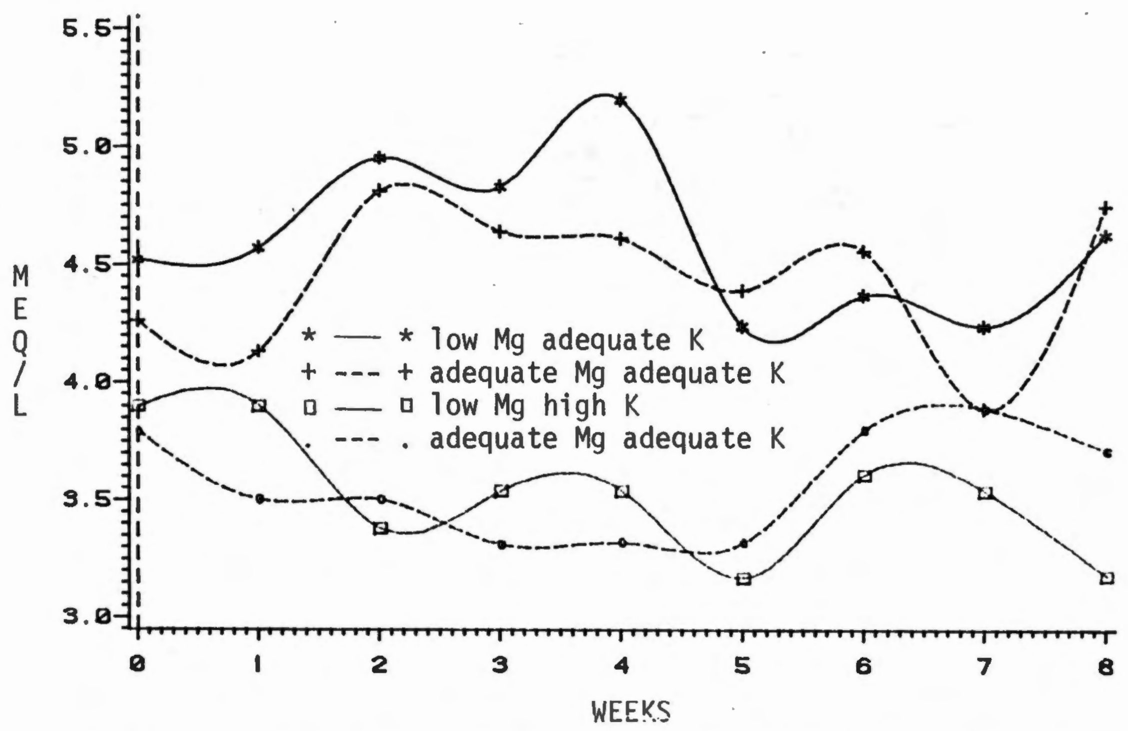

FIGURE 9. PLASMA POTASSIUM CONCENTRATION IN PREGNANT EWES FED SEMIPURIFIED DIETS DURING FIRST AND SECOND EXPERIMENT.

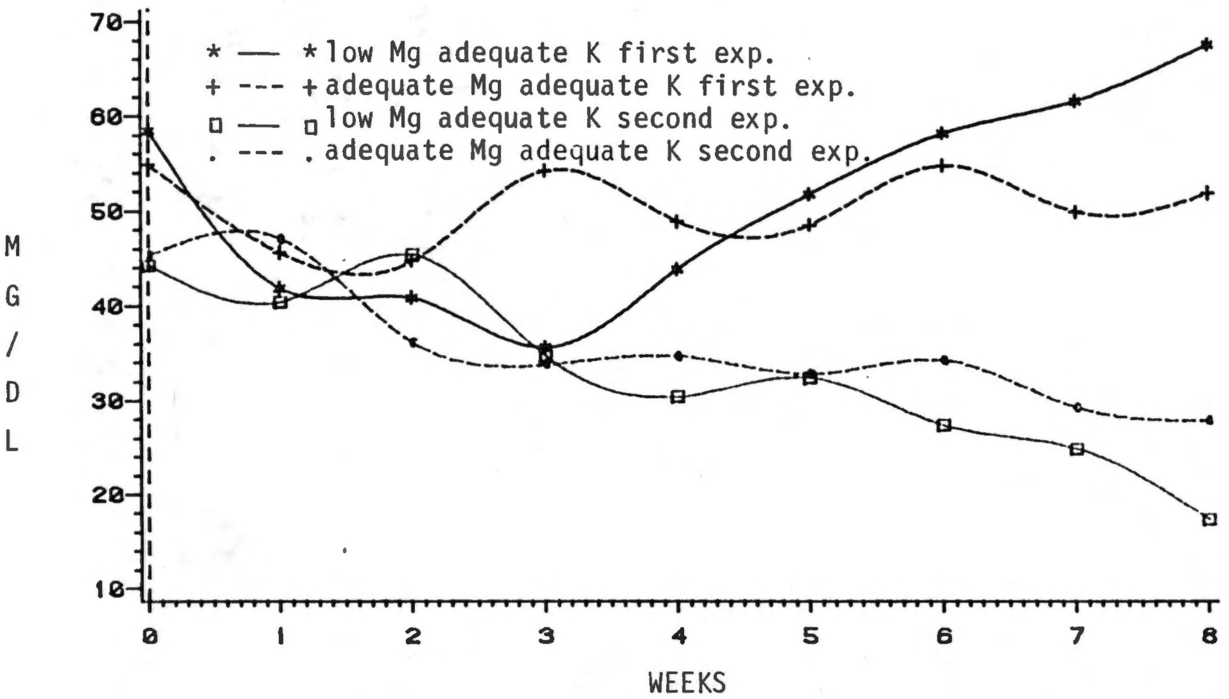

FIGURE 10. BLOOD GLUCOSE CONCENTRATION IN PREGNANT EWES FED SEMIPURIFIED DIETS DURING FIRST AND SECOND EXPERIMENT.

TABLE 12. LEAST SQUARE MEANS COMPARISON OF 8 WEEKS FOR PREGNANT EWES[1] IN RESPONSE TO PREVIOUS Mg STATUS DURING SECOND EXPERIMENT

Variable	Mg Status[2] Previous/Present				SE[3]
	A/A	A/D	D/A	D/D	
Plasma Mg (m Eq/L)	2.60	1.58^{b}	2.26	1.61^{b}	0.04
Plasma Ca (m Eq/L)	4.27	4.52^{b}	4.30	4.60^{b}	0.02
Plasma K (m Eq/L)	3.84	3.87	3.53	3.55^{c}	0.03
Systolic (mm Hg)	113.23	123.82	116.51	121.87	1.47
Diastolic (mm Hg)	69.73	80.28^{a}	66.89	75.64^{a}	1.07
Mean Arterial Pressure (mm Hg)	83.35	95.97	82.85	93.43	1.28
Heart Rate Per Minute	124.41	121.12	136.31	121.61^{b}	1.83
Packed Cell Volume (%)	37.20	38.49	39.96	43.82^{d}	0.42

[1]Two mature ewes and two ewe lambs were used in each treatment.

[2]A = Adequate Mg, D = Deficient Mg, First experiment/Second experiment.

[3]Standard error of mean.

[a,b]Present Mg deficient differed significantly from present Mg adequate (a, $P<.05$; b, $P<.01$).

[c,d]Previous Mg deficient differed significantly from previously Mg adequate (c, $P<.05$; d, $P<.01$).

deficiency lowered plasma Mg ($P<.01$) and increased plasma Ca ($P<.01$) but had no effect on plasma K (Table 12). Diastolic pressure ($P<.05$) was higher and heart rate ($P<.01$) was lower in ewes receiving Mg deficient than Mg adequate diets (Table 12). Present Mg deficiency slightly increased systolic, mean arterial pressures and packed cell volume but differences were not statistically significant (Table 12).

Previous Mg deficiency lowered ($P<.05$) plasma K and increased ($P<.01$) packed cell volume but had no effect on plasma Mg, Ca or blood pressures and heart rate (Table 12).

CHAPTER V

DISCUSSION

Ewes fed Mg deficient diets in the first experiment had lower plasma Mg concentrations than those fed Mg adequate diets containing either adequate or high K. Within 4 weeks plasma Mg began to fall in ewes receiving the Mg deficient diets (Figure 1, page 40). This decline continued until the twelfth week of the experimental period and plasma Mg averaged lower for the whole period. Similar findings (Fontenot et al., 1960; Suttle and Field, 1967, 1969; Newton et al., 1972; Miller et al., 1980; McKim, 1983; Reynolds, 1985) indicated a direct relationship of low blood Mg concentration to low dietary Mg content and increased fecal Mg loss accompanying elevated K intake. When excess K was added to a low Mg diet of wether lambs (Ramsey et al., 1982) the rate of plasma Mg decline increased over that due to low Mg alone. However, in this experiment, supplementing diets with high K did not influence plasma Mg levels of ewes that received the Mg deficient diet but caused a decrease in plasma Mg of ewes fed the Mg adequate over those fed the Mg adequate and supplemented with adequate K. When insulin was elevated (Reynolds, 1985) observed a similar depression in plasma Mg of calves fed adequate Mg excess K diets and indicated that dietary K at levels often contained in early spring grasses may alter insulin and glucagon metabolism such that K, Mg and carbohydrate metabolism is influenced. House and Van Campen (1971) attributed the decline in plasma Mg partially to decreased Mg

absorption, but mainly to a direct depressing effect of K on the circulating Mg. The absorption and retention of K increases in response to increased intake of the cation and it has been postulated that cells take up and retain more Mg as the level of K in the cell increases. Lentz et al. (1976) demonstrated that intravenous infusion of K will increase insulin and glucose in Mg deficient calves. They suggested circulating Mg was transferred into tissue, thus lowering serum Mg. Therefore, high K could exert an effect by altering intermediary metabolism in some way. Plasma K averaged higher in ewes fed high dietary K than in those receiving adequate K regardless of Mg treatment (Table 9, page 39). Higher absorption of K in response to increased intake could contribute to the faster decline in plasma Mg of ewes fed adequate Mg when dietary K was excessive compared to adequate. Similar findings have been reported (Kunkel et al., 1953; Fontenot et al., 1960; Bohman et al., 1969; Yano et al., 1982). However, there was no difference in plasma Ca of ewes due to Mg or K treatments (Table 9, page 39). Chicco et al. (1973) found that an increased level of Mg (500 to 7,750 ppm) fed to wethers did not influence fecal excretion or bone concentrations of Ca but reduced both urinary and plasma Ca. They indicated that increased Ca decreased Mg utilization while Mg had the same effect on Ca. This effect could be explained by competition between Ca and Mg for the same absorption mechanism involving a limited carrier system for both minerals (Care and Van't Klooster, 1965). In the first experiment Ca content of the concentrate diets were increased from

0.28% during gestation to 0.46% after parturition (Table 3, page 27) to meet recommended allowances for lactation (NRC, 1975). Increasing Mg content of the diet from 0.03% (diet 1 and 3) to 0.25% (diet 2 and 4) and supplementing them with adequate K level 0.73 and 0.69% in diet 1 and 2 or high K 3.55 and 3.23% in diets 3 and 4 (Table 3, page 27) apparently was not enough to cause a reduction in plasma Ca of ewes (Table 9, page 39). Fluctuations in plasma Ca throughout the experimental period could not be explained by dietary Mg or K intake (Figure 3, page 27).

Whole blood glucose of the ewes averaged 54 mg/dl for the experimental period and was not affected by Mg or K treatments (Table 9, page 39). There were no clinical signs of hypomagnesemia or metabolic disorders in ewes fed the Mg deficient diet during gestation and all diets were adequate in supporting normal pregnancy and successful lambing. However, ewes fed the Mg deficient diet had lower survival rate of lambs (33%) than ewes receiving the Mg adequate diet (100%) when K was not excessive. When high K was included in the diet, survival rate of lambs dropped from 75% in ewes fed the Mg deficient diet to 50% for ewes receiving the Mg adequate diet. However, numbers of animals are too small to determine whether these differences are meaningful. Wilson (1964) reported that the symptoms of nervous origin are likely to occur if plasma Mg concentration falls below 1.0 to 1.2 mg/dl. In this experiment plasma Mg concentration averaged 1.9 mg/dl in ewes fed Mg deficient diets with either adequate or high K. This could explain the lack of clinical signs of hypomagnesemia in this experiment.

During 8 weeks comparison in the second experiment (Table 11, page 50), plasma Mg was lower but plasma K and Ca were not affected in ewes fed Mg deficient compared to those receiving Mg adequate diet. Comparison of both experiments indicated that Mg deficient diet resulted in a similar effect on plasma Mg but had no effect on plasma Ca (Table 11, page 50) in the two experiments. However, plasma K and blood glucose were lower in the second than in the first experiment (Table 11, page 50). During the late stage of gestation in the second experiment, ewes fed both experimental diets developed abnormal plasma K (as low as 6.2 mg/dl), glucose (as low as 13.8 mg/dl), acetoacetate (>500 M/L) and β-hydroxy butyrate (>5,000 M/L). Changes were more severe in sheep previously Mg deficient but were not affected by present Mg status. Although some of the ewes displayed signs of pregnancy toxemia, none responded to treatment with propylene glycol or removal of fetuses by caesarean section. The problem was recognized as copper toxicity due to high Cu content of corn gluten meal too late to prolong the comparison through lactation. Impaired gluconeogenesis due to liver and kidney damage in Cu poisoned ewes could cause hypoglycemia and explain why it failed to respond to propylene glycol given orally (Baird et al., 1974; Perry, 1979; Prior and Christenson, 1978). Hypokalemia could have resulted from excessive K loss by damaged kidneys. The diet used in the second experiment was similar to the one used satisfactorily in the first experiment except that 4% $KHCO_3$ was used to provide excess K in diet 3 and 4 in the first experiment (Table 2, page 26) so the same amount

of HCO_3^- as $NaHCO_3$ was added to diets 1 and 2 (Adequate K). In the second experiment, K was not a variable so 1.1% $KHCO_3$ and 3.2% corn cobs replaced 3.4% $NaHCO_3$ and 0.9% KCl in the normal K diet (Tables 4 and 5 pages, 29,30). In a subsequent comparison (unpublished), fecal recovery of oral ^{64}Cu in a 4-day balance trail averaged 73% in four wethers fed the first experiment diet but only 46% for four wethers on the second experimental diet. Lower absorption of Cu may have protected the ewes from Cu toxicity in the first experiment.

Hereditary susceptibilty to clinical hypomagnesemia has been suggested on the basis of repeated occurrence in individual animals. Previous Mg deficiency, either in utero or as adult ewes in the second experiment (Table 12, page 55) did not affect plasma Mg and Ca, but decreased plasma K and increased packed cell volume. Plasma Mg and Ca were affected primarily by present Mg status (Table 12, page 55).

In the first experiment blood pressure and heart rate during pregnancy and lactation were lower and animals were calmer during blood sampling and blood pressure measurement when dietary Mg was adequate but K was not excessive (Table 9, page 39). Similar results were observed during pregnancy in the second experiment (Table 11, page 50). Blood pressure and heart rate averaged slightly lower in adult ewes fed Mg adequate diets than those receiving Mg deficient diets (Table 11, page 50). When ewe lambs were included in the comparison of the second experiment, systolic and diastolic blood pressure were slightly lower while mean arterial pressure was

statistically lower ($P<.05$) and heart rate was higher ($P<.05$) in ewes fed the Mg adequate diet (Table 13). There was no indication that previous Mg status had an affect on blood pressure or heart rate (Table 12, page 55). However, blood pressure and heart rate were higher in ewes fed adequate Mg than in Mg deficient ewes when excess K was fed in the first experiment (Table 9, page 39). The results of both experiments indicate that supplementing diets with Mg would lower blood pressure when diets contain adequate but not excessive K. Gant and Worley (1980) suggested the use of $MgSO_4 \cdot 7H_2O$ as a treatment for controlling hypertension in pregnant women. They noted a transient, mild reduction in blood pressure after administration of $MgSO_4 \cdot 7H_2O$ as an intravenous bolus and reported that therapeutic anticonvulsant blood levels were achieved when the concentration of Mg reached 4 to 4.5 M Eq/Liter. Altura and Altura (1982) indicated that extracellular Mg was important in control of coronary arterial tone via regulation of vascular membrane Mg-Ca exchange sites. It is possible then, that deficient Mg diets could have contributed to increased vascular tension in a number of ways: 1) by eliciting contractile activity indirectly by increasing membrane permeability to Ca, 2) potentiating the effects of vasoconstrictors, and 3) depressing the effects of vasodilators. This would explain the higher blood pressure found in ewes fed Mg deficient diets in both experiments.

To investigate the role of dietary Mg on platelet function in the first experiment, in vitro platelet reactivity to burro collagen

TABLE 13. LEAST SQUARE MEANS COMPARISON OF 8 WEEKS FOR PREGNEANT EWES[1] FED SEMIPURIFIED DIETS DURING SECOND EXPERIMENT

Variable	Adequate K Mg-deficient	Mg-adequate	SE[2]
Plasma Mg (m Eq/L)	1.58	2.40[b]	0.04
Plasma Ca (m Eq/L)	4.56	4.30[b]	0.02
Plasma K (m Eq/L)	3.70	3.68[a]	0.03
Systolic (mm Hg)	123.14	114.92	1.47
Diastolic (mm Hg)	77.82	68.30	1.07
Mean Arterial Pressure (mm Hg)	94.85	84.04[a]	1.28
Heart Rate Per Minute	118.52	129.20[a]	1.83
Packed Cell Volume (%)	41.17	38.78	0.42

[1]Four mature ewes and four ewe lambs were used in each treatment.

[2]Standard error of mean.

[a,b]Indicates significant difference among treatment means (a, P<.05; b, P<.01).

was measured in plasma of ewes before breeding, during gestation, after parturition, and during lactation. In the second experiment, blood samples were not obtained for platelet study due to the health problem of the ewes. During the preliminary period, platelets in plasma tended to have slightly longer delay time than during the gestation period (Table 10, page 44). This would indicate that the stress imposed by pregnancy increased reactivity of platelets and led to shorter delay time (Figure 4, page 45). However, contrary to expectation, maximal aggregation velocity was higher during preliminary than during gestation periods and there were no differences between the two periods in maximal aggregation intensity (Table 10, page 44, Figure 4, page 45). No differences were found in delay time, velocity, and intensity, due to Mg or K treatments after parturition (Figure 5, page 46) or during lactation (Figure 6, page 47).

Considered together, results of in vitro tests indicated that in response to burro collagen, platelets from ewes fed the Mg deficient diet either were less reactive or did not differ from platelets from ewes fed the Mg adequate diet when supplemented with adequate or high K. However, when total and aggregated platelets were counted in plasma of ewes after parturition to determine the percentage of platelets present in aggregates to be used as an index of activation in vivo, the results indicated that ewes fed the Mg deficient diets 1 and 3 had a higher percentage of platelets clumped (18 and 22%) than those receiving the Mg adequate diets 2 and 4 (8 and 6%). This is consistent with an ability of Mg in delaying

clumping of platelets. A similar finding was reported by Hughes and Tonks (1965) who found that addition of Mg to platelet rich plasma in vitro delayed clumping of platelets. Ardlie et al. (1970) also pointed out that Mg is necessary for maintenance of the disc shape and is required for platelet disaggregation.

Recent studies focused on isolation and characterization of powerful, biologically active, highly labile, hormone-like compounds endogenously formed from prostaglandin endoperoxides suggest that changes in the vascular endothelium as well as in the platelets should be considered (Hamberg et al., 1974a,b; Moncada and Vane, 1977, 1979; Samuelsson et al., 1978). Endothelium and smooth muscle cells in blood vessel walls and lung synthesize a powerful anti-and de-aggregatory and vasodilative prostaglandin, prostacyclin (PGI_2) (Moncada and Vane, 1977). Platelets produce an equally powerful proaggregatory and vasoconstrictive compound, thromboxane A_2 (Samuelsson and Hamberg 1974). These opposing substances equalize each others actions on blood vessel walls, lung and heart in healthy animals receiving adequate Mg.

In the first experiment the stable hydrolysis products of prostacyclin and thromboxane A_2 in plasma of ewes during preliminary and gestation periods were measured by radioimmunoassay, since both these substances are unstable and have short half-lives (Granstrom et al., 1976; Olson et al. 1982). The levels of those products (6-keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2) should reflect the levels of their precursors prostacyclin and thromboxane A_2,

respectively. Thromboxane B_2 in plasma of ewes increased ($P<.05$) from 1.80 ng/ml in preliminary periods to 4.90 ng/ml during gestation (Figure 11) while 6-keto-prostaglandin $F_{1\alpha}$ concentration in plasma during those periods were 21.25 and 20.00 pg/ml (Figure 11). These results suggest that although the decline in 6-keto-prostaglandin $F_{1\alpha}$ during gestation is not statistically lower than during the preliminary period the increase in thromboxane B_2 would lead to elevation in thromboxane A_2: prostacyclin ratio. Elevated thromboxane A_2: prostacyclin may predispose hypertension during the gestation period. These results were in agreement with the finding of Remuzzi et al. (1981) who reported that plasmatic activity of prostacyclin-stimulating factor during early pregnancy was comparable with that in non-pregnant women. However, in late pregnancy they found the activity was significantly depressed. Blood pressures were higher and platelet counts declined in plasma of women patients with pre-eclampsia compared with women in early or late normal pregnancy (Remuzzi et al., 1981). This could be attributed to lower activity of prostacyclin and elevation in prostacyclin: thromboxane A_2 ratio which led to higher platelet clumping and higher blood pressure. Prostacyclin production by endothelial cells was increased by 2.3 fold when cells were incubated with plasma obtained from pre-eclamptic patients on $MgSO_4$ therapy compared to pretherapy plasma (Watson et al., 1984).

In this experiment, the effect of Mg and K treatments on thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ could not be tested because the limited amount of plasma stored was used to establish

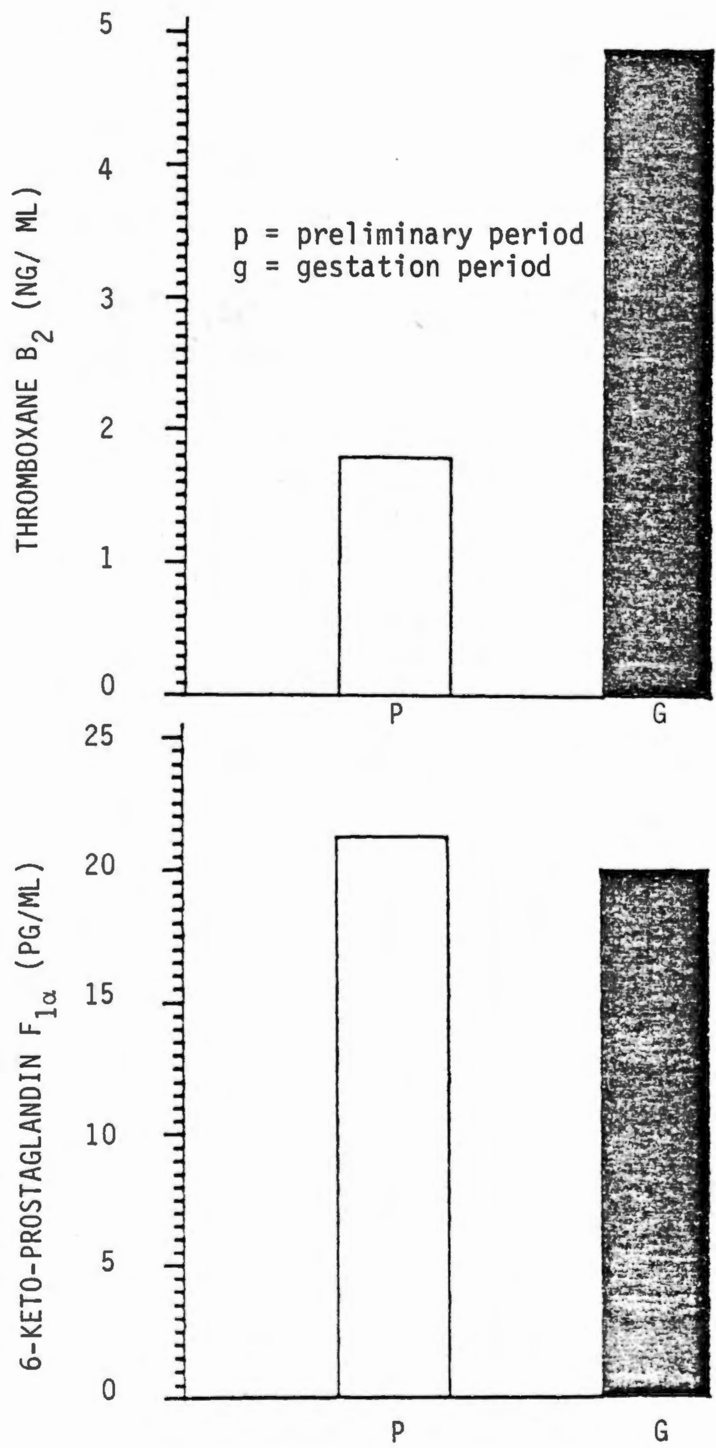

FIGURE 11. PLASMA CONCENTRATION OF 6-KETO-PROSTAGLANDIN $F_{1\alpha}$ AND THROMBOXANE B_2 FOR EWES DURING PRELIMINARY AND GESTATION PERIODS IN FIRST EXPERIMENT.

the correct dilution rate for radioimmunoassay. However, the comparison of preliminary and gestation periods agreed with previous findings (Bonnar et al., 1977; Gant and Worley, 1980; Remuzzi et al., 1980, 1981; Watson et al., 1984).

Further study is needed to test the hypothesis that vascular endothelial damage in Mg deficient ewes could reduce formation of prostacyclin while thromboxane A_2 formation by hyperactive platelets is unaffected or even increased. This would tip the balance in favor of thromboxane, thus favoring thrombus formation and vasospasm which could be a significant mortality risk factors in Mg deficient ewes.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Two experiments were designed to investigate the relationships between Mg, previous Mg status, K, and platelet reactivity during pregnancy and lactation. In the first experiment 12 Finnish cross bred ewes were fed one of four diets: deficient Mg, adequate K (diet 1), adequate Mg plus adequqte K (diet 2), deficient Mg, high K (diet 3) or adequate Mg plus high K (diet 4). In the second experiment, eight mature ewes and eight ewe lambs from the first experiment were used to investigate effects of previous Mg deficiency, either prenatally or as adults. Ewes were fed either a Mg deficient (diet 1) or Mg adequate (diet 2).

In the first experiment plasma Mg was higher ($P<.05$) in ewes fed Mg adequate diets than in those fed the Mg deficient diet supplemented with adequate K but not high K. High dietary K increased ($P<.05$) plasma K and lowered ($P<.05$) plasma Mg in ewes fed Mg-adequate but not Mg-deficient diets. Plasma Ca and blood glucose were not affected by treatment. Ewes fed diets adequate in both Mg and K had lower ($P<.05$) systolic and diastolic blood pressure than those fed the diet deficient in Mg but adequate in K. High K intakes lowered ($P<.05$) systolic, diastolic, and mean arterial pressures when Mg-deficient but not Mg-adequate were fed. In vitro platelet delay time was longer and % aggregation velocity was higher ($P<.05$) during gestation but % aggregation intensity was not different from

measurements made before the ewes were bred. Magnesium and K treatments after parturition or during lactation did not affect in vitro platelet reactivity measurements in ewes. Plasma thromboxane B_2 was higher ($P<.05$) in ewes during gestation than during preliminary period but 6-keto-prostaglandin $F_{1\alpha}$ in plasma was not affected. An elevated thromboxane A_2 to prostacyclin ratio could contribute to higher blood pressure often noted during pregnancy.

Ewes fed the Mg-deficient diet in the second experiment had lower ($P<.01$) plasma Mg, higher ($P<.01$) plasma Ca and higher ($P<.05$) plasma K than ewes fed the Mg-adequate diet. Mean arterial pressure was higher ($P<.05$) and heart rate was lower ($P<.05$) in ewes fed Mg-deficient compared to Mg-adequate diets. Previous Mg deficiency lowered ($P<.05$) plasma K and increased ($P<.01$) packed cell volume but did not affect plasma Mg and Ca. Previous Mg status did not affect blood pressures or heart rate. The ewes plasma K and blood glucose were lower ($P<.01$) in the second than in the first experiment.

Ewes in the second experiment developed abnormal health problems which forced early termination of the comparison before parturition. The problem was recognized later as Cu toxicity due to high Cu content of corn gluten meal and possibly low Mo in the diets.

In conclusion, the deficient Mg diets lowered plasma Mg when supplemented with adequate K. High dietary K lowered plasma Mg when Mg was fed at adequate levels but not deficient. There was an inverse relationship between plasma Mg and blood pressure when dietary K was not excessive. In vitro platelet reactivity measurements indicated

platelet were more reactive to collagen during gestation than during the preliminary period, but were not affected by Mg or K treatments after parturition or during lactation. Thromboxane B_2 concentration in plasma of ewes was higher during gestation than during the preliminary period but 6-keto-prostaglandin $F_{1\alpha}$ concentration was not affected. Further study is needed to determine the effect of Mg deficiency on hormone activity in ruminants.

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