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Effects of Female Sex Hormones and Dietary Magnesium Levels in Sheep

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To the Graduate Council:

I am submitting herewith a thesis written by Kenny S. Marbury entitled "Effects of Female Sex Hormones and Dietary Magnesium Levels in Sheep." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

M. C. Bell, Major Professor

We have read this thesis and recommend its acceptance:

R. L. Murphee, G. M. Merriman

Accepted for the Council:
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Vice Provost and Dean of the Graduate School

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

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R L Murphree

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EFFECTS OF FEMALE SEX HORMONES AND DIETARY MAGNESIUM LEVELS IN SHEEP

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Kenny S. Marbury

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ABSTRACT

The major objectives of this study were to evaluate (a) the effects of diet on magnesium, calcium and potassium metabolism in yearling wethers and (b) the effects of progesterone, estradiol and diethylstilbestrol (DES) on the metabolism of the minerals.

A two by two factorial design was selected whereby twelve yearling crossbred wether lambs were divided into four groups of three. One group was fed a basal diet plus magnesium along with hormones, one group was fed a basal diet plus magnesium without hormones, one group was fed a magnesium-deficient diet along with hormones, and one group was fed a magnesium-deficient diet without hormones.

During trial one, two of the groups were injected intramuscularly with progesterone every twelve hours for four consecutive days. Then, forty-eight hours after the last injection of progesterone, 100 µg of estradiol was injected intramuscularly in two separate injections (50 µg per injection) six hours apart. This was intended to simulate the hormonal milieu of an ewe coming into estrus. At the time of the initial injection of estradiol, 61.3 µc of magnesium-28 and 701 µc of calcium-45 were administered orally via a lubricated balling gun to each of the wethers. Whereas during trial two, four mg DES was administered orally for twenty-one days while consuming either the basal diet plus magnesium or a magnesium-deficient diet.

The major finding of this research was that when animals were fed a magnesium-deficient diet along with a high-potassium level in the diet

there was a greater retention of potassium than in animals fed a basal diet plus magnesium and a high level of potassium.

The progesterone and estradiol stimulated appetite, increased calcium and magnesium intake, increased calcium and magnesium excretion in the urine and feces, increased the retention of potassium, increased plasma potassium, decreased plasma calcium, increased the calcium-45 in urine and increased the calcium-45 in the plasma at peak levels ($p < 0.05$). The interaction of these two hormones with diet showed that the animals that were fed the basal diet plus magnesium and injected with hormones excreted more magnesium-28 for the twenty-four and forty-eight hour test period, and for the complete test period.

The animals fed DES consumed less feed, consumed less calcium and magnesium, excreted less calcium and magnesium in the feces, excreted more magnesium in the urine, and excreted less calcium in the urine and had a greater concentration of magnesium in the plasma. The interaction of DES X diet showed that the animals that were fed DES and maintained on a magnesium-deficient diet had the least amount of plasma potassium of any group.

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CHAPTER I

INTRODUCTION

Even though interest in various aspects of hypomagnesemia and tetany in adult cattle and sheep have been the subject of intensive studies, it has not been possible to answer many of the questions related to the origin, etiology, nature, and occurance of the disease. However, it has become evident that the problem of hypomagnesemia is not a simple one. Apparently, hypomagnesemia is a metabolic disorder of ruminants which can be precipitated by a variety of cause and effect mechanisms. It occurs world-wide in the temperate zones and particulary in animals on spring pastures under intensive management practices (Mayland and Grunes, 1974; Wilcox and Hoff, 1974).

Many theories have been postulated as to the factor or factors involved in the mechanism or mechanisms that act to trigger hypomagnesemia. However there is still much to be learned about hypomagnesemia.

The objectives of this study were to evaluate (a) the effects of diet on magnesium, calcium and potassium metabolism in wethers and (b) the effects of progesterone, estradiol, and diethylstilbestrol (DES) on the metabolism of these minerals.

CHAPTER II

REVIEW OF LITERATURE

I. HISTORY OF HYPOMAGNESEMIA

Dairymen in the Netherlands have known for over one-hundred years that cows may develop tetany soon after being turned out from the stalls to rapidly growing lush green pastures. According to Grunes et al. (1970), Sjollema in 1928, first related the disease to low serum magnesium. Duncan et al. (1935) reported that calves maintained for several weeks on a whole milk diet suffered from tetany associated with low blood serum magnesium, presumably due to a magnesium deficiency. In the United States, the first documented cases of hypomagnesemi tetany occurred in cattle grazing cereal forages (Fontenot, 1972).

Generally only sexually mature ruminants (cows, ewes, and goats) in the late stage of pregnancy or nursing young are affected with grass tetany (Voisin, 1963; Merchon and Custer, 1958). However, the disturbance has been reported in calves and steers (Aikawa, 1971; and Rook and Storry, 1962).

Animals affected with hypomagnesemic tetany exhibit symptoms which include hyperexcitability, muscular twitching, opisthotonus and convulsions which may be very acute in onset or of long duration (Fontenot, 1972; Aikawa, 1971; Grunes et al. 1970; Voisin, 1963; Hughes and Cornelius, 1960; Stewart, 1954; and Sjollema, 1932).

II. ABSORPTION OF MAGNESIUM

There have been conflicting reports on the site of magnesium absorption and the mechanism involved in magnesium absorption in ruminants. Smith (1969) stated in his review on magnesium that many workers, using some very impressive in vivo studies with multiple markers and reentry cannula and sampling procedures, found that up to about one month of age calves absorb magnesium from the large as well as small intestine, giving efficient overall net absorption (70%-90% intake). This ability is lost with increasing age until by about 3-4 months the small intestine is the only important absorption site. Additional work by Field (1961) in sheep and Kemp et al. (1973) with cows showed that the small intestine was the major site of magnesium absorption in older animals. However, Axford et al. (1975), Ben-Ghedalia et al. (1975), and Marongiu (1972) reported that the forestomach of the sheep was the main absorption site of magnesium.

As stated earlier, factors contributing to the mechanism or mechanisms of magnesium absorption are poorly understood. Wilson (1964), in his review on magnesium metabolism, stated that absorption can take place in a number of different ways; simple diffusion, facilitated diffusion or active transport. Other authors (Care and Van't Klooster, 1965; Allcock and MacIntyre, 1962) claim that calcium and magnesium are absorbed competetively by a single mechanism. However, it appears unlikely that magnesium absorption is by the same system that absorbs calcium. Working with sheep and calves, Rook and Storry (1962), Smith (1962 and Phillipson

and Storry (1965) pointed out that calcium and magnesium absorption characteristics are different. For example, magnesium absorption does not respond to vitamin D as does that of calcium (O'Dell et al., 1960). However, supplemental vitamin D increased apparent availability and retention of calcium and magnesium in animals consuming low nitrogen containing forage, but had no effect when given to animals consuming high nitrogen containing forages (Stillings et al., 1964).

III. FACTORS INFLUENCING MAGNESIUM ABSORPTION

Many factors have been postulated to affect magnesium absorption in the various species. These include the chemical form of magnesium, amount of various minerals in the diet, season and temperature, dietary nitrogen and nitrogen related compounds, endocrine hormones, age of the animal and possibly many other factors. However, the literature is quite confusing and conflicting on how many of the above affect magnesium absorption. Some of these are listed below.

Form of Magnesium

Based on plasma magnesium levels in calves, Huffman et al. (1941) found that carbonate, chloride, phosphate and oxide compounds of magnesium were better utilized than sulfate, citrate, and silicate forms. However, Storry and Rook (1963), using the change in urinary excretion to measure magnesium absorption in two cows, found that citrate was better utilized than the oxide. Thomas (1959), working with calves, and Ammerman et al. (1972), working with sheep, reported that there were no differences

in availability among carbonate, sulfate, and acetate forms of magnesium. Meyer and Grund (1963) reported a greater absorption of magnesium from the chloride and oxide forms than from the carbonate by cattle. Magnesium as the oxide was better absorbed and resulted in greater serum magnesium than that in dolomitic limestone when tested with cattle (Gerken and Fontenot, 1967).

Potassium

Within the cell, magnesium is the next most abundant cation to potassium (Wilson, 1964). Therefore one would expect a close association between the two cations. However, potassium is a monovalent ion and magnesium is a divalent ion (Selwood, 1965) and potassium will be absorbed more readily than magnesium as indicated by high plasma concentration of potassium and a low plasma concentration of magnesium in animals consuming a high potassium diet (Frye, 1975; Sanwal, 1974; Newton et al., 1972; Sanwal and Hansard, 1972; House and Campen, 1971; Suttle and Field, 1967, 1969; and Odell et al., 1952). However, Camp et al. (1968), Hemingway et al. (1963), Smyth et al. (1958), and Pearson et al. (1949), reported that potassium had no significant effect on the plasma concentration of magnesium.

Phosphorus

A review by Fontenot (1969) stated that a sizable amount of the phosphorus in plants is in the phytate form. In nonruminants it appears that feeding high levels of phytic acid decreases magnesium absorption. Dietary organic phosphorus was compared to an inorganic form of phosphorus

and no significant effect was found on magnesium excretion, absorption, or retention nor on blood serum magnesium of sheep fed two levels of magnesium. However, Wise et al. (1963) reported a significant reduction in serum magnesium in calves from increasing the phosphorus level in the presence of low calcium in the diet.

Calcium

Seki (1972) reported that when rats were fed a diet high in calcium there was an increased utilization of phophorus and magnesium. Work reported by O'Dell et al. (1960) showed that a high level of calcium fed to guinea pigs and rats impaired the utilization of magnesium.

In the ruminant, as the calcium content of the diet increases the magnesium requirement of the animals increases (Voisin, 1963). Allcroft and Ivins (1964) reported that the addition of calcium lactate to milk accelerates the manifestation of hypomagnesemia in milk fed calves.

Season and Temperature

Allcroft (1954) has reported that a tetany episode often occurs on cold wet mornings after the animal has been changed over from its winter diet to a rapidly growing pasture in the spring. Grunes et al. (1970) indicated that forages in cool weather have a lower concentration of magnesium and a higher concentration of potassium compared to forages grown during warm weather. Therefore, animals consuming these forages could develop hypomagnesemia.

As to how environment affects the magnesium absorption, it has been known that the one characteristic biochemical finding in hibernation

which occurs in cold weather, is an elevation in the serum concentration of magnesium (170% higher during hibernation) (Aikawa, 1971). However, Sanwal (1974) and Sykes et al. (1969) reported a depression in plasma magnesium after sheep were exposed to cold temperature.

Nitrogen and Nitrogen Related Compounds

Voisin (1963) stated that the more protein the forage contains, the greater the amount of ammonia that will be produced in the rumen. High concentrations of ammonia in the rumen increases the pH and further reduces the magnesium and calcium that will be absorbed, resulting in a low magnesium in the blood serum, and interacts to produce hypomagnesemic tetany in an animal (Wilcox and Hoff, 1974). Similar results in sheep were found by Grace and Macrae (1972) and Stillings et al. (1964).

Age

Voisin (1963) pointed out that older cows are more susceptible to grass tetany. He showed that cows six years old or older have the highest incidence of tetany. He also showed that young ewes had a mean content of magnesium in the blood serum of 2.26 mg/100 cc, whereas, the older ewes had a mean content of only 1.62 mg/100 cc; therefore, this drop in the blood serum magnesium level obviously renders older ewes more susceptible to tetany. Voisin attributed the decline to the mechanisms regulating the magnesium content of the blood serum becoming less efficient with older age.

Hormones

It has been reported by many researchers that hormonal effects may be involved in hypomagnesemia or magnesium metabolism (Grunes et al., 1970; Care, 1969; Wilson, 1964; Sellers et al., 1951; and LeBlond and Gross, 1943). As suggested by Grunes et al. (1970), the increased incidence of grass tetany during cold, wet, and windy weather may be associated with changes in thyroid activity. Thyroid, parathyroid, and adrenal hormones may have regulatory effects on absorption of magnesium from the alimentary tract and on plasma magnesium concentration, as suggested by Sanwal (1974) in his review on the effects of temperature and diet on magnesium, calcium and potassium metabolism in sheep and rats.

Wilson (1964) suggested that, since there is a marked difference in the production of endogenous magnesium among wethers, there may be a variety of "animal factors" involved in the amount of endogenous magnesium in the digestive juices which may be a factor in the development of hypomagnesemia. He speculated that it could be possible that factors in spring herbage may exert pharmacological effects increasing the endogenous secretion. He also reported that Ross and Care in 1961 found that aldosterone inhibited the uptake of magnesium by the cells of the small intestine and that aldosterone was increased in response to a high potassium and low sodium intake. Thus, a high potassium and a low sodium intake would consequently decrease the magnesium uptake by the intestine. Other workers have also reported that aldosterone may decrease the availability of magnesium (Dobson et al., 1966; and Scott and Dobson, 1965).

As has been reported, the onset of estrus can precipitate clinical symptoms in cattle in which some degree of hypomagnesemia is already present (Stewart, 1954).

Yuthasastrakosol et al. (1975), working with ewes showed that preceding estrus there is a decline of progesterone from a peak of 4 ng/ml to 0.25 ng/ml and an increase in estrogen from 4.40 pg/ml to 13.3 pg/ml in the blood plasma. Perhaps this increase in estrogen can contribute to the decrease in appetite at estrus that has been observed in sheep by Tarttelin (1968) and in cows by Swan and Jamieson (1956). Low doses of synthetic estrogens are used commercially to stimulate food intake and growth in ruminants (Hale and Ray, 1973; and Hutcheson and Preston, 1968). It has been pointed out that, at high levels, synthetic estrogens can cause a depression in food intake in adult female rats (Bull et al., 1974), in cows (Muir et al., 1970, 1972), and in wether sheep (Forbes, 1973, 1975). In 1973 Forbes used wethers that had been surgically prepared so the estrogen compound could be directly injected intraventricularly in the brain. He found that intraventricular injection of 10 or 20 µg estradiol benzoate significantly increased the weight of food eaten. Therefore, he speculated that the long term increase in food intake with subcutaneous diethylstilbestrol is due to a direct effect on the centers which control feeding. He also found that the partial suppression of feeding with higher levels of estradiol occurred more rapidly with intraventricular administration than followed intravenous injections. Therefore, he stated, "this is further support for a central rather than a peripheral, action of estrogen on food intake."

When considering the effects of the sex hormones on the blood chemistry, Swan and Jamieson (1956) stated:

> Oestrus is commonly accompanied by sudden changes in milk production, especially in highly strung cows, as well as by the increased physical activity and interference with grazing time resulting from bulling. The effect of oestrus on cows past the third month of lactation varied according to the intensity and duration of homosexual activity. Cows showing slight symptoms were unaffected. Those with marked sexual excitement and attraction grazed up to 50% less, lay down up to 60% less, showed a higher body temperature, a lower milk yield, higher fat content in the milk, and higher butterfat yield, and lost up to 10% in body weight on the day or days of oestrus as compared with the days before and after. The most active cow of a group of eight watched cows on which blood chemical studies were made, showed the only significant blood changes observed. Her serum magnesium and calcium content on the days before, during, and after oestrus were: 2.7, 2.0, 2.7, and 10.5, 10.0, 10.4 mg.% respectively. A fall in the serum calcium at the time of oestrus has been common in our animals. No evidence has been found in the literature that oestrogenic hormones cause a fall in serum calcium in lactating cows. It may be assumed in the absence of such evidence that this occurrence is also a result of the physical disturbances of oestrus.

Goldsmith and Johnston (1976) have also reported that young women who are currently using high estrogen OCs have 3% more bone mineral than nonusers, 6-16% less magnesium, calcium and phosphorous in serum and 40% less magnesium and calcium in the urine. However, Bargeloh et al. (1975) found that when melengestrol acetate (MGA) at the rate of 1 mg daily or estradiol-17β at the rate of .05 mg/kg body weight was given to mature cows at prepartum, the magnesium level in the blood was 2.32 mg/100 ml for the MGA treated group, 2.14 mg/100 ml for the estradiol-17β treated group and 1.95 mg/100 ml for the controls. When the same amounts of the hormones were given postpartum, the MGA treated group had a magnesium level of 2.55 mg/100 ml, the estrogen group had a magnesium level of

2.11 mg/100 ml, and the control animals had a 2.11 mg/100 ml of magnesium in the plasma.

IV. FUNCTION OF MAGNESIUM

As pointed out previously, magnesium is the second most plentiful cation inside the cell, therefore, it is an important cofactor in the activity of many enzyme systems, particularly those involving adenosine triphosphate (ATP). That is the system concerned in the utilization of energy for synthesis, and transport in and out of cells (Kiesel et al., 1969; Wilson, 1964). Lehninger (1975) pointed out that magnesium was also necessary for protein systhesis, gluconeogenesis, glycolysis, and practically all systems involved in catabolism and anabolism.

Outside the cell, 1% of the body's magnesium is in the extracellular fluid (Wilson, 1964). Miller et al. (1972), Aikawa (1971) and Wilson (1964) pointed out that the magnesium in the extracellular fluid is involved in bathing all body cells, cellular adhesion, nerve conduction, transmission at myoneural function and muscular contractions. However, the major percentage (50-70%) of the magnesium in the body is in the skeleton (Wilson, 1964). Therefore, it is considered to be one of the structural elements of an individual.

CHAPTER III

EXPERIMENTAL PROCEDURE

I. GENERAL PLAN OF THE INVESTIGATION

A two by two factorial arrangement was selected to test the effects of the female sex hormones (pregesterone, estradiol, and DES) on the metabolism of calcium, magnesium, and potassium in yearling wether lambs consuming a basal diet plus magnesium or a basal diet low in magnesium.

This study was divided into two trials. The first trial was designed to evaluate the effects of progesterone and estradiol on the metabolism of calcium, magnesium, and potassium, and radioactive calcium and magnesium. The second trial was used to evaluate the effects of DES has on the metabolism of these minerals.

II. TRIAL I

Procedure with Lambs During Trial I

From a flock of lambs maintained at Blount farm of the University of Tennessee, 15 wethers which averaged 43.4 kg in weight were selected and placed in dual-unit type metabolism stalls as described by Briggs and Gallup (1949). The animals were given an adjustment period of fourteen days during which seven of the animals were fed a control diet calculated to contain 0.2% magnesium and the remaining eight of the animals were fed a diet calculated to contain 0.055% magnesium. The composition and

analysis of the diets are presented in Table I and II respectively. Animals were given regular tap water twice daily, with a magnesium content of .378 mg/100 ml.

After the blood magnesium level had dropped below 2.0 mg/100 ml for the animals on the magnesium-deficient diet, the animals were divided into four groups of three each based on uniformity and performance in the collection stalls during the preliminary period. During the twelve day test period groups I and II were fed the basal diet plus magnesium and groups III and IV were fed the basal diet only. On day one and every twelve hours for five consecutive days, groups II and IV also received an intramuscular injection of 10 mg progesterone dissolved in Wesson oil. On day six, 48 hours after the last injection of progesterone all the animals were dosed with 61.3 μc of magnesium-28, which was obtained from the Brookhaven National Laboratory and 701 μc of calcium-45 which was obtained from New England Nuclear. Appropriate dilutions of calcium-45 and magnesium-28 were made and placed into a gelatin capsule containing an absorbant tissue. The specific activity of the magnesium-28 was 444 mc per mg of magnesium and calcium-45 was 14 mc per mg of calcium. Capsules containing both isotopes were immediately given via a lubricated balling gun to each of the wethers. Animals in groups II and IV also received 50 μg estradiol dissolved in Wesson oil intramuscularly at the time of dosing and a second injection of 50 μg estradiol six hours later.

TABLE I

SHEEP RATION COMPOSITION

Ingredients	Composition (%)
Corn cobs, gr	60
Corn gluten meal, 60%	12
Dextrose	15
Starch	4.2
Corn oil	2
Urea	1
Mineral mix*	5.6
Vitamin mix	0.2
	100.0

*Mineral mix	Kilograms per 1000 kg of diet
$CaHPO_4 \cdot 2H_2O$	7.1
$CaCO_3$	1.8
NaCl	5.0
KCL	42.2
KI	.00013
$CuSO_4 \cdot H_2O$	.014
$CuSO_4 \cdot 5H_2O$	.00042
$ZnCl_2$	.1043
$MnSO_4$	.0137
$FeSO_4 \cdot 7H_2O$	.2363
TOTAL	56.469
Control mix with added MgO TOTAL	2.42
	58.889

TABLE II

PERCENTAGE ANALYSIS OF SHEEP DIETS

Nutrient	Diet	
	Control	Low magnesium
Magnesium	0.124	0.028
Calcium	0.376	0.371
Potassium	2.487	2.695
Crude protein	13.41	13.41

III. TRIAL II

Procedure with Lambs During Trial II

Between trials I and II the lambs were given a 30 day rest period in pens bedded with shredded corn cobs. The four groups of wethers remained on their respective rations throughout the rest period and during trial II. Before the lambs were returned to their collection stalls, one of the wethers in group I was removed from the experiment due to low feed consumption during trial I and a substitute animal that had previously been on the same diet was used. The wethers were given eleven days for readjustment to the confined condition. Then the lambs in groups II and IV were fed 4 mg DES daily for 21 consecutive days along with their regular diet while groups I and III served as controls. The DES was dissolved in Wesson Oil and thoroughly mixed with the respective diets.

IV. SAMPLING

Blood samples were taken periodically throughout the preliminary period and the rest period. During trial I blood samples were collected at 1, 4, 8, 12, 24, 48, 72 and 96 hours after dosing with magnesium-28 and calcium-45 and during trial II blood was taken every 2 to 3 days. All blood samples were taken by jugular puncture in heparanized tubes. Since magnesium-28 has a half life of only 21 hours, measured amounts of whole blood were used for counting immediately after sampling. The remainder of the blood from trial I and throughout the entire experimental period was centrifuged and the plasma removed and frozen for later stable mineral analysis and radiocalcium counting.

Throughout the period of confinement in metabolism stalls the feed intake, total fecal excretion and total urinary excretion were measured at 24 hour intervals for each lamb. Samples of feed, urine, and feces were collected from each lamb each day during the experimental period and stored in a cooler at four degrees celsius until stable minerals analyses were completed.

V. ANALYSIS

Plasma and urine were diluted to known volumns for stable mineral analyses by the atomic absorption spectrophotometer. Calcium and potassium were determined in addition to magnesium because of their close relationship to hypomagnesemia and grass tetany (Fonenot, 1972). The feces and feed were ashed for 12 hours at 550°C, put into solution with 2 or 3 ml of 6N HCL, transferred to graduated tubes, and made to known volume with deionized water previous to the determination of calcium, magnesium and potassium by routine atomic absorption spectrophotometric methods.

For the magnesium-28 analysis, weighed samples of feces and measured volumes of whole blood and urine were counted immediately after sampling in a Nuclear-Chicago model 181A gamma counter and results were expressed as the percentage of the administered dose by comparing with an appropriately diluted standard.

Since calcium-45 is a weak beta emitter with a half life of 163 days, plasma samples were stored for 120 days, urine samples were stored for 90 days and feces samples were stored for 80 days before calcium-45 counting.

Before these samples could be counted, measured volumes of plasma and urine were adsorbed onto tissue, and ashed at 550°C for 12 hours; while the feces were ashed directly. After ashing all samples, they were put into solution with one or two ml of 6N HCL, transferred to graduated tubes and diluted to known volumns with deionized water. Then a measured volume of the sample was placed in a planchet and dried, and counted in the Nuclear-Chicago model 181A beta counter and the results were expressed as a percentage of the administered dose.

CHAPTER IV

RESULTS

The following data are presented on the effects of progesterone, estradiol and DES on the metabolism of magnesium, calcium and potassium.

I. TRIAL I

Feed Intake

The results of trial I shows that the level of magnesium in the diet had no significant effect ($p < 0.05$) on the total amount of feed consumed by the animals each day or for the total period. The animals on the control diet consumed an average of 630 grams of feed per animal per day compared to 615 grams for the animals on the magnesium deficient diet. However, one of the animals that was fed the basal diet plus magnesium refused to eat eight of the twelve days. The injection of hormones did not affect feed consumption for any individual day. However, for the complete test period, the animals injected with hormones consumed 669 grams per animal per day compared to 576 grams for the animals that were not injected with hormones.

Balance Data

All the treatment groups were in both negative calcium and magnesium balances as indicated by Tables III, IV; and XIV and XV in the appendix. However, the animals on the basal diet plus magnesium were in more ($p < 0.05$) of a negative balance in calcium and in less of a negative

TABLE III

AVERAGE DAILY CALCIUM BALANCE (GRAMS), TRIAL I

Ration and treatment	Calcium consumed	Calcium excreted			Calcium balance
		urine	feces	total	
Control	2.205	0.532	2.653	3.185	-0.980
Control + hormones[1]	2.692	0.729	2.957	3.686	-0.994
Deficient	2.550	0.256	2.857	3.113	-0.563
Deficient + hormones[1]	2.671	0.402	3.057	3.459	-0.788

[1]Animals in this group were injected intramuscularly with 10 mg progesterone every 12 hours for five consecutive days, and with 50 µg of estradiol benzoate 48 and 56 hours after the last injection of progesterone.

TABLE IV

AVERAGE DAILY MAGNESIUM BALANCE (GRAMS), TRIAL I

Ration and treatment	Magnesium consumed	Magnesium excreted			Magnesium balance
		urine	feces	total	
Control	0.715	0.179	0.556	0.735	-0.020
Control + hormones[1]	0.876	0.229	0.720	0.949	-0.073
Deficient	0.171	0.024	0.266	0.290	-0.119
Deficient + hormones[1]	0.179	0.018	0.288	0.306	-0.127

[1]Animals in this group were injected intramuscularly with 10 mg progesterone every 12 hours for five consecutive days, and with 50 µg of estradiol benzoate 48 and 56 hours after the last injection of progesterone.

balance in magnesium for the entire treatment period. In looking at each individual day, there were no significant differences among the treatments. Because the animals injected with hormones consumed more of the total diet, the intake of both calcium and magnesium was higher ($p < 0.05$) for the animals injected with hormones. Thus, the magnesium and calcium content of the feces was greater ($p < 0.05$) for these animals injected with hormones. Even though there was no significant difference in calcium consumption, the animals on the basal diet plus magnesium excreted more ($p < 0.05$) calcium in the urine. The animals injected with hormones, also excreted more ($p < 0.05$) calcium and magnesium in the urine.

The potassium balance data is summarized for animals on each treatment in Table V and each individual animal in Table XVI in the Appendix. Both hormones and diet had a significant effect ($p < 0.05$) on the intake of potassium. The animals that were injected with hormones consumed an average of 16.43 grams of potassium compared to 15.46 grams for the wethers that did not receive any hormones. The animals fed the basal diet plus magnesium consumed less potassium even though they consumed more of the total diet because the control diet was slightly lower in potassium than the magnesium deficient diet (Table II, page 15). Therefore, it was possible for the animals on the control diet to consume slightly more of the total diet but less potassium.

The wethers fed the basal diet plus magnesium excreted more potassium in the feces and urine with the major excretory pathway in the urine. Even though the animals injected with progesterone and estradiol consumed more of the potassium, there was less ($p < 0.05$) excreted in the

TABLE V

AVERAGE DAILY POTASSIUM BALANCE (GRAMS), TRIAL I

Ration and treatment	Potassium consumed	Potassium excreted			Potassium balance
		urine	feces	total	
Control	13.479	10.826	1.584	12.410	1.069
Control + hormones[1]	16.670	12.653	1.889	14.542	2.128
Deficient	17.428	8.849	1.759	10.608	6.820
Deficient + hormones[1]	18.256	10.114	2.291	12.405	5.851

[1]Animals in this group were injected intramuscularly with 10 mg progesterone every 12 hours for five consecutive days, and with 50 µg of estradiol benzoate 48 and 56 hours afater the last injection of progesterone.

feces of these animals. This indicates that the hormones aided in the absorption of potassium from the gut. Hormone administration did not affect ($p < 0.05$) the excretion of potassium in the urine. Therefore, the increased absorption indicated by the decreased fecal excretion shows that more of the potassium was retained by the animals injected with hormones. The animals consuming the basal diet plus magnesium also retained less ($p < 0.05$) of the potassium.

Plasma Data

Blood plasma data are summarized in Table VI. After the animals had been on their respective diets for thirty days, there was an increase in the potassium and a decrease in the magnesium level as compared to pretrial levels for all four groups. In the lambs fed the basal diet plus magnesium, the plasma magnesium value decreased from an initial average of 2.41 mg/100 ml to an average of 2.08 mg/100 ml. The average potassium value in the plasma was 15.74 mg/100 ml initially and increased to average of 18.22 mg/100 ml for all groups. The magnesium level in the plasma dropped to an average of 1.09 mg/100 ml for the animals fed the basal diet. In considering the calcium, the animals on the basal diet plus magnesium had an average of 9.98 mg/100 ml compared to an average of 10-11 mg/100 ml for the animals fed the magnesium-deficient diet ($p < 0.05$).

The hormones had no significant effect ($p < 0.05$) on the calcium and magnesium content of the plasma. However, there was an increase ($p < 0.05$) in the potassium content for the animals injected with progesterone and estradiol.

TABLE VI

AVERAGE MINERAL CONTENT OF BLOOD PLASMA, TRIAL I

Ration and treatment	Calcium	Magnesium	Potassium
	----------	mg/100ml ----------	
Pretrial (all animals)	10.39	2.35	15.74
Control	10.08	1.98	17.88
Control + hormones[1]	9.88	2.18	19.08
Deficient	10.27	1.24	17.97
Deficient + hormones[1]	9.96	0.95	19.00

[1]Animals in this group were injected intramuscularly with 10 mg progesterone every 12 hours for five consecutive days, and with 50 µg of estradiol benzoate 48 and 56 hours after the last injection of progesterone.

Radioisotope Data

The effects of diet and hormones on calcium and magnesium metabolism as measured by whole blood magnesium-28, plasma calcium-45, excreted magnesium-28 and excreted calcium-45 are presented in Tables XI, XII and XIII in the Appendix, and graphically in Figures 1, 2, 3, and 4. Neither diet nor hormones had a significant effect on plasma calcium-45, total excreted calcium-45 and feces calcium-45 for the 96 hour period. However, at peak level (4 hours) the plasma calcium-45 was greatest for animals that were injected with progesterone and estradiol. The animals fed the basal diet plus magnesium and animals injected with hormones also excreted more ($p < 0.05$) calcium-45 in the urine.

The animals fed the basal diet plus magnesium had a greater ($p < 0.05$) uptake of magnesium-28 in the whole blood and excreted more in the urine as compared to the animals fed the basal diet only. However, there was no significant effect on the total amount of magnesium-28 excreted in the feces or total amount of magnesium-28 excreted during the 96 hours period. Whereas, the diet X hormones showed that the animals that were fed the basal diet plus magnesium and injected with hormones excreted more ($p < 0.05$) magnesium-28 for the twenty-four and forty-eight hour test period and for the complete test period.

II. TRIAL II

Feed Intake

As indicated earlier, groups II and IV were fed 4 mg DES dissolved in Wesson oil and thoroughly mixed with the respective diets for a total

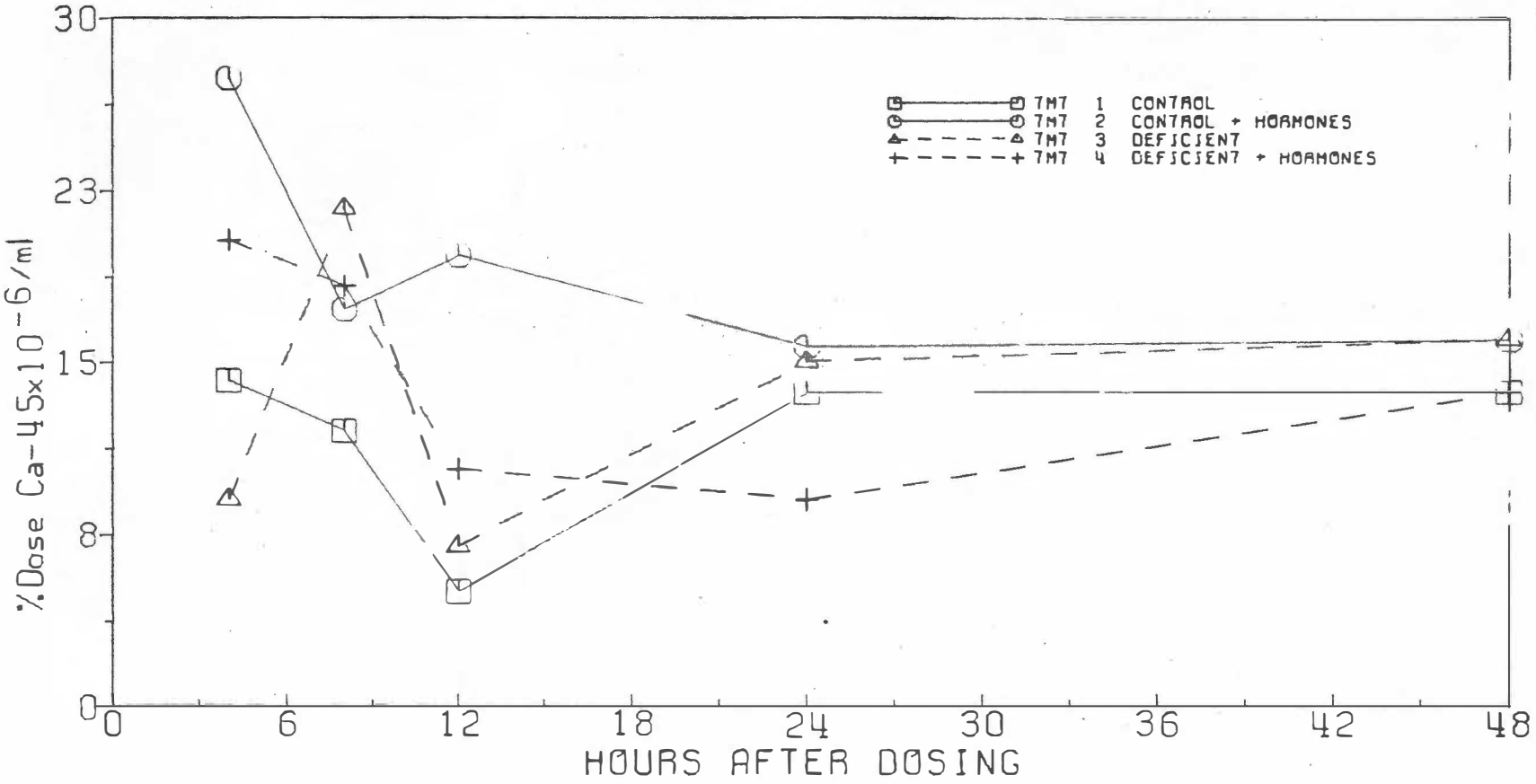

Figure 1. Calcium-45 in the plasma of control, control plus hormones, magnesium-deficient and magnesium-deficient plus hormones to wether lambs as a function of time following oral administration.

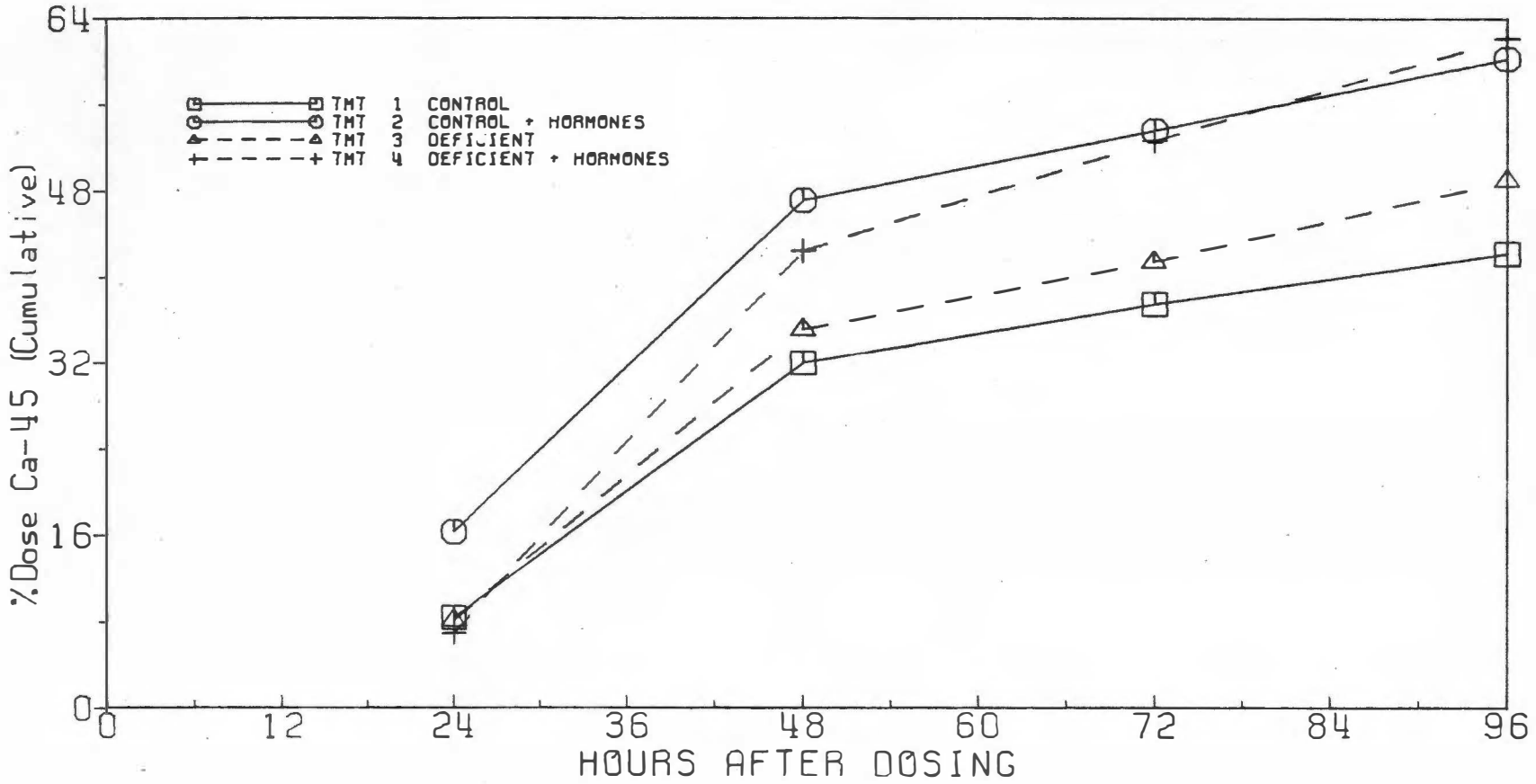

Figure 2. Accumulative fecal and urinary excretion of orally administered calcium-45 by wether lambs fed a control diet, control diet plus hormones, magnesium-deficient diet and magnesium-deficient diet plus hormones.

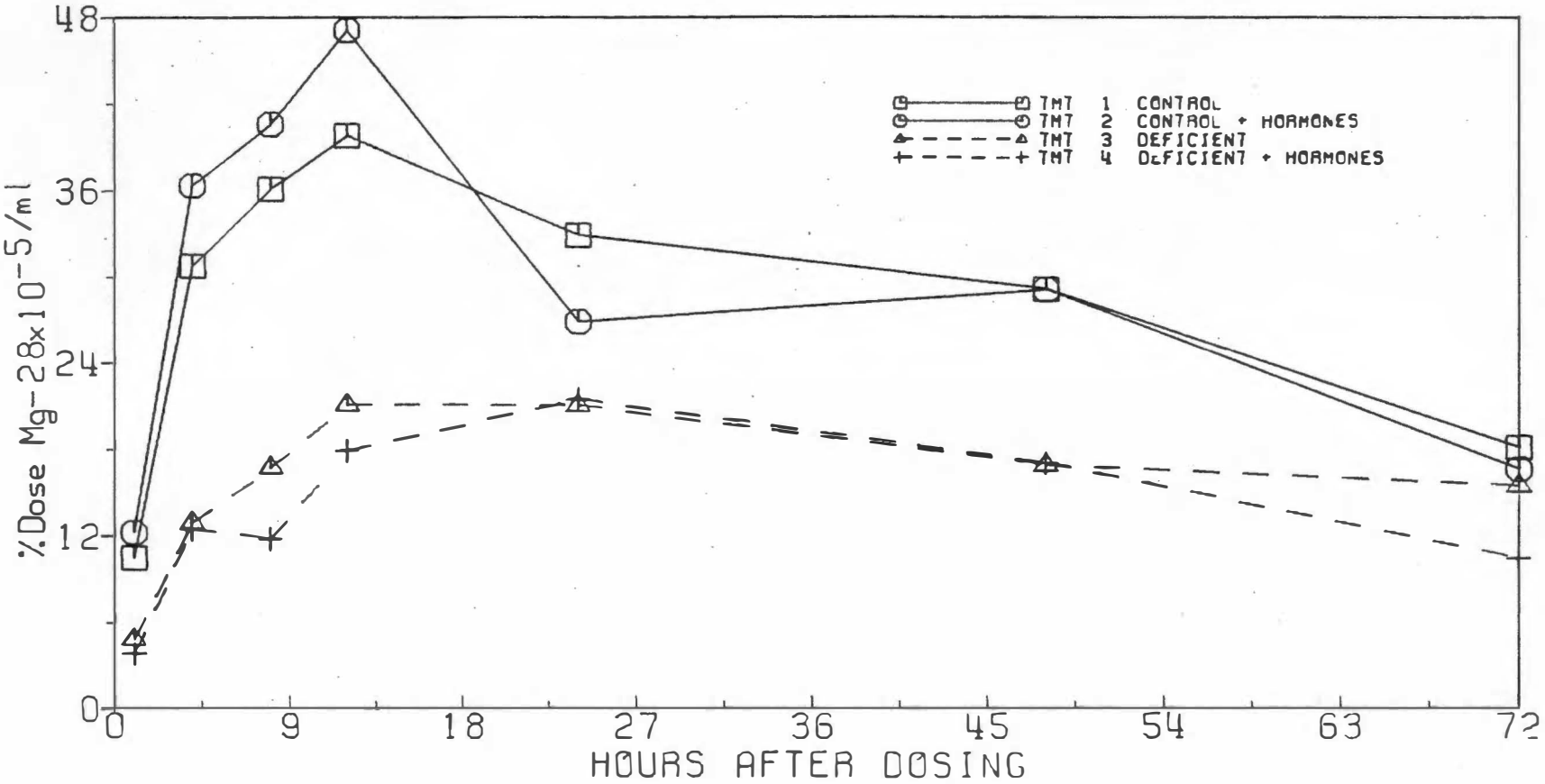

Figure 3. Magnesium-28 in the whole blood of control, control plus hormones, magnesium-deficient and magnesium-deficient plus hormones to wether lambs as a function of time following oral administration.

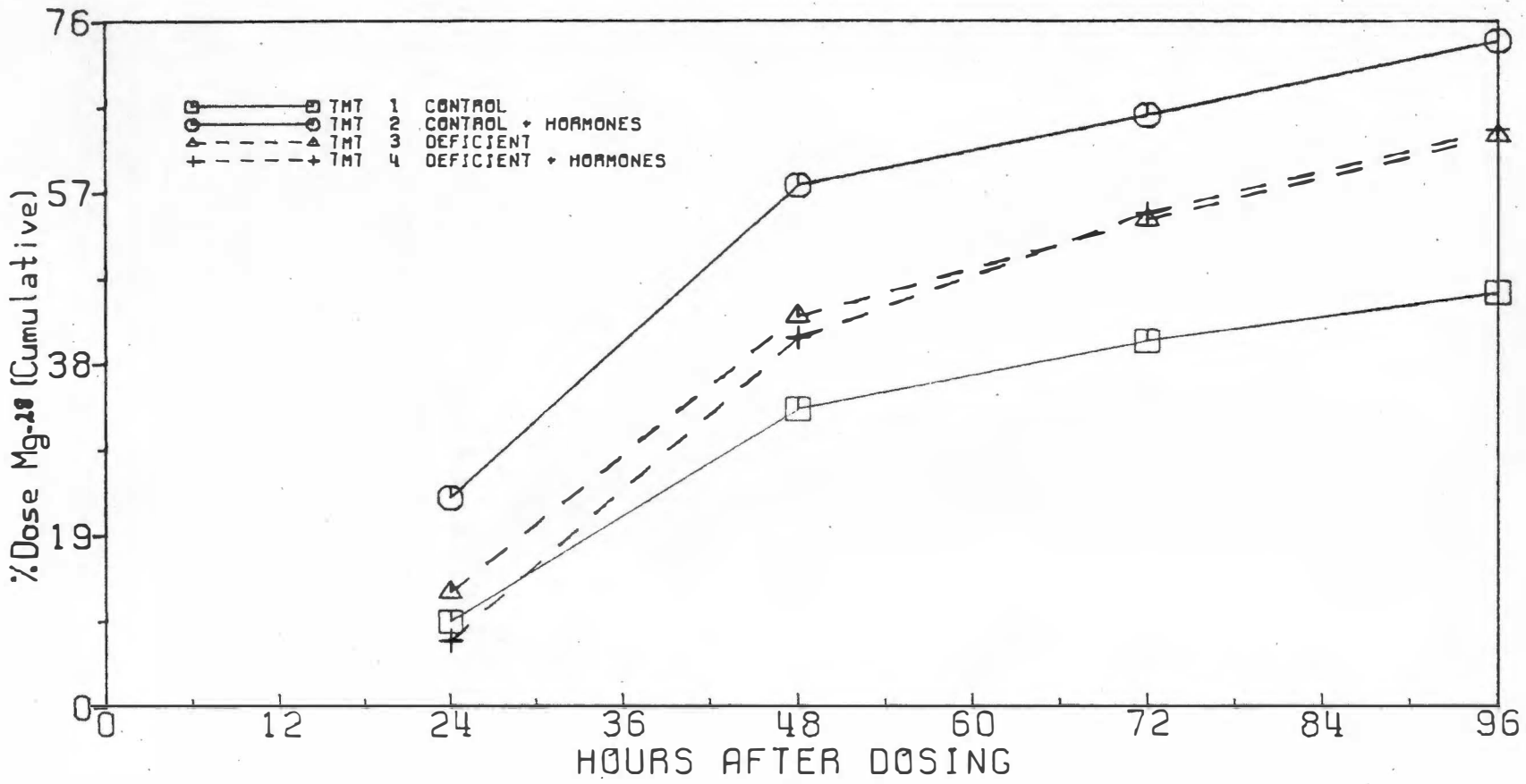

Figure 4. Accumulative fecal and urinary excretion of orally administered magnesium-28 by wether lambs fed a control diet, control diet plus hormones, magnesium-deficient diet and magnesium-deficient diet plus hormones.

of twenty-one days. The wethers that were fed the DES consumed an average of 321 grams per day compared to 392 grams ($p < 0.05$) for the animals that did not receive any hormones. The lambs fed the basal diet plus magnesium consumed more (454 grams per day)($p < 0.05$) total feed compared to the animals on the magnesium deficient diet (258 grams). In considering the hormones X diet interaction, the animals fed the basal diet without magnesium and injected with hormones consumed the least total diet of any treatment group.

Balance Data

Summaries of the balance data for animals on each treatment are presented in Tables VII, VIII, IX and for each individual wether in Tables XVII, XVIII, XIX, in the Appendix. The animals on the basal diet plus magnesium consumed more ($p < 0.05$) potassium in the feed and excreted less in both the urine and feces. The animals fed the magnesium-deficient diet followed the same trend as in trial I and retained more ($p < 0.05$) potassium.

Again, all the animals were in a negative magnesium and calcium balance. The animals that were fed the basal diet plus magnesium were in more of a negative magnesium balance and less of a negative calcium balance compared to animals fed the basal diet only. The feces was the major excretory pathway for magnesium and calcium with the animals injected with hormones excreting less calcium and magnesium in feces. Furthermore, the animals that received the hormones excreted more magnesium in the urine but less calcium in the urine.

TABLE VII

AVERAGE DAILY CALCIUM BALANCE (GRAMS), TRIAL II

Ration and treatment	Calcium consumed	Calcium excreted			Calcium balance
		urine	feces	total	
Control	2.233	0.530	2.209	2.739	-0.506
Control + hormones[1]	2.045	0.304	1.807	2.111	-0.066
Deficient	1.530	0.111	1.791	1.902	-0.372
Deficient + hormones[1]	1.011	0.182	1.130	1.312	-0.301

[1]Stilbestrol fed at the rate of 4 mg per day for 21 days.

TABLE VIII

AVERAGE DAILY MAGNESIUM BALANCE (GRAMS), TRIAL II

Ration and treatment	Magnesium consumed	Magnesium excreted			Magnesium balance
		urine	feces	total	
Control	0.707	0.248	0.659	0.907	-0.200
Control + hormones[1]	0.592	0.191	0.592	0.783	-0.191
Deficient	0.136	0.014	0.219	0.233	-0.097
Deficient + hormones[1]	0.090	0.004	0.168	0.172	-0.082

[1]Stilbestrol fed at the rate of 4 mg per day for 21 days.

TABLE IX

AVERAGE DAILY POTASSIUM BALANCE (GRAMS), TRIAL II

Ration and treatment	Potassium consumed	Potassium excreted			Potassium balance
		urine	feces	total	
Control	15.317	12.526	1.818	14.344	0.973
Control + hormones[1]	14.066	10.517	1.489	12.006	2.060
Deficient	12.903	6.883	1.037	7.920	4.983
Deficient + hormones[1]	8.322	5.925	0.961	6.886	1.436

[1]Stilbestrol fed at the rate of 4 mg per day for 21 days.

Plasma Data

The magnesium, calcium and potassium plasma level remained the same for the animals fed the basal diet plus magnesium. However, there was a decrease in all these minerals in the plasma for the animals fed the magnesium deficient diet. The average magnesium, calcium and potassium levels in the plasma were 1.09, 10.14 and 18.49 mg/100 ml respectively during trial I compared to an average of 0.59, 7.69 and 17.11 mg/100 ml during trial II in the magnesium deficient groups.

During the 21 day test period, the magnesium in the plasma (Table X) was greater ($p < 0.05$) for the animals that were fed the DES than that of the wethers not fed DES. Even though it was not statistically significant, there was more total calcium and potassium in the plasma of the animals that were not fed DES. The effect of diet showed that the animals that were on the basal diet plus magnesium had the greatest amount of magnesium, calcium and potassium in the plasma. During trial II both the plasma magnesium and calcium decreased in all groups compared to trial I. However, the animals that were fed the basal diet plus magnesium continued to have more ($p < 0.05$) of these two minerals in the plasma. The interaction of diet and hormones showed that the animals that were fed the DES had the least ($p < 0.05$) amount of plasma potassium of any group.

TABLE X

AVERAGE MINERAL CONTENT OF BLOOD PLASMA, TRIAL II

Ration and treatment	Calcium	Magnesium	Potassium
	----------	mg/100ml	----------
Control	9.79	1.83	17.32
Control + hormones[1]	9.96	2.07	17.66
Deficient	8.79	0.52	17.74
Deficient + hormones[1]	8.59	0.64	16.48

[1]Stilbestrol fed at the rate of 4 mg per day for 21 days.

CHAPTER V

DISCUSSION

During trial I progesterone and estradiol were used at levels intended to simulate the hormonal effect of an animal coming into estrus. The diet contained a high potassium content and in one treatment of each trial a low magnesium content to stimulate the diet the animal would be consuming during the spring of the year, since many of the spring grasses are high in potassium and low in magnesium (Grunes et al., 1970). As indicated by Forbes (1973), the female sex hormones can cause wethers to consume more of the total feed. This was observed during trial I of our experiment. An increase in the potassium and a decrease in the magnesium in the plasma were also observed. These findings were in agreement with other authors (Frye, 1973; Hemingway et al., 1963; Kunkel et al., 1953) who contributed their decline in plasma magnesium to the high potassium level in the diet. However, during trial II the animals fed the DES consumed less of the total feed. This work does not agree with the finding of Bell et al. (1957), Hutchenson and Preston (1968) and Hale and Ray (1973). However, during the trial reported herein, all the animals were on the high potassium diet for 60 days and one of the animals in group four consumed only a small amount of feed each day. This animal coughed and was anemic throughout trial II.

As indicated earlier all the animals on the magnesium deficient diet retained more of the potassium. However, Sanwal (1974) reported, that when wethers were fed a diet which contained 0.40 percent potassium and

a control or low magnesium diet, the animals on the control diet retained more of the potassium. Perhaps the increased retention of potassium in the wethers fed the magnesium deficient diet found in this work is expected, since there is only a small amount of magnesium to compete for absorption from the alimentary track. Even though there was an increased potassium retention for the animals fed the magnesium-deficient diet, the plasma potassium was essentially the same in both groups indicating that the potassium did not stay in the blood stream, but went on into the tissue fluid and different cells of the body.

As expected, the animals that were fed the magnesium deficient diet developed hypomagnesemia. However, there were no clinical signs observed even though during trial II the plasma magnesium dropped to 0.17 mg/100 ml for one of the wethers. Perhaps the reasons no clinical signs were observed were because all the wethers were consuming water that contained 0.378 mg/100 ml of magnesium, this experiment was only a simulated condition and the animals had not gone through gestation and parturation and obviously were not lactating.

Voisin (1963) reported that animals affected with hypomagnesemic tetany have a low calcium and magnesium, and a high potassium level in the plasma. Perhaps the low magnesium high potassium found in the diet and in the plasma for the animals in groups three and four of our experiment caused the plasma calcium to decrease during trials I and II.

In trial I hormones had no significant effect on either plasma calcium or magnesium. However, the animals injected with hormones consumed more potassium in the diet; therefore, this could possibly

cause an increase in the plasma potassium observed in groups two and four. The interaction of diet X hormones caused the animals fed the deficient diet and injected with hormones to have the lowest plasma magnesium level. However, during trial II the animals fed the DES had the greatest concentration of magnesium in the plasma. The interaction of diet X hormones caused the animals fed the magnesium-deficient diet along with DES to be the lowest in plasma magnesium. Bargeloh et al. (1975) reported that the animals injected with progesterone had a high magnesium content of the plasma, which is in contrast to the work done by Swan and Jamieson (1956) who reported that the magnesium content of the blood would drop at estrus.

The uptake and disappearance of the orally administered magnesium-28 and calcium-45 in the blood follow characteristic curves. The magnesium-28 disappearance followed the same pattern as that reported by McAleese et al. (1961) and the calcium-45 followed the pattern reported by Shroder and Hansard (1958). The animals that were fed the basal diet plus magnesium retained more magnesium-28 in the whole blood which was in agreement with the work done by McAleese et al. (1961). This indicates that when an animal is on a magnesium-deficient diet, the magnesium that is consumed will be taken up by the tissue fluid and will not stay in the blood. The hormones did not affect the whole blood magnesium-28.

For the plasma calcium-45, the animals that were injected with hormones had the greatest concentration of calcium-45 in the plasma at peak levels (4 hours). However, for the total period and for any other test period neither the diet nor the hormones had no effect on

the plasma calcium-45. Shroder and Hansard (1958) reported, "that dietary stilbestrol was without effect in altering the rate of movement of calcium or phosphorous between the gastrointestinal tract, blood, bone, and tissues."

McAleese et al. (1961) reported that when lambs were fed a magnesium-deficient diet only 25 to 30% of the magnesium-28 ingested was excreted in the urine and feces and when an animal was supplemented with magnesium 50% was excreted. They also reported the peak level of radiomagnesium in the whole blood was 2.5×10^{-4} per ml for the animals consuming the magnesium-deficient diet and 5.5×10^{-4} per ml for the animals consuming the control diet. However, the data reported here shows that around 64% was excreted in the 96 hours collection period for the animals consuming the basal diet and 51% for the animals fed the basal diet plus magnesium. Furthermore, better than 30% was excreted within 48 hours. The whole blood magnesium-28 values at peak levels were around 4.4×10^{-4} for the animals on the basal diet plus magnesium and 2.1×10^{-4} for the wethers fed the magnesium-deficient diet. Since the major excretory pathway of magnesium-28 was by the feces and the animals in this trial did not have as high an uptake of radiomagnesium and excreted more magnesium-28 in the feces and urine than reported by McAleese et al. (1961), apparently the high potassium in the diet impaired the absorption of magnesium from the gastrointestinal tract. In group one where the animals were fed the basal diet, one of the animals did not eat for eight of the twelve day test period, and this brought the average excreted down from 74% for group three to 51% for both groups one and three.

For the total period, the animals injected with hormones and the animals fed the basal diet plus magnesium excreted more of the radiomagnesium in the urine. Since the animals that were consuming the basal diet plus magnesium and the animals injected with hormones consumed more magnesium, the radiomagnesium that was absorbed was apparently not needed by the tissues; therefore, it was excreted in the urine.

The excreted calcium-45 followed essentially the same pattern as that reported by Shroder and Hansard (1958). Even though it was not significant, the total amount of radiocalcium excreted by the animals that were injected with progesterone and estradiol was more than the animals that did not receive any hormones. Again, as with magnesium-28 there was a significant increase in the urinary excretion of calcium-45 for the animals injected with hormones. Perhaps, this follows the same line of reasoning as explained earlier for the urinary magnesium-28.

Since all the animals were in both a calcium and magnesium negative balance, any comments related to these stable elements would be speculatory. Therefore, this area will not be discussed in relation to this work. However, Bell et al. (1957) using balance data reported that stilbestrol feeding significantly increased calcium and phosphorus retention in wethers fed a fattening-type ration. Shroder and Hansard (1958) using calcium-45 and phosphorus-32 in wether lambs fed stilbestrol reported that the primary effect of stilbestrol administration was that of growth stimulation. They assumed this by a reduction in the fecal endogenous calcium with little apparent influence upon calcium absorption from the gastrointestinal tract. As to the effect of stilbestrol on

radiophosphorus, they reported that stilbesterol increased the phosphorus absorption, but that fecal endogenous phosphorus was only slightly decreased.

Even though the urinary magnesium and calcium was increased using the radioisotopes, the present study does not suggest that progesterone, estradiol and diethylstilbesterol are involved in hypomagnesemic tetany. Of course, the magnesium deficient diet caused the animals to get into a hypomagnesemic state as was indicated by the low magnesium level in the blood of the animals fed the magnesium deficient diet. Frye (1975) reported that a high-dietary potassium increased the fecal and urine magnesium excretion and decreased apparent magnesium absorption. Therefore, it is possible that potassium plays a greater role in grass tetany than does the female sex hormones. However, additional work will be necessary in order that one might understand the mechanism or mechanisms that are involved in mineral metabolism that causes tetany in cattle, sheep, and nonruminants.

CHAPTER VI

SUMMARY

The major objectives of this study were to evaluate (a) the effects of diet on magnesium, calcium and potassium metabolism in yearling wethers and (b) the effects of progesterone, estradiol and DES have on the metabolism of these minerals.

A two by two factorial design was selected whereby twelve yearling crossbred wether lambs were divided into four groups of three each. One group was fed a basal diet plus magnesium along with hormones, one group was fed a basal diet plus magnesium without hormones, one group was fed a magnesium deficient diet along with hormones, and one group was fed a magnesium deficient diet without hormones.

During trial one, two of the groups were injected with progesterone every 12 hours for 4 consecutive days. Then, 48 hours after the last injection of progesterone, 100 µg of estradiol was injected in two separate subcutaneous injections (50 µg per injection) 6 hours apart. This was intended to simulate the hormonal milieu of a ewe coming into estrus. At the time of the initial injection of estradiol, 61.3 µc of magnesium-28 and 701 µc of calcium-45 were administered orally via a lubricated balling gun to each of the wethers. Whereas during trial two, four mg of DES was administered orally for twenty-one days while consuming either the basal diet plus magnesium or a magnesium-deficient diet.

The major finding of this research was that when animals were fed a magnesium-deficient diet along with a high-potassium level in the diet there was a greater retention of potassium than in animals fed a basal diet plus magnesium along with a high potassium diet.

The progesterone and estradiol stimulated appetite, increased calcium and magnesium intake, increased calcium and magnesium excretion in the urine and feces, increased the retention of potassium, increased plasma potassium, decreased plasma calcium, increased the calcium-45 in urine and increased the calcium-45 in the plasma at peak levels ($\underline{p} < 0.05$). The interaction of these two hormones with diet showed that the animals that were fed the basal diet plus magnesium and injected with hormones excreted more magnesium-28 for the twenty-four and forty-eight hour test period, and for the complete test period.

The animals fed DES consumed less feed, consumed less calcium and magnesium, excreted less calcium and magnesium in the feces, excreted more magnesium in the urine, excreted less calcium in the urine, and had a greater concentration of magnesium in the plasma. The interaction of DES X diet showed that animals fed DES and maintained on a magnesium-deficient diet had the least amount of plasma potassium of any group.

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APPENDIX

TABLE XI

PLASMA CALCIUM-45 AND WHOLE BLOOD MAGNESIUM-28,
TRIAL I

Ration and treatment	Hours						
	1	4	8	12	24	48	72
Control		1.42[2]	1.20	0.50	1.37	1.37	
	10.44	30.76	36.10	39.77	32.86	29.13	18.10
Control + hormones[1]		2.73[2]	1.73	1.97	1.57	1.60	
	12.20[3]	36.32	40.56	47.12	26.86	29.10	16.58
Deficient		0.90[2]	2.17	0.70	1.50	1.60	
	4.76[3]	12.87	16.67	21.09	21.00	16.87	15.42
Deficient + hormones[1]		2.03[2]	1.83	1.03	0.90	1.36	
	3.77[3]	12.43	11.77	17.86	21.43	17.00	10.41

[1]Animals in this group were injected intramuscularly with 10 mg pregesterone every 12 hours for five consecutive days, and with 50 µg of estradiol benzoate 48 and 56 hours after the last injection of progesterone.

[2]Plasma calcium-45, percent of dose 10^{-6}/ml.

[3]Whole blood magnesium-28, percent of dose 10^{-5}/ml.

TABLE XII

FECAL AND URINARY EXCRETION OF CALCIUM-45

Ration and treatment	Collection period (hours) 24	48	72	96	Total
	% of Dose				
Control	7.74[1]	22.68	4.88	4.36	39.66
	0.65[2]	0.94	0.60	0.32	2.51
Control + hormones[3]	14.11[1]	29.20	5.48	6.19	54.98
	2.20[2]	1.60	0.96	0.50	5.26
Deficient	7.58[1]	26.43	6.05	7.30	47.36
	0.53[2]	0.57	0.29	0.19	1.58
Deficient + hormones[3]	6.31[1]	34.78	9.64	9.11	59.84
	0.66[2]	0.67	0.54	0.40	2.27

[1]Feces

[2]Urine

[3]Animals in this group were injected intramuscularly with 10 mg progesterone every 12 hours for five consecutive days, and with 50 µg of estradiol benzoate 48 and 56 hours after the last injection of progesterone.

TABLE XIII

FECAL AND URINARY EXCRETION OF MAGNESIUM-28

Ration and treatment	Collection period (hours) 24	48	72	96	Total
	---------- % of Dose ----------				
Control	6.69[1]	20.89	5.71	4.28	37.57
	2.70[2]	2.64	1.81	1.12	8.27
Control + hormones[3]	17.23[1]	31.32	5.48	6.82	60.85
	5.86[2]	3.31	2.24	1.44	12.85
Deficient	12.50[1]	30.40	10.42	9.15	62.47
	0.14[2]	0.14	0.29	0.14	0.71
Deficient + hormones[3]	7.14[1]	33.33	13.50	8.96	62.93
	0.15[2]	0.19	0.31	0.28	0.93

[1]Feces

[2]Urine

[3]Animals in this group were injected intramuscularly with 10 mg progesterone every 12 hours for five consecutive days, and with 50 µg of estradiol benzoate 48 and 56 hours after the last injection of progesterone.

TABLE XIV

AVERAGE DAILY CALCIUM BALANCE OF INDIVIDUAL WETHERS (GRAMS), TRIAL I

Ration and treatment	Animal number	Calcium consumed	Calcium excreted			Calcium balance
			urine	feces	total	
Control	519	1.634	0.399	1.874	2.273	-0.639
	502	2.692	0.634	2.928	3.562	-0.870
	518	2.759	0.562	3.158	3.720	-0.961
Control + hormones[1]	223	2.788	0.794	2.616	3.410	-0.622
	526	2.503	0.892	3.039	3.931	-1.428
	517	2.788	0.499	3.217	3.716	-0.928
Deficient	533	2.020	0.171	2.118	2.289	-0.269
	532	2.784	0.235	3.422	3.657	-0.873
	510	2.845	0.363	3.030	3.393	-0.548
Deficient + hormones[1]	513	2.696	0.462	3.394	3.856	-1.160
	523	2.886	0.479	2.943	3.422	-0.536
	110	2.433	0.267	2.832	3.099	-0.666

[1]Animals in this group were injected intramuscularly with 10 mg progesterone every 12 hours for five consecutive days, and with 50 µg of estradiol benzoate 48 and 56 hours after the last injection of progesterone.

TABLE XV

AVERAGE DAILY MAGNESIUM BALANCE OF INDIVIDUAL WETHERS (GRAMS), TRIAL I

Ration and treatment	Animal number	Magnesium consumed	Magnesium excreted			Magnesium balance
			urine	feces	total	
Control	519	0.374	0.119	0.334	0.453	-0.079
	502	0.875	0.181	0.651	0.832	+0.043
	518	0.897	0.238	0.684	0.922	-0.025
Control + hormones[1]	223	0.907	0.161	0.872	1.033	-0.126
	526	0.814	0.249	0.611	0.860	-0.046
	517	0.906	0.279	0.677	0.956	-0.050
Deficient	533	0.136	0.014	0.204	0.218	-0.082
	532	0.187	0.017	0.299	0.316	-0.129
	510	0.192	0.041	0.294	0.335	-0.143
Deficient + hormones[1]	513	0.181	0.021	0.288	0.309	-0.128
	523	0.194	0.017	0.299	0.316	-0.122
	110	0.164	0.017	0.275	0.292	-0.128

[1]Animals in this group were injected intramuscularly with 10 mg progesterone every 12 hours for five consecutive days, and with 50 µg of estradiol benzoate 48 and 56 hours after the last injection of progesterone.

TABLE XVI

AVERAGE DAILY POTASSIUM BALANCE OF INDIVIDUAL WETHERS (GRAMS), TRIAL I

Ration and treatment	Animal number	Potassium consumed	Potassium excreted			Potassium balance
			urine	feces	total	
Control	519	6.707	5.434	0.864	6.298	0.409
	502	16.663	14.807	1.570	16.377	0.286
	518	17.067	12.236	2.319	14.555	2.512
Control + hormones[1]	223	17.259	13.077	2.292	15.369	1.890
	526	15.508	11.876	1.706	13.582	1.926
	517	17.259	13.005	1.669	14.674	2.585
Deficient	533	19.046	10.440	1.742	12.182	6.864
	532	13.780	6.046	0.874	6.920	6.860
	510	19.456	10.060	2.654	12.714	6.742
Deficient + hormones[1]	513	18.435	9.794	2.198	11.992	6.443
	523	19.735	9.651	2.607	12.258	7.477
	110	16.597	10.897	2.069	12.966	3.631

[1]Animals in this group were injected intramuscularly with 10 mg progesterone every 12 hours for five consecutive days, and with 50 µg of estradiol benzoate 48 and 56 hours after the last injection of progesterone.

TABLE XVII

AVERAGE DAILY CALCIUM BALANCE OF INDIVIDUAL WETHERS (GRAMS), TRIAL II

Ration and treatment	Animal number	Calcium consumed	Calcium excreted			Calcium balance
			urine	feces	total	
Control	192	2.351	0.424	2.595	3.019	-0.668
	502	1.990	0.395	1.987	2.382	-0.392
	518	2.357	0.771	2.046	2.817	-0.460
Control + hormones[1]	223	1.835	0.247	1.424	1.671	+0.164
	526	1.943	1.796	0.435	2.231	-0.288
	517	2.357	0.230	2.202	2.432	-0.075
Deficient	533	2.204	0.165	2.389	2.554	-0.350
	532	1.720	0.152	2.270	2.422	-0.702
	510	0.672	0.018	0.737	0.755	-0.083
Deficient + hormones[1]	513	0.716	0.141	0.946	1.087	-0.371
	523	1.679	0.372	1.578	1.950	-0.271
	110	0.637	0.033	0.864	0.897	-0.260

[1]Stilbestrol fed at the rate of 4 mg per day for 21 days.

TABLE XVIII

AVERAGE DAILY MAGNESIUM BALANCE OF INDIVIDUAL WETHERS (GRAMS), TRIAL II

Ration and treatment	Animal number	Magnesium consumed	Magnesium excreted			Magnesium balance
			urine	feces	total	
Control	192	0.748	0.272	0.708	0.980	-0.232
	502	0.623	0.193	0.595	0.788	-0.165
	518	0.749	0.279	0.673	0.952	-0.203
Control + hormones[1]	223	0.566	0.247	0.506	0.753	-0.187
	526	0.614	0.169	0.582	0.751	-0.137
	517	0.749	0.242	0.690	0.932	-0.183
Deficient	533	0.195	0.006	0.290	0.296	-0.101
	532	0.152	0.033	0.246	0.279	-0.127
	510	0.059	0.004	0.122	0.126	-0.067
Deficient + hormones[1]	513	0.063	0.002	0.133	0.135	-0.072
	523	0.148	0.006	0.253	0.259	-0.111
	110	0.056	0.003	0.118	0.121	-0.065

[1]Stilbestrol fed at the rate of 4 mg per day for 21 days.

TABLE XIX

AVERAGE DAILY POTASSIUM BALANCE OF INDIVIDUAL WETHERS (GRAMS), TRIAL II

Ration and treatment	Animal number	Potassium consumed	Potassium excreted			Potassium balance
			urine	feces	total	
Control	192	16.089	14.133	1.664	15.797	0.292
	502	13.729	10.398	1.377	11.775	1.954
	518	16.134	13.045	2.412	15.457	0.677
Control + hormones[1]	223	12.722	8.231	1.648	9.879	2.843
	526	13.342	9.475	1.397	10.872	2.470
	517	16.134	13.843	1.420	15.263	0.871
Deficient	533	18.168	8.542	1.395	9.937	8.231
	532	14.204	8.366	1.095	9.461	4.743
	510	5.515	3.811	0.639	4.450	1.065
Deficient + hormones[1]	513	5.920	4.503	0.610	5.113	0.807
	523	13.813	9.596	1.688	11.284	2.529
	110	5.243	3.674	0.584	4.258	0.985

[1]Stilbestrol fed at the rate of 4 mg per day for 21 days.

VITA

Kenny Sullivan Marbury, the son of Mrs. Herbert Lee Marbury and the late Mr. Marbury, was born in Haywood County, Tennessee, on December 14, 1947. He attended public school in Haywood and Crockett Counties and was graduated from Bells High School in May 1966. In September of that year he enrolled in the University of Tennessee at Martin, and studied there for two years before transferring to the University of Tennessee at Knoxville. In June 1970, he was awarded a Bachelor of Science degree in Agriculture with a major in Animal Husbandry from the University of Tennessee at Knoxville. In July 1970, he accepted a position as an Assistant Extension Agent in McNairy County. To pursue his education further, he took a study leave in January 1974, to enter the graduate school, University of Tennessee, and began work toward a Master of Science degree in Animal Science.