

THE EFFECTS OF GYPSUM ON RUMEN FERMENTATION
AND THIAMIN STATUS

TIMOTHY FRANK BROWN

B.S., Virginia Polytechnic Institute
and State University, 1976

-

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas
1979

Approved by:



Major Professor

Spec. Coll.
LD
2668
.T4
1979
E777
C.2

TABLE OF CONTENTS

<u>Chapter</u>	<u>Page</u>
I. INTRODUCTION.	1
II. REVIEW OF LITERATURE	3
PEM as a Thiamin Deficiency	3
Ruminal Thiamin Synthesis	3
Thiamin Destruction	4
Impaired Absorption or Phosphorylation of Thiamin	7
Thiochrome Method for Thiamin Determination	8
III. INTRODUCTION TO RESEARCH.	11
IV. MATERIALS AND METHODS	12
Trial I	13
Trial II	13
V. RESULTS AND DISCUSSION	15
Trial I	15
Trial II	29
VI. BIBLIOGRAPHY	39
APPENDIX	

ACKNOWLEDGEMENT

I express my appreciation to Dr. B.E. Brent for guidance throughout my master's program. Sincere thanks to Martin Stokes and Don Sapienza for their help, without which I couldn't have done it. Thanks also to fellow grad students for their friendship, which made the whole thing easier. Special thanks to my parents for their continuous love and support.

INTRODUCTION

Agricultural gypsum has been used for years to limit supplement intake for ruminants. However, gypsum used for this purpose has been associated with signs similar to the central nervous system disorder, polioencephalomalacia (PEM). So far, research has not positively linked gypsum consumption to PEM.

Wagnon (1960) reported illness in several heifers which had been eating a supplement containing gypsum. All except the most severely afflicted returned to normal after gypsum was removed from the ration. The one severely ill heifer was described as having a permanent coordination disorder. Such disorders are often seen in early stages of PEM. Terlecki and Markson (1961) described PEM signs as aimless wandering, progressing to circling, accompanied by rapidly ensuing blindness. Simultaneous ataxia developed and rapidly becomes worse. Affected animals stagger, sway, stand precariously with feet wide apart, with head drawn back in convulsions and spasms. Loew, Roberts and Dunlop (1969) described the onset of symptoms as very rapid, with the usual manifestations being disorientation and blindness. Head pressing was frequently observed.

Gypsum is the common name for calcium sulphate dihydrate ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$). Solubility in cold water is 0.24 grams per 100 ml. (Handbook of Chemistry and Physics, 1971-1972).

Gypsum occurs in extensive deposits in several areas of the United States and is used principally in making plaster of Paris and Portland cement. It is not recommended for use as a calcium supplement because of the low availability of calcium in the compound. (Barrentine and Ruffin, 1958).

REVIEW OF LITERATURE

PEM as a Thiamin Deficiency

PEM symptoms were first described by Jensen, Griner and Adams (1956). Because PEM responded to thiamin (IV), it was presumed to be thiamin deficiency. Benevenga et al. (1967) supported this theory by showing that blood pyruvate increased in PEM, due to a lack of cocarboxylase (phosphorylated thiamin), a coenzyme needed for oxidative decarboxylation of pyruvate. Other supporting evidence was lowered transketolase activity in red blood cells and lowered thiamin in cerebral cortex and liver.

Herrick (1971) showed that parenteral injection of thiamin into cattle with PEM relieved the signs, but they returned within 2 to 3 days. Feeding 250 mg. thiamin every other day prevented recurrence. When supplemental thiamin was removed from the ration, the disease reappeared within a few days.

Lusby and Brent (1972) induced PEM in sheep using an intra-uminally infused liquid diet. Each intramuscular thiamin injection (200 mg) prevented signs for 28 hours. Adding 150 mg. thiamin daily to the infusion also prevented PEM signs. Removal of thiamin from the diet resulted in PEM within 28 hours.

Ruminal Thiamin Synthesis

In the normally developed rumen, microbes should supply the host with adequate thiamin. Kon and Porter (1954) found

substantial thiamin (0.3 ug/g) in rumen contents of animals fed a thiamin-free purified diet. These animals grew and thrived, proving thiamin synthesis.

Buziassy and Tribe (1960) showed that when high thiamin rations were fed, ruminal synthesis was very low, but when low thiamin rations were fed, synthesis was high. They found rumen thiamin concentrations of 0.29 ug/ml rumen fluid. Porter (1960) reported most rumen thiamin was in solution; not associated with bacteria.

Herrick (1971) estimated that an 800 pound finishing steer requires only 15.6 mg. thiamin per day. Synthesis amounts to about 8 mg. daily, pointing out that the thiamin status of a normally functioning ruminant should be adequate.

Thiamin Destruction

An animal receiving adequate thiamin excretes any excess in the urine (Heinrick, Hornig and Wiss, 1973). Broberg (1960) noted a precipitous decline in urinary thiamin excretion during grain-induced lactic acidosis, attributed this to destruction of thiamin synthesizing bacteria by ruminal accumulation of lactic acid.

Edwin, Lewis and Allcroft (1968a) found that rumen fluid from PEM-affected animals destroyed thiamin. In sheep, thiamin destruction (percent thiamin destroyed per ml rumen fluid) was 12.0 to 18.4 for healthy sheep and 83.6 to 100 for animals confirmed as having PEM. Similar comparisons for cattle found

0 percent and 63 to 100 percent respectively. This implicated rumen thiaminase as a cause of PEM.

When Sapienza and Brent (1972) induced PEM by continuous infusion of a liquid diet, thiamin half-life in rumen fluid was 14 minutes, compared to a 9 minute half-life for thiamin in rumen fluid from a spontaneous bovine PEM case, and no thiamin hydrolysis in rumen fluid from normal animals. Although Loew, Dunlop and Christian (1970) reported similar results, they also found some apparently normal cattle with high ruminal thiamin-destroying activity. They concluded that poor thiamin synthesis might be significant in some PEM cases, whereas thiamin destroying activity might be more important in others.

Edwin and Jackman (1973) showed that cerebrocortical necrosis (CCN), the British term for PEM, is associated with low levels of liver and brain thiamin as well as high rumen thiaminase. They concluded that CCN is due in part to a failure of thiamin supply from the gut because of thiaminase.

Evans () discussed two general classifications of thiaminases. The first, thiaminase I, is generally of bacterial origin and catalysed thiamin decomposition by a base exchange reaction involving a nucleophilic displacement of the methylene group of the pyrimidine moiety. This nucleophilic displacement usually takes place in the presence of an amine and, the pyrimidine moiety of thiamin is transferred to the amine. Edwin, Spence and Woods (1968a) suggested that rumen temperature and pH are conducive to formation of such antimetabolites, and many

amines such as ammonia, methylamine, choline and nicotinamide are present.

Antimetabolites structurally resemble the metabolite and relate their actions on living things to those of the metabolite. (Woolley, 1952). Evans () stated that thiaminases act on thiamin in such a way that the products formed no longer possess the metabolic activity of thiamin. Some antimetabolites, however, compete more strongly against the metabolite than others.

Pyriethiamin, oxythiamin and Amprolium are some common thiamin antimetabolites. The antithiamin activity of pyriethiamin is greater than that of oxythiamin, which is greater than that of Amprolium. Markson et al. (1972) gave pyriethiamin (180 mg/day), oxythiamin (27 mg/day), and Amprolium (16.2 gm/day) to pre-ruminant calves and found lowered brain (cerebrum) thiamin. Doses were different for each drug because of the different antithiamin activities of each. Only the animal receiving Amprolium developed PEM signs; none developed in animals given Amprolium plus supplemental thiamin.

Evans () suggested that because of the synthetic activity of rumen microflora, induction of thiamin deficiency requires enough thiaminase I to destroy the vitamin not only in the rumen, but also at the sites of absorption and digestion in the abomasum and small intestine.

Thiaminase can also come from fungi. However, Loew, and Dunlop (1972) suggested that bacterial thiamin destruction,

possibly in conjunction with decreased thiamin synthesis, is a more likely cause of PEM. Fungi may, however, alter the rumen bacterial flora to result in thiamin inadequacy.

Thiaminase II catalyses the simple hydrolysis of thiamin at the methylene bridge. Another substance which cleaves thiamin at the methylene bridge is the sulphite ion. In fact, sulphite is used to prepare thiamin-free blanks in thiamin analysis (Gyorgy, 1950). Leichter and Joslyn (1969) showed appreciable thiamin cleavage required considerable excess of sulphite and pH of approximately 5. Hendrickx (1961) showed sulphite was produced in the rumen, however, little sulphite accumulates because it is rapidly reduced to sulphide. Edwin et al. (1968a) claimed that 9.0 gm. per day sodium sulphite fed to young ruminant sheep failed to produce thiamin deficiency, although urinary thiamin excretion was markedly lower.

Impaired Absorption or Phosphorylation of Thiamin

Another reason for metabolic thiamin deficiency might be impaired thiamin absorption and/or phosphorylation. Rerat, LeBars and Malle (1958a) found no thiamin absorption from the rumen wall in normally fed sheep. In another experiment, however, Rerat, Malle and LeBars (1958b) observed that free thiamin declined in the perfused, isolated rumen of anesthetized sheep and that thiamin concentrations in the portal blood draining the rumen increased. Hoeller, Fecke and Scholler (1977) found no appreciable thiamin absorption across sheep rumen wall in vitro

at levels normally found in vivo. They also found no indication of active thiamin transport across rumen wall.

Lewis et al. (1966) found 0.5 to 1.0 ug thiamin per gram rumen contents in normal animals and 0.3 to 0.7 ug per gram in animals with PEM. Thiamin levels were lower, however, in the liver and urine of animals with PEM. They concluded that no failure of rumen thiamin synthesis occurred during PEM, but suggested that a failure in absorption or some aberration in the thiamin metabolism was responsible for PEM sign.

Edwin et al. (1968a) concluded that thiamin malabsorption is unlikely because administering thiamin by stomach tube was as effective as parenteral injection in treating PEM.

Loew and Dunlop (1972) induced PEM in mature ruminants by feeding Amprolium [1-(4-Amino-2-n-propyl-5-pyrimidinyl methyl)-2-picolinium chloride hydrochloride], a drug known to inhibit thiamin phosphorylation to thiamin pyrophosphate (TPP). Since there was no difference in total blood thiamin between animals with PEM and those with other diseases, they suggested that PEM was caused not by inadequate rumen synthesis, but by a metabolic lack of TPP.

Thiochrome Method for Thiamin Determination

Jansen (1936) showed that oxidation of thiamin to thiochrome could be used to determine thiamin in natural materials. This reaction has been the basis of several thiamin assays.

Several forms of thiamin can be found in biological

systems (Pearson, 1968). Considerable thiamin occurs bound to protein and phosphorylated, thus unavailable for analysis. Mild acid hydrolysis is usually sufficient to free thiamin from proteins. However, treatment with phosphatase such as the fungal enzymes Takadiastase¹, Diastase², Mylase³, Clarase⁴, and Paladase⁵ is needed to release thiamin from its phosphorylated form. It is generally agreed (Freed, 1966; Pippon and Potter, 1975; A.O.A.C., 1970) that incubation of samples in a dual enzyme mixture of papain and a phosphatase in sodium acetate buffer is sufficient to free thiamin.

Next, fluorescent impurities are removed from the thiamin solution by adsorbing thiamin onto thiochrome Decalso⁶. Freed (1960) suggested that although one gram of Decalso will adsorb 40 mg thiamin, other substances, such as amino acids will also fill adsorption sites, greatly reducing the thiamin adsorbing capacity of the Decalso.

Thiamin can be eluted from the Decalso free of contaminating substances with either hot or cold acid potassium chloride.

1. Parke-Davis Co., Detroit, Mich.
2. Merck & Co., Rahway, N.J.
3. Wallerstein Laboratories, New York, N.Y.
4. Takamine Laboratories Inc., Clifton, N.J.
5. Schwarz Laboratories Inc., Mt. Vernon, N.Y.
6. Fisher Scientific

However, Pippon and Potter (1975) found that elution is more complete when the solution is hot.

Once elution is complete, thiamin can be oxidized to thiochrome with alkaline potassium ferricyanide (A.O.A.C., 1970; Freed, 1966), alkaline mercuric chloride (Morita, 1969; Edwin, Jackman and Hebert, 1975), or cyanogen bromide (Fujiwara, 1949; Edwin et al., 1975), extracted into isobutyl alcohol and relative intensity of fluorescence can be determined spectrofluorometrically (A.O.A.C., 1970).

INTRODUCTION TO RESEARCH

Beef and sheep producers throughout the United States have used various feed additives in attempts to limit supplement intake by range animals. One such additive, used with varying success, is agricultural gypsum. By varying the gypsum level, producers attempt to control daily supplement intakes. However, some producers using gypsum have observed signs of PEM.

PEM is usually found in animals on high grain rations. Thus, PEM in grazing animals fed limited supplement appears unlikely. If, however, extenuating circumstances prevented access to the supplement for longer than normal, extreme appetite may cause the animals to over-eat supplement. As yet, it is unknown whether this possible overeating of concentrate is causing PEM or if there is some metabolic disturbance resulting from gypsum intake.

The following experiments were devised to study rumen parameters of sheep under two different conditions; feeding concentrate containing increasing levels of gypsum, or allowing access to a concentrate containing gypsum after a period of starvation. We hypothesized that sulphate from gypsum would be reduced to sulphite in sufficient quantities to create thiamin deficiency and PEM signs.

MATERIALS AND METHODS

Two trials were conducted to study the effects of feeding agricultural gypsum in a concentrate mix. Mature fistulated wethers weighing approximately 55 kg were housed in metabolism cages and fed a ration calculated to meet maintenance nutrient requirements. All animals were fed this ration for 2 weeks prior to each trial as a preliminary adaptation period. Water was available at all times, although intake was not measured.

All rumen samples were immediately strained through 4 layers of cheesecloth and pH was measured. Samples of rumen fluid (36 ml) were acidified with 4 ml 6 N HCl to stop fermentation and to preserve thiamin. H_2SO_4 is normally used when acidifying rumen fluid to preserve volatile fatty acids, however, H_2SO_4 can lower thiamin concentrations (Gyorgy, 1950). Approximately 40 ml rumen fluid were left unacidified. Both acidified and unacidified fluid was spun at 42,000x g for 30 minutes in a refrigerated centrifuge. Supernatant fluid was poured into glass scintillation vials and stored at 2°C.

Lactic acid was determined by the method of Barker and Summerson (1941). Volatile fatty acids were determined in a gas chromatograph⁷ equipped with a flame ionization detector. The 1.83 M glass column (64 mmOD and 2 mm ID) was packed with

7. Hewlett Packard Model 7672A

Chromosorb 101⁸ (100-120 mesh). Nitrogen was the carrier gas. Thiamin was determined by a modification of the Thiochrome method (See Appendix).

Trial I

Eight animals were involved in this trial. All were fed 300 grams concentrate plus 300 grams hay at 0700 hrs. each day. Three hundred grams hay was also fed at 1600 hrs. This scheme was followed for 21 days. On day 21, rumen samples were taken at 0600 hrs. and every two hours thereafter up to 2200 hrs. On day 22, 10% gypsum was added to the concentrate mix for 6 of the sheep and 15% gypsum was added for 2 of the sheep. This ration was fed for another 21 days at which time animals were again sampled. This procedure was followed throughout the trial, with gypsum level increasing 5% every 21 days until 30% gypsum was being added. After animals had been sampled on the 30% level, gypsum was removed from the concentrate mix and animals were fed the base ration for another 21 days, at which time they were sampled and removed from trial. Daily intakes of hay and concentrate were recorded.

Trial II

Three animals were used in this trial. All three were fed the same base ration as the animals in trial I. The concentrate contained no gypsum. After 2 weeks on this ration,

8. Johns-Manville, Denver, Colorado

the animals were fasted, one for 48 hours, one for 72 hours, and one for 96 hours. At 0800 hours following each fasting period, concentrate containing 30% gypsum was offered ad libidum. Animal B6 (48 hr fast) ate 830 grams, animal B2 (72 hr fast) ate 610 grams and animal R5 (96 hr fast) ate 860 grams.

Three rumen samples were taken on the day the concentrate was offered (1030, 1430 and 1930 hrs.), three were taken the following day and one was taken on the third day. Animals were removed from trial after the last sample was taken.

RESULTS AND DISCUSSION

Trial I

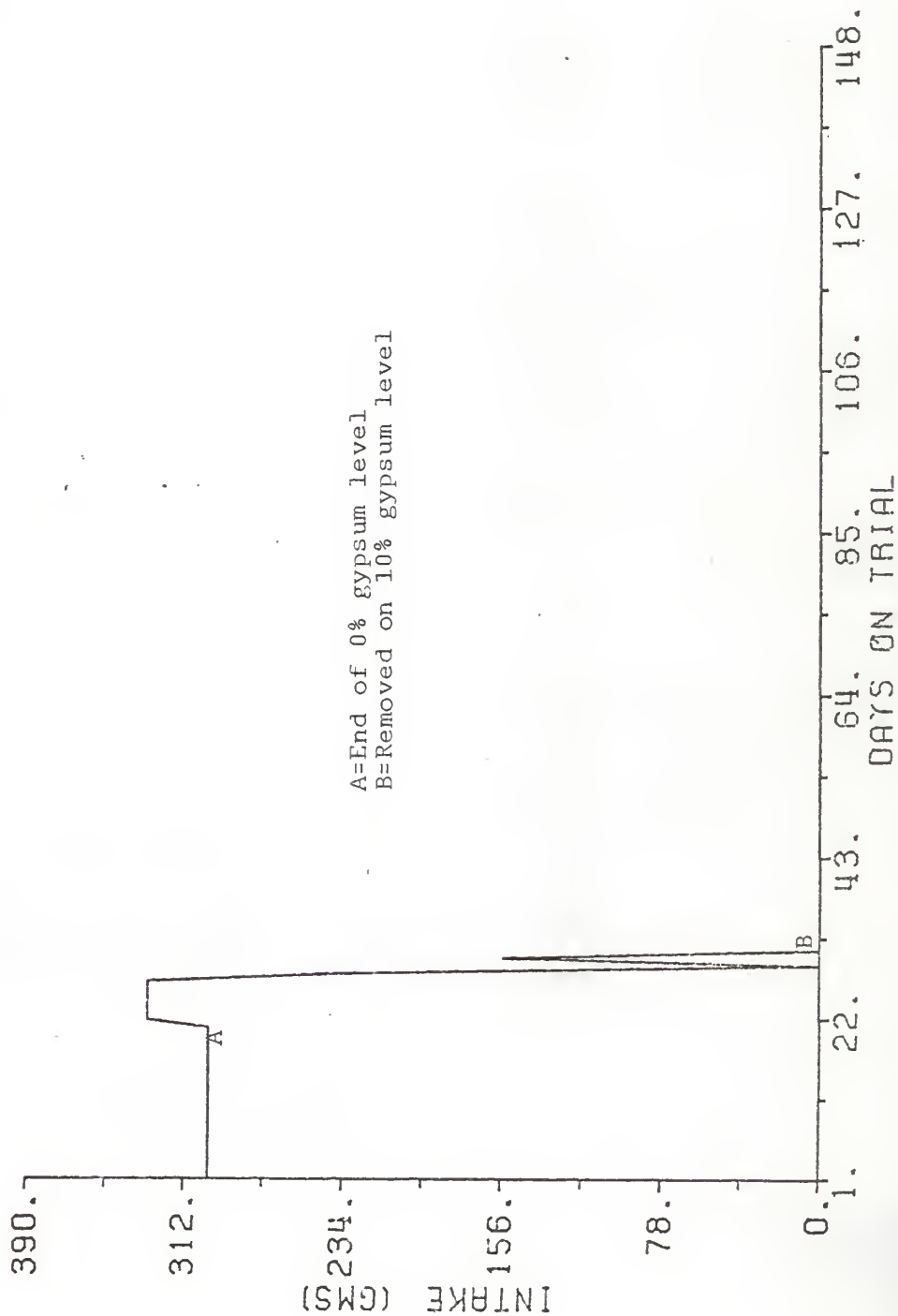
Incorporating gypsum into the concentrate mix caused variable reduction of feed intake. Two animals accepted 10% gypsum with no decrease in intake, while all others decreased their intakes. All animal's intakes were decreased by 15% gypsum.

No specific concentrate intake pattern was established, although intake generally decreased as gypsum increased. (graphs 1-5). Animals developed a technique of sorting gypsum from the concentrate mix, so daily concentrate intake does not accurately reflect gypsum consumed. Had sorting been prevented, by proper feed processing method, (pelletting, etc.), daily intake fluctuations may have been reduced.

Hay intake was also reduced in some animals as gypsum increased, and intake was variable. (graphs 6-10). Reduced hay intake indicates that metabolic factors rather than palatability limited feed intake. Total intake was reduced in 4 animals sufficient to cause removal from trial. One was removed while on 10% gypsum, two while on 15% gypsum, and one while on 30% gypsum. Only three animals remained on trial throughout the 30% level, after which gypsum was removed from the concentrate. Two of these animals resumed full intake immediately, the third, three days later. Upton, L'Estrange and McAleese

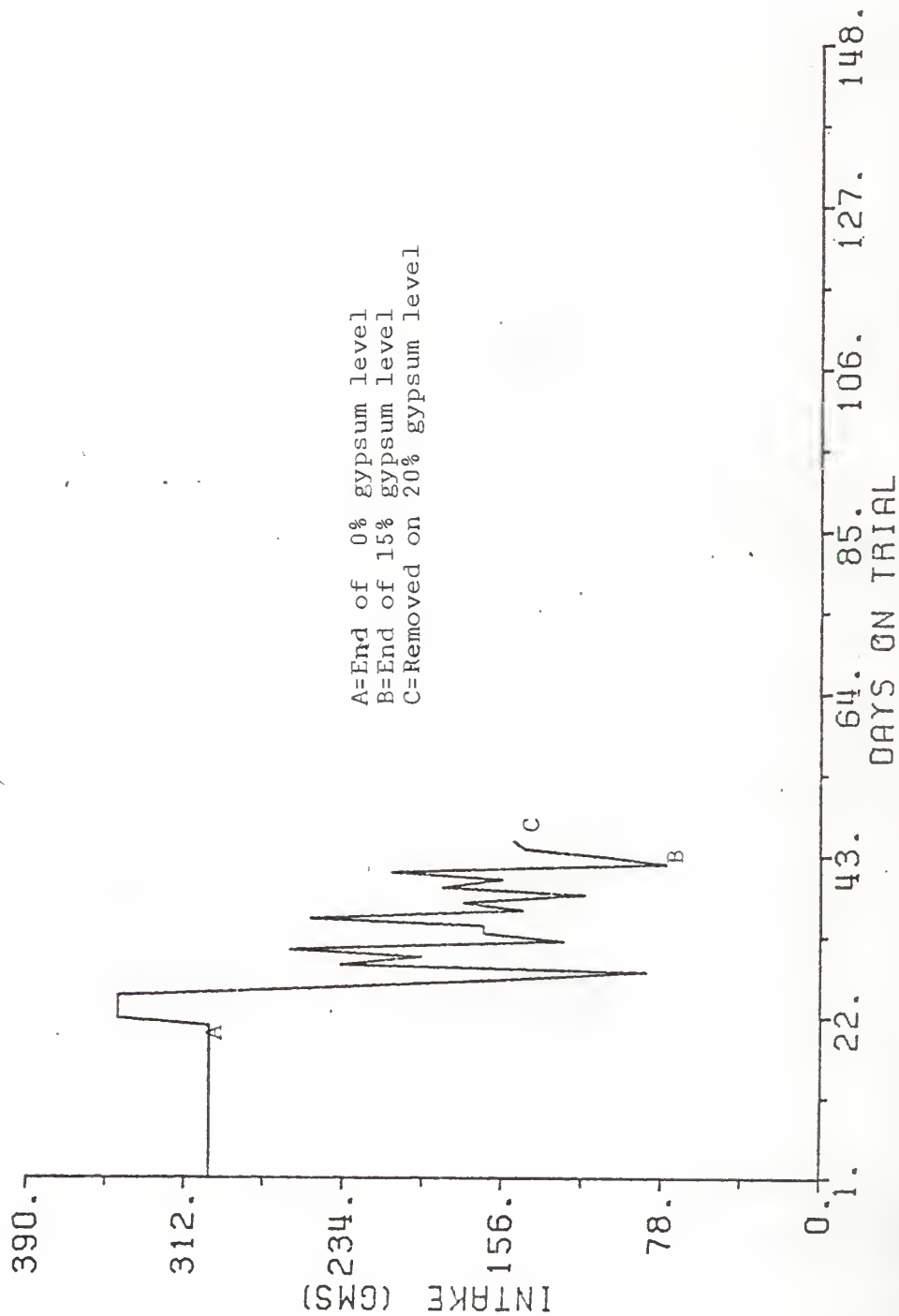
TRIAL I DAILY CONCENTRATE INTAKE ANIMAL R4

Graph 1



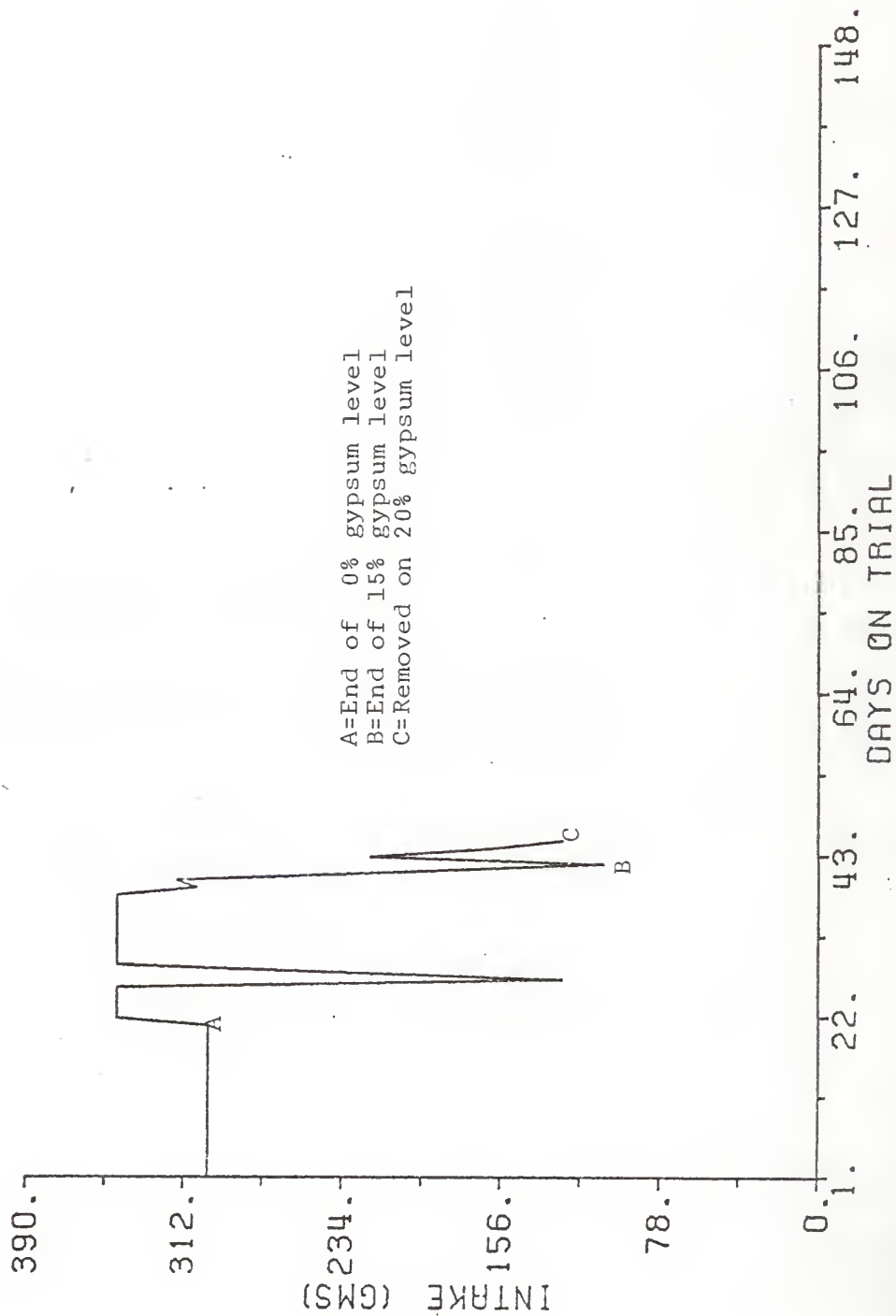
TRIAL I DAILY CONCENTRATE INTAKE ANIMAL R3

Graph 2



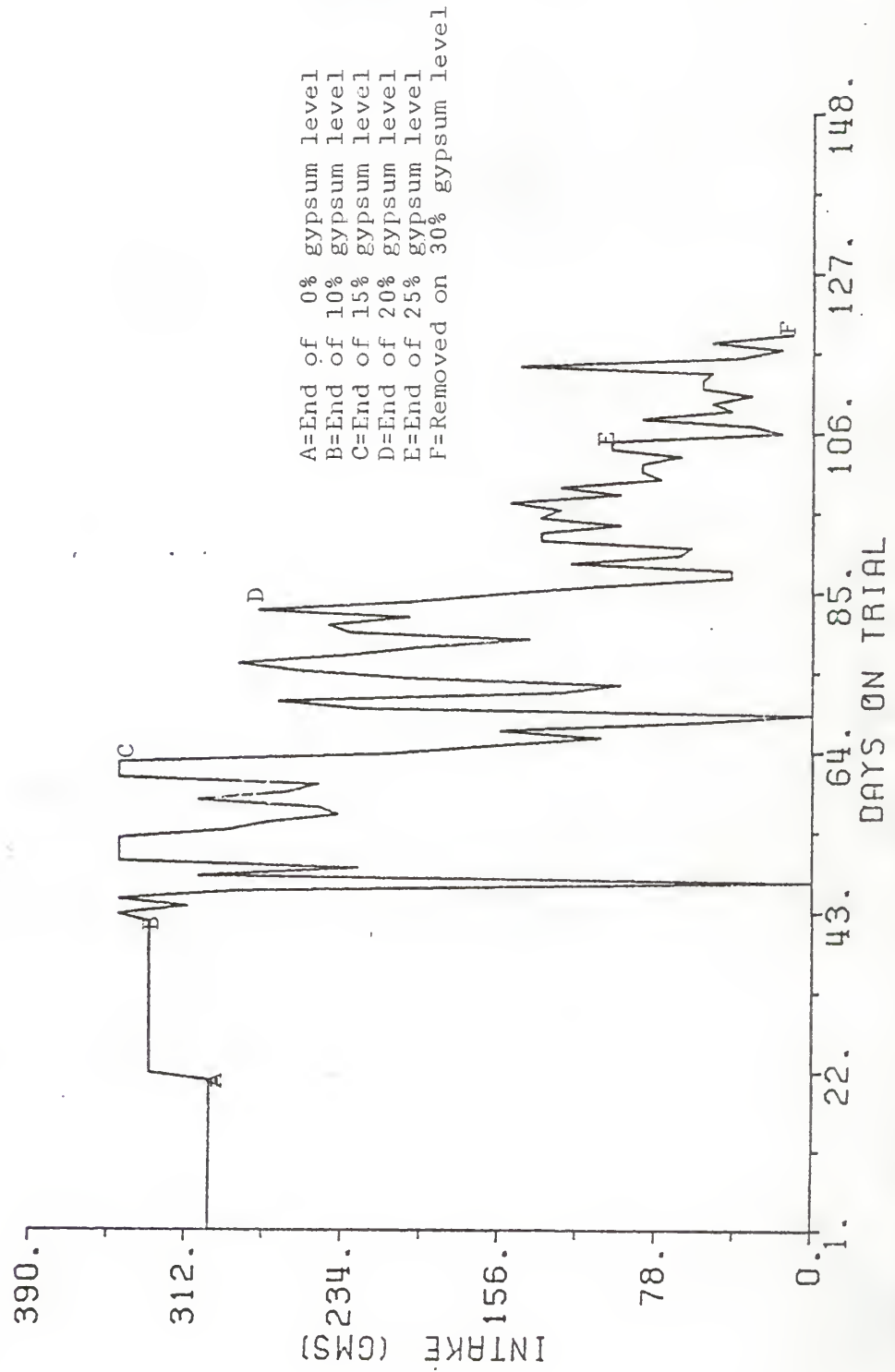
TRIAL I DAILY CONCENTRATE INTAKE ANIMAL B5

Graph 3



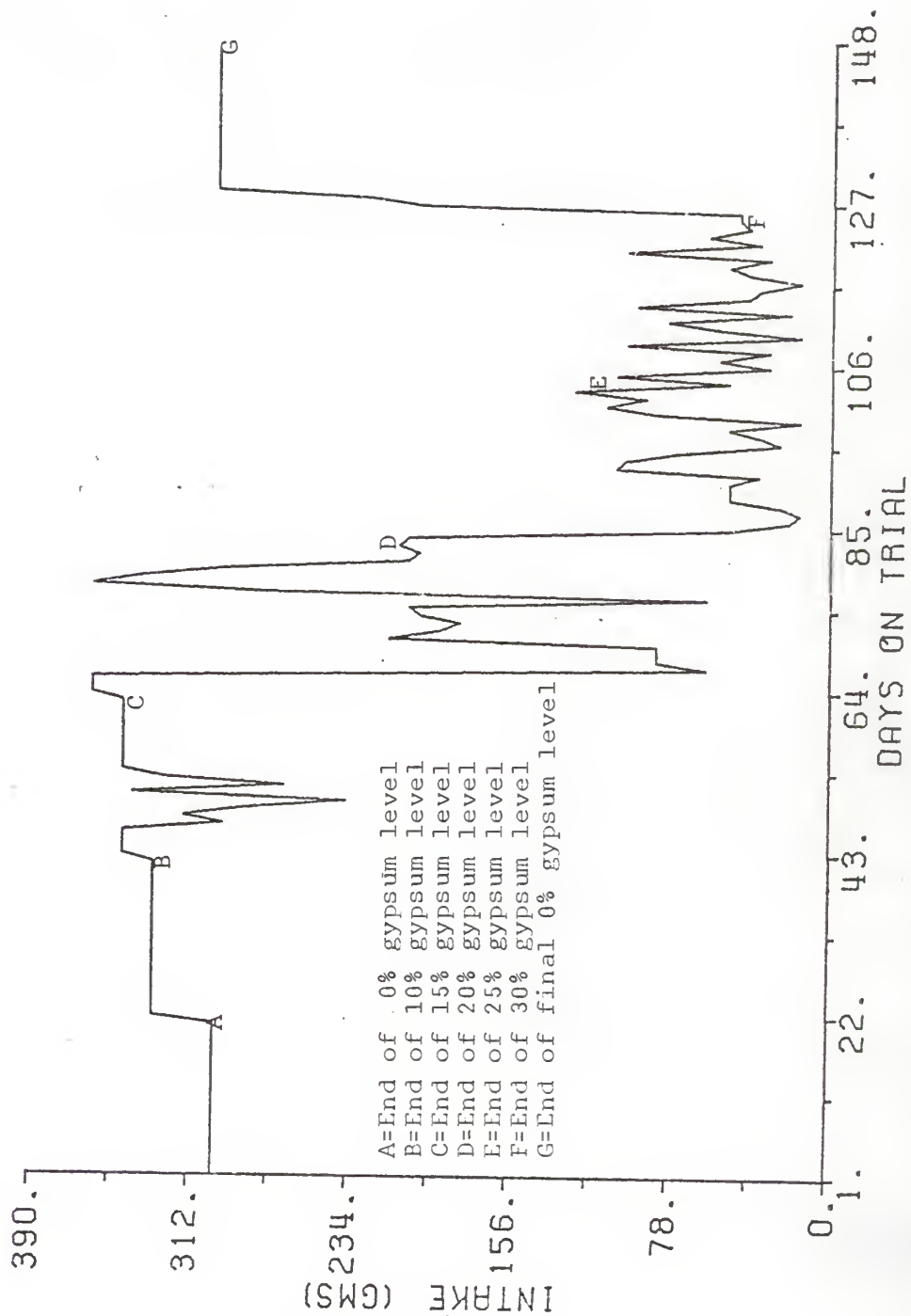
TRIAL I DAILY CONCENTRATE INTAKE ANIMAL B4

Graph 4



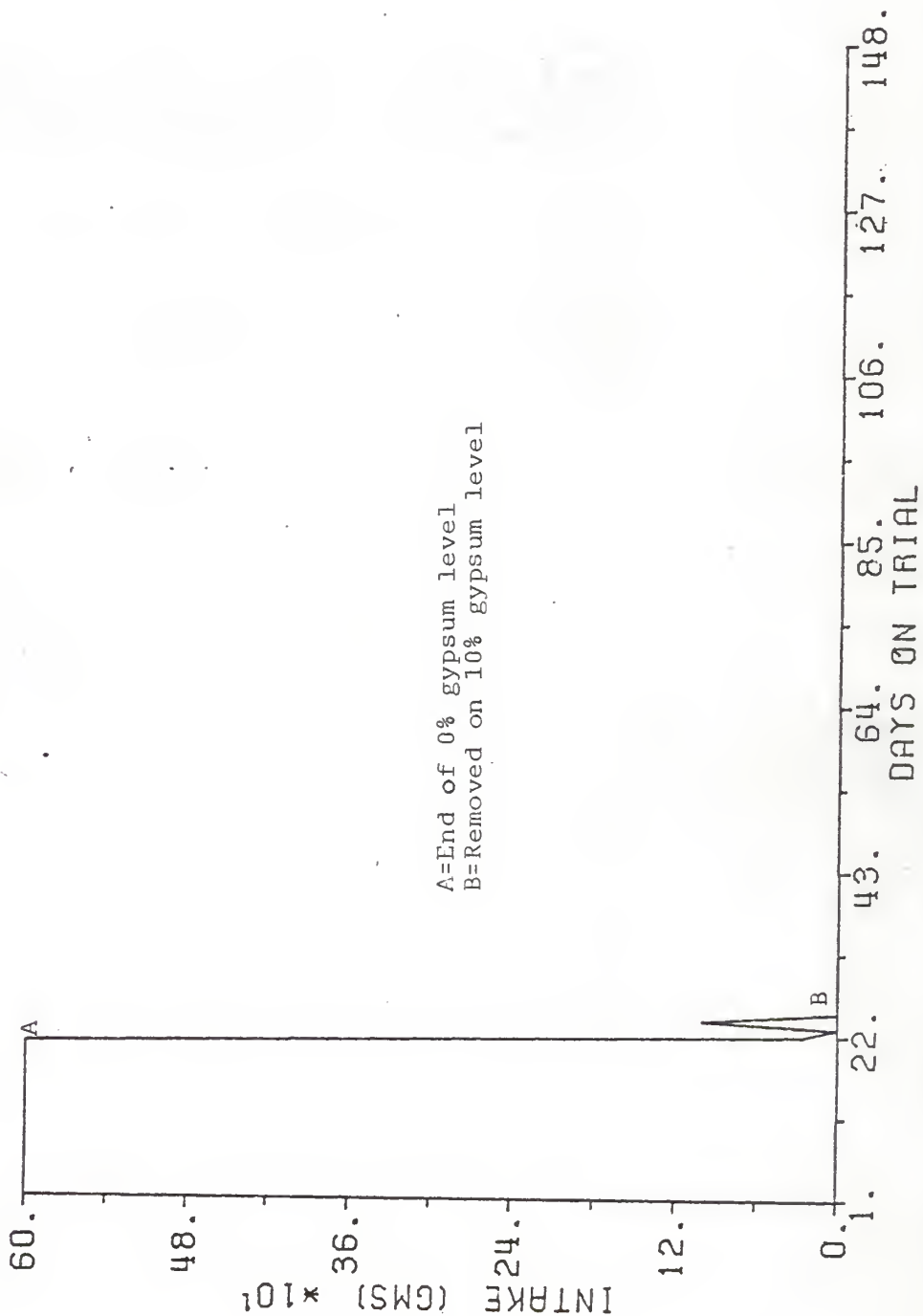
TRIAL I DAILY CONCENTRATE INTAKE ANIMAL R2

Graph 5



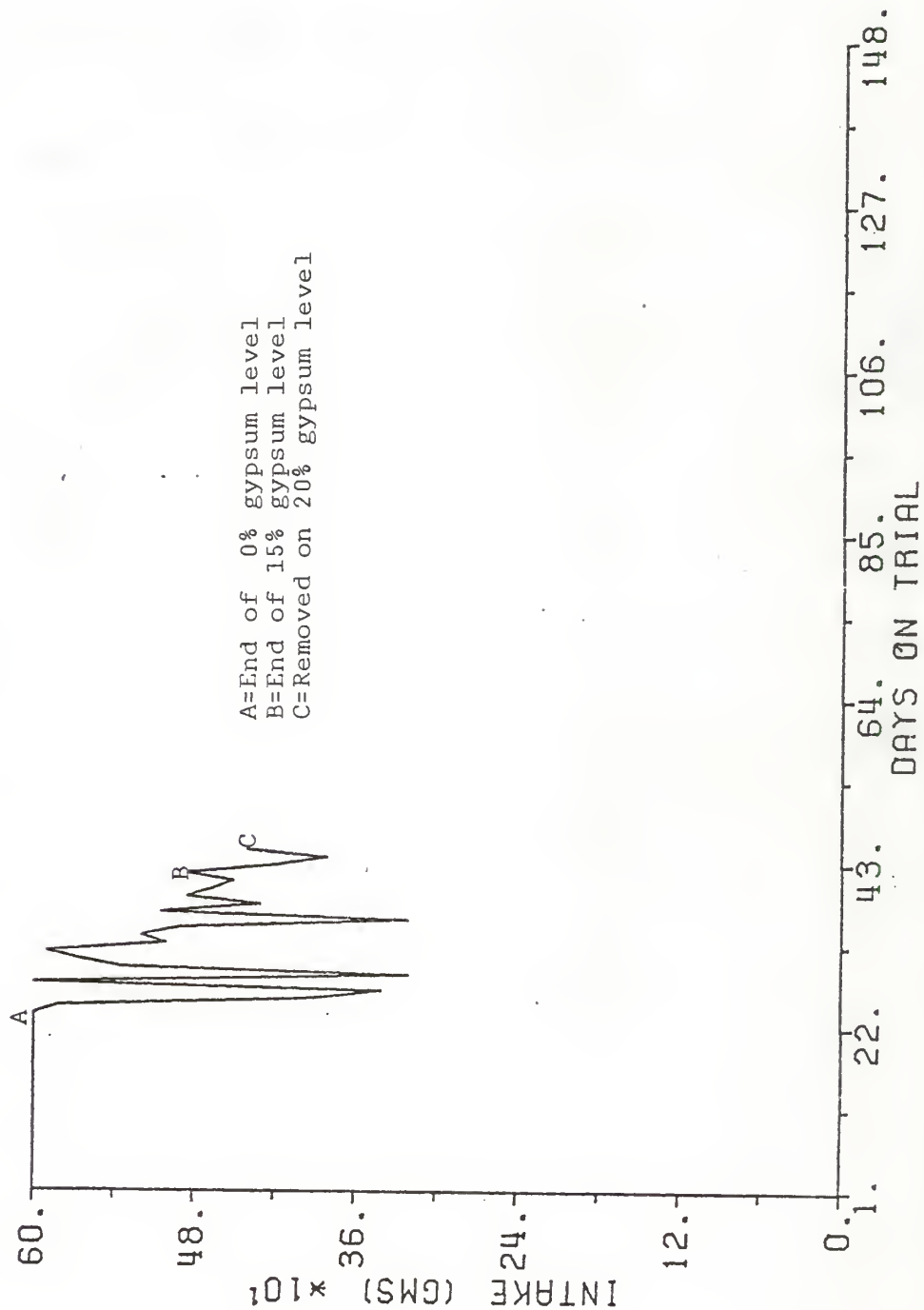
TRIAL I DAILY HAY INTAKE ANIMAL R4

Graph 6



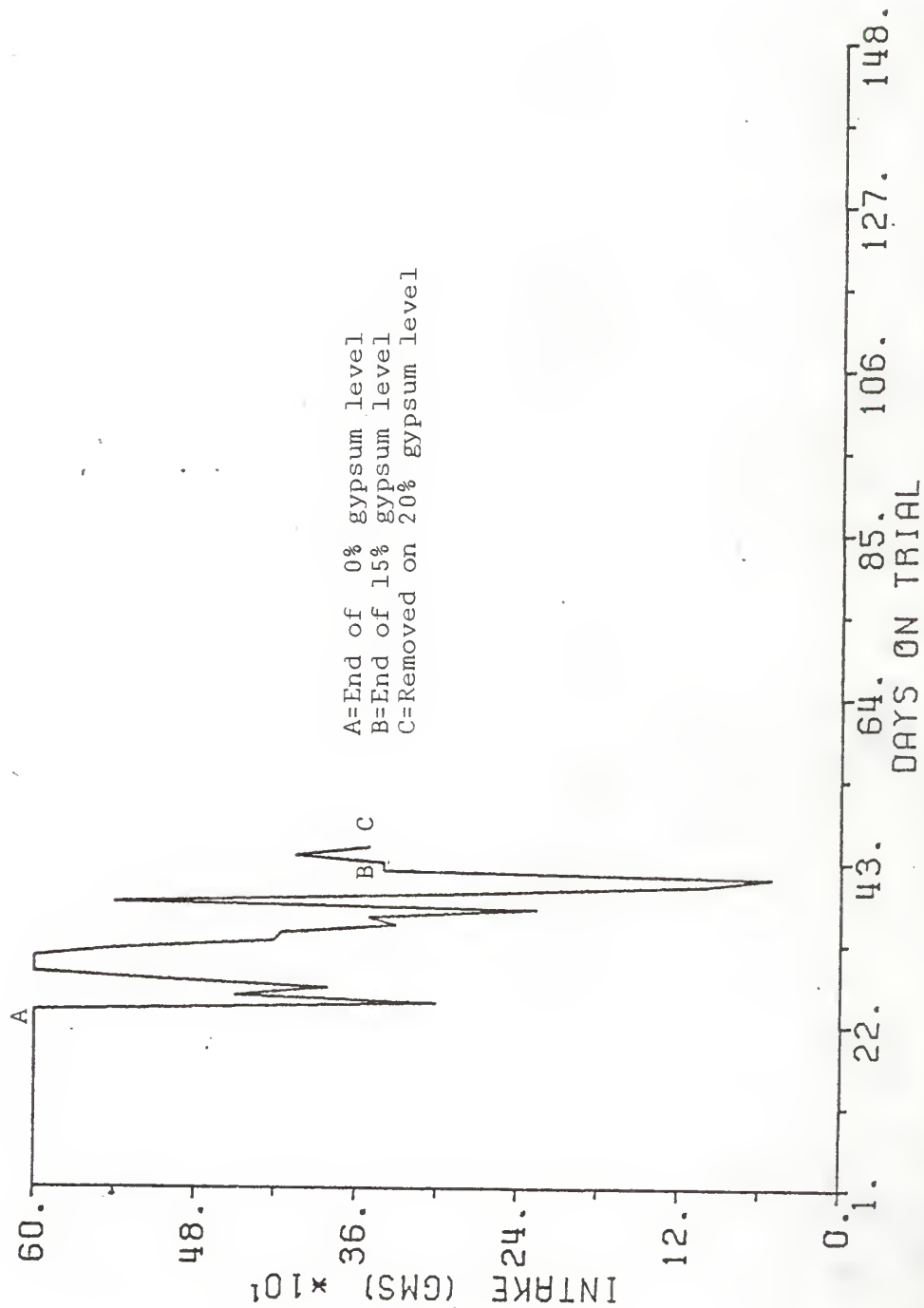
TRIAL I DAILY HAY INTAKE ANIMAL R3

Graph 7



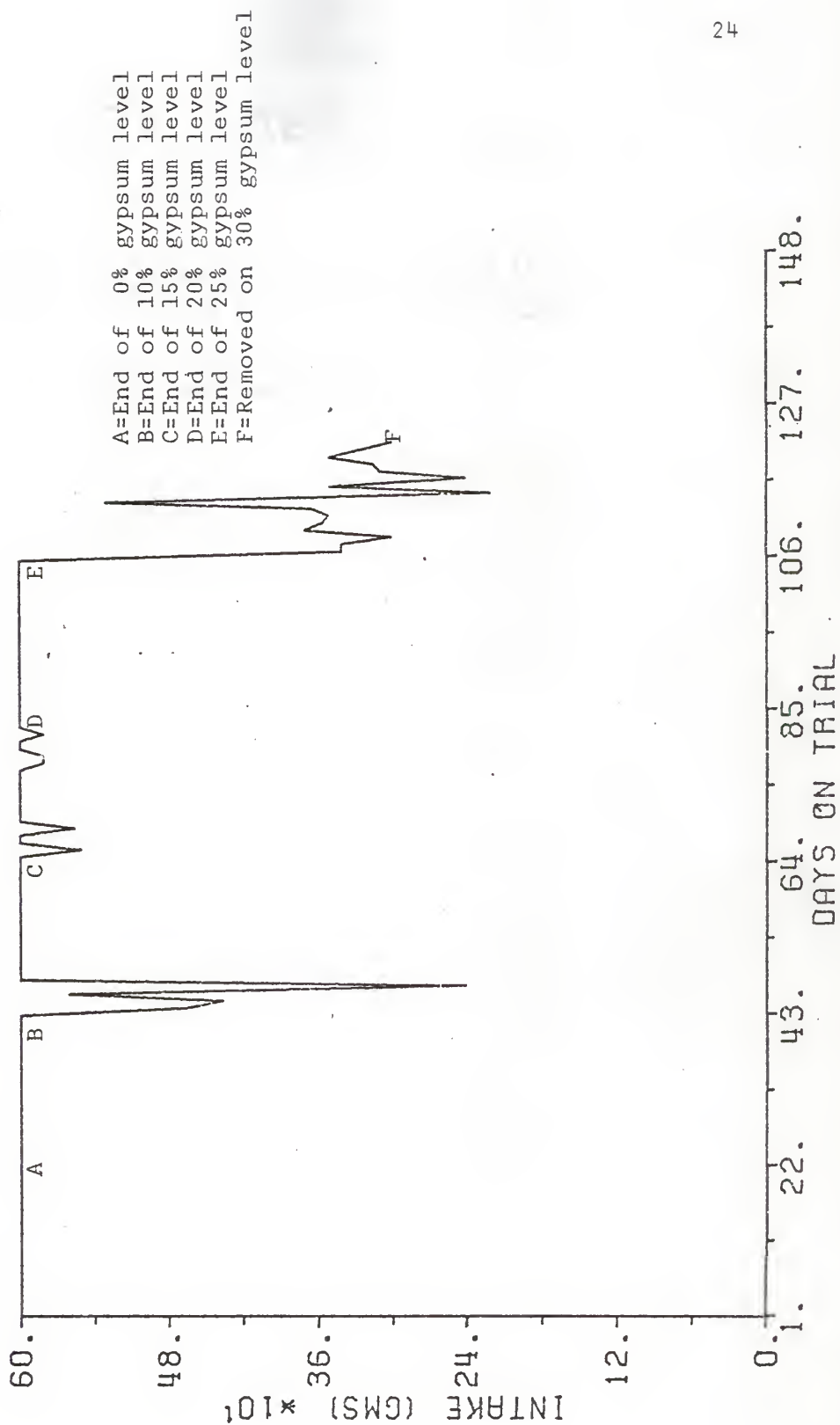
TRIAL I DAILY HAY INTAKE ANIMAL B5

Graph 8



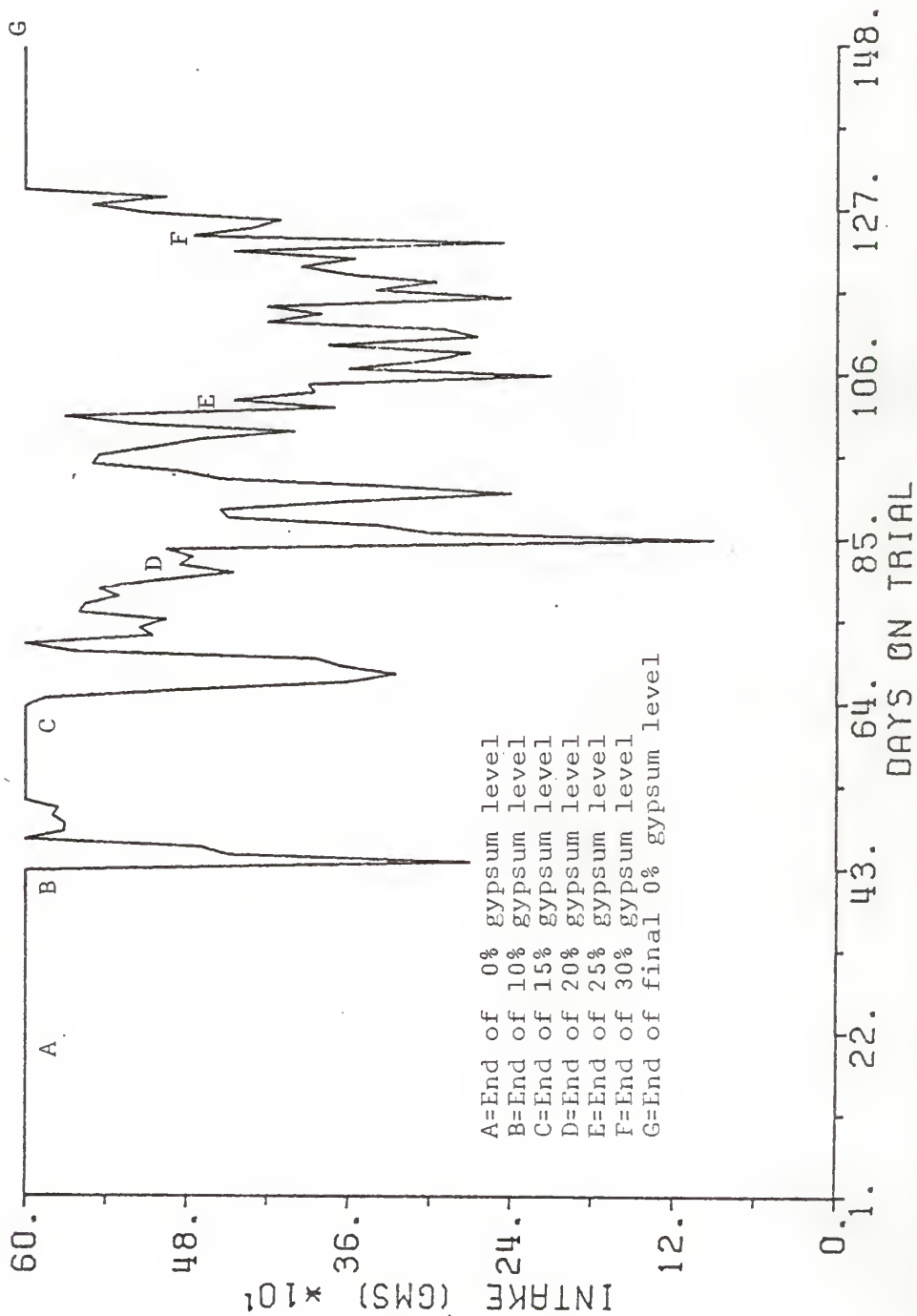
TRIAL I DAILY HAY INTAKE ANIMAL B4

Graph 9



TRIAL I DAILY HAY INTAKE ANIMAL R2

Graph 10



(1972) stated that the influence of sulphate on intake in sheep was the same whether the sulphate was given in the diet or intraruminally, indicating that sulphate exerts a metabolic and not a palatability effect. Immediate resumption of full intake by two sheep in this trial indicates that if the effect is metabolic, it is short-lived.

VFA production throughout the trial was affected only in absolute amount. As gypsum increased and feed intake decreased, total VFA production decreased. VFA molar ratios remained constant. Molar percent ranges for all animals were: Acetate, 56-70; propionate, 14-21; butyrate, 9-18; valerate, 1.5-2.5; isobutyrate, 1-3; isovalerate, 1-5.

Rumen lactate remained low at all gypsum levels. (Table 1). Rumen pH varied from normal daily fluctuation only when animals became ill, when it dropped slightly. Upton (1972) reported no change in rumen fluid pH or VFA production in animals fed sulphate to limit intake.

Gypsum did not effect rumen thiamin (Table 2). Only one animal had low rumen thiamin, and this animal was removed because of low feed intake. All other animals maintained rumen thiamin levels within the range set on 0% gypsum. It is possible that conditions for thiamin cleavage by sulphite described by Leichter and Joslyn (1969) were not met. They found that considerable excess sulphite and pH of approximately 5.0 were needed. Rumen sulphite was not determined in this trial,

Table 1

RUMEN LACTIC ACID VS VARIED GYPSUM LEVELS

<u>Gypsum level</u>	<u>0%</u>	<u>10%</u>	<u>15%</u>	<u>20%</u>	<u>25%</u>	<u>30%</u>	<u>0%</u>
Animal R4	28 ^a	29	---	---	---	---	---
Animal B4	41	41	15	26	41	32	---
Animal R3	27	---	36	---	---	---	---
Animal B5	45	---	53	---	---	---	---
Animal B1	22	63	52	20	48	35	39

a-Rumen lactic acid (ug/ml rumen fluid)

Table 2

RUMEN THIAMIN VS VARIED GYPSUM LEVELS

<u>Gypsum level--</u>	<u>0%</u>	<u>10%</u>	<u>15%</u>	<u>20%</u>	<u>25%</u>	<u>30%</u>	<u>0%</u>
Animal R4	.68 ^a	.13	---	---	---	---	---
Animal B4	1.29	.38	.60	.70	.42	.26	---
Animal R3	.26	---	.42	---	---	---	---
Animal B5	.34	---	.78	---	---	---	---
Animal B1	.47	.61	.53	.36	.40	.59	.50

a- Rumen thiamin (ug/ml rumen fluid)

however, low concentrate intake and gypsum sorting leads one to expect low sulphite. Henderickx (1961) explained that little sulphite is found in the rumen because it is rapidly reduced to sulphide. Rumen pH in this trial was higher than the optimum for thiamin cleavage according to Leichter and Joslyn (1969).

Animals with low feed intake did not have low rumen thiamin. Kon and Porter (1954) and Buziassy and Tribe (1960) reported similar results in animals with low dietary thiamin. No animals showed signs of PEM, however, rumen thiamin level may not be a good indicator of metabolic thiamin status. Kon and Porter (1954) and Buziassy and Tribe (1960) reported thiamin levels of 0.3 ug/gm and 0.29 ug/gm in rumen contents of normal animals. Lewis et al. (1966) reported 0.3 to 0.7 ug thiamin per gram rumen contents in animals with PEM. The present study showed 0.26 to 1.29 ug thiamin per ml rumen fluid in normal animals. The overlapping ranges of rumen thiamin in normal animals and in those with PEM points to something other than simple rumen thiamin deficiency as a cause of PEM.

Trial II

Concentrate containing 30% gypsum was offered ad lib following periods of fasting. Consumption for each animal was: B6, 830 grams; B2, 610 grams; R5, 860 grams. Within 2 hours lactic acid reached high levels and remained high throughout the first sampling day. (Table 3). All lactic

Table 3

RUMEN LACTIC ACID VS STARVATION

<u>Animal Number---</u>		<u>B6^a</u>	<u>B2^b</u>	<u>R5^c</u>
<u>Concentrate mix</u>				
<u>Presentation</u>	+ 2 hrs	2352 ^d	2545	2017
	+ 4 hrs	3605	3605	3078
	+ 9 hrs	3605	3374	3605
	+ 26 hrs	78	0	14
	+ 30 hrs	17	27	4
	+ 35 hrs	19	62	0
	+ 50 hrs	0	66	0

a- B6 fasted 48 hours prior to concentrate mix presentation

b- B2 fasted 72 hours prior to concentrate mix presentation

c- R5 fasted 96 hours prior to concentrate mix presentation

d- Rumen lactic acid (ug/ml rumen fluid)

acid levels had returned to normal the following day, but but all animals exhibited lactic acidosis signs of reduced eructation and reduced rumen motility.

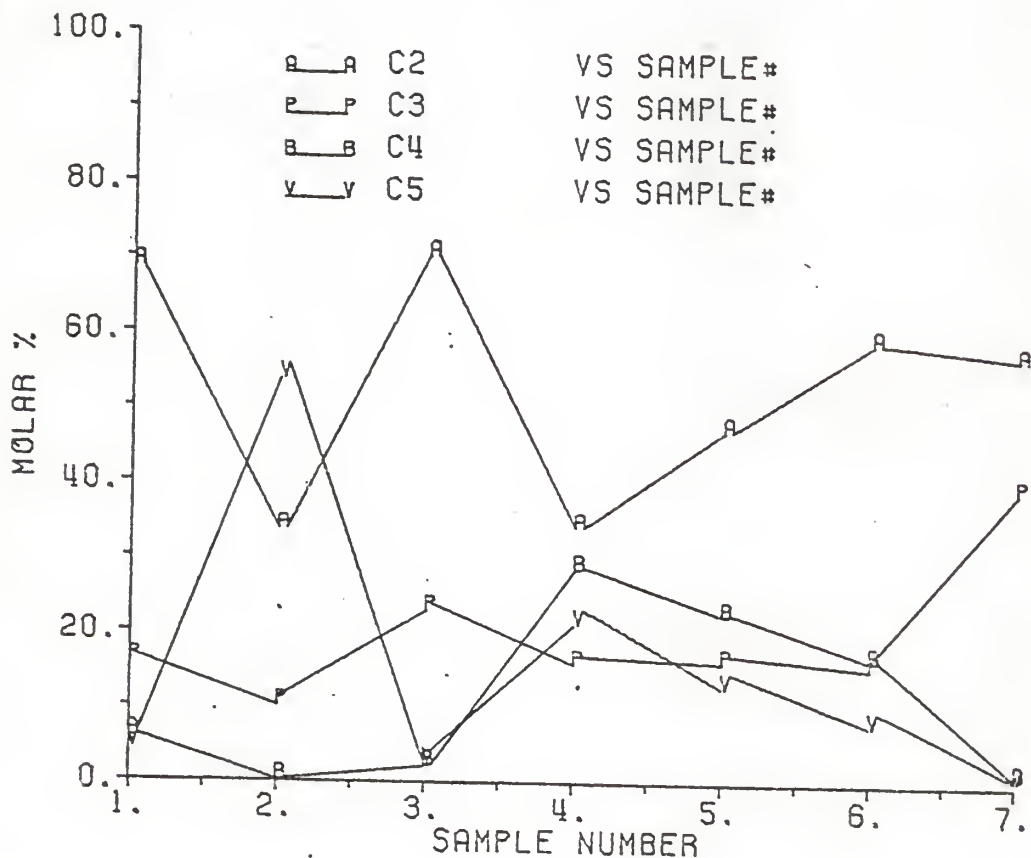
Molar percents of acetic, propionic, butyric and valeric acids varied greatly. (graphs 11-13). No change was seen in molar ratios of isobutyric and isovaleric acids. Total VFA levels varied slightly for all three animals, although it followed the same general pattern for all animals. (Table 4).

Rumen thiamin levels (Table 5) remained normal throughout the first sampling day for all sheep. By 1030 the second sampling day, only the animal from the two day fast had normal rumen thiamin levels, but this dropped to near zero by 1430. All levels remained near zero until the animals were removed from trial. It is interesting that as rumen thiamin dropped to zero on day 2, total VFA production increased and rumen lactic acid decreased. With bacterial activity sufficient to cause increases in VFA production, it seems likely that thiamin synthesis would increase. It appears that all animals had survived lactic acidosis and were regaining normal rumen function, although animals refused to eat concentrate containing 30% gypsum and showed no increase in rumen thiamin.

Perhaps the continued low rumen thiamin is due to destruction by sulphite. Less problem with sorting of gypsum was encountered in this trial because of the extreme appetite of the animals. Therefore, animals consumed approximately 200

Graph 11

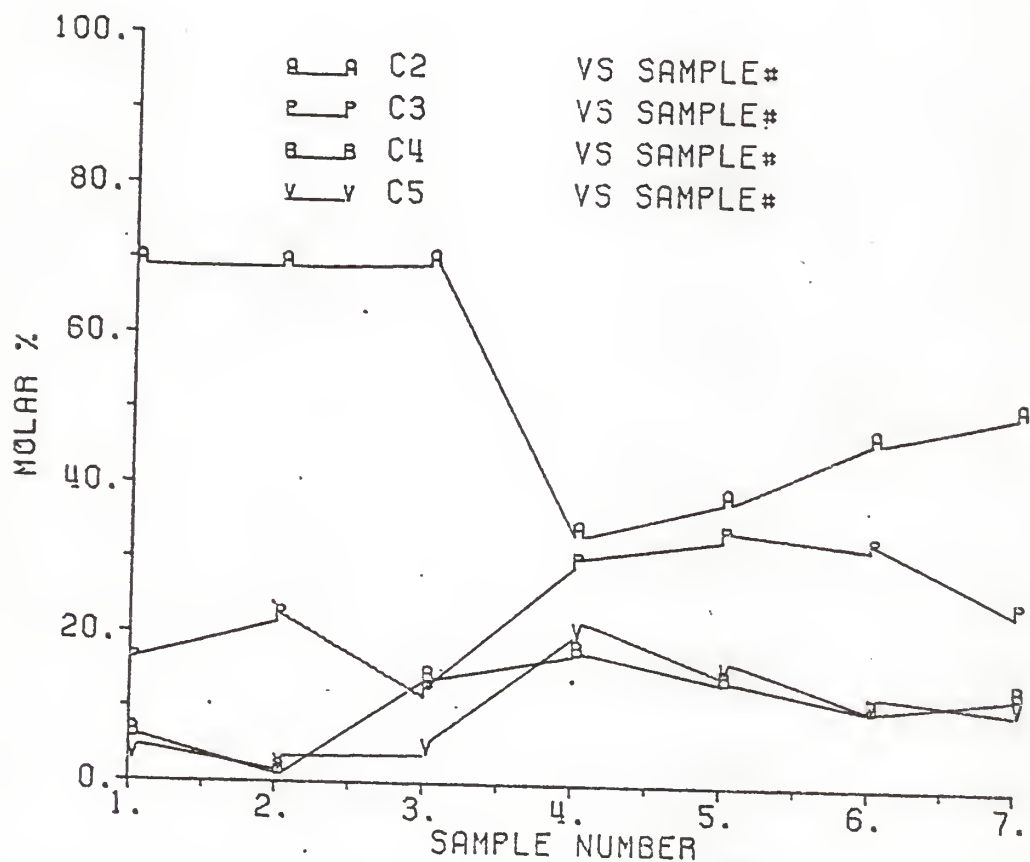
TRIAL II RUMEN VFA ANIMAL B6



Sample # 1 taken 2 hrs after concentrate mix presentation
 Sample # 2 taken 4 hrs after concentrate mix presentation
 Sample # 3 taken 9 hrs after concentrate mix presentation
 Sample # 4 taken 26 hrs after concentrate mix presentation
 Sample # 5 taken 30 hrs after concentrate mix presentation
 Sample # 6 taken 35 hrs after concentrate mix presentation
 Sample # 7 taken 50 hrs after concentrate mix presentation

Graph 12

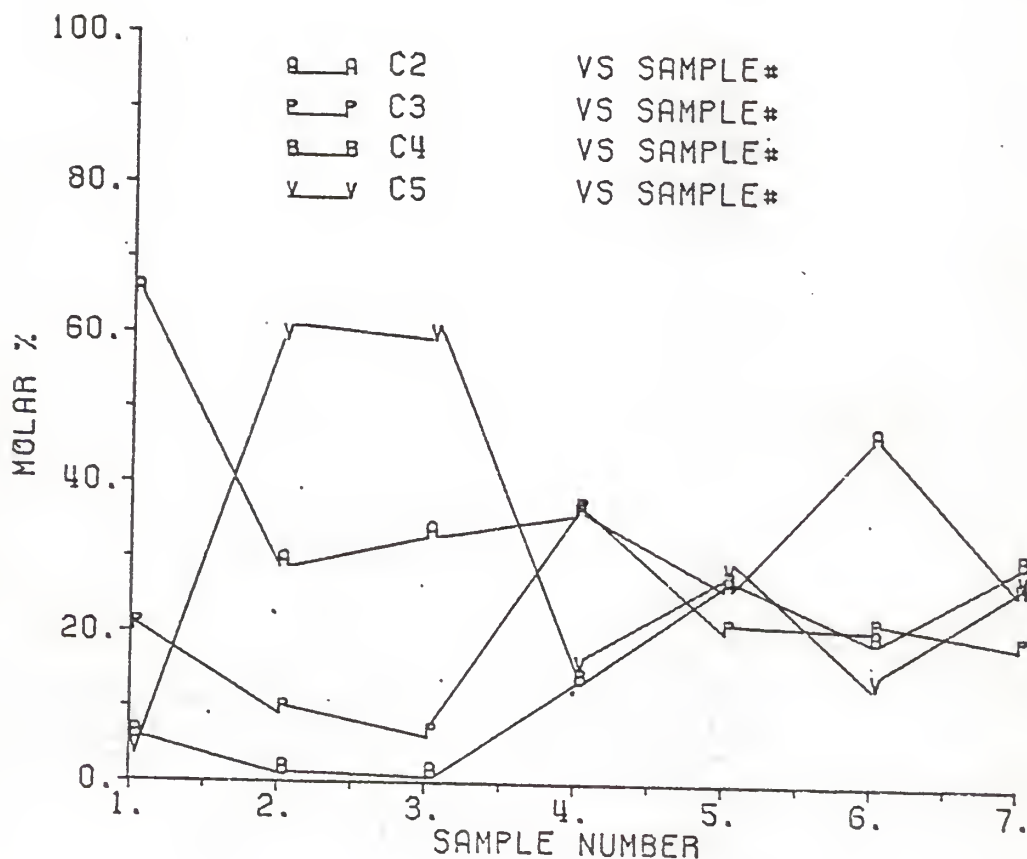
TRIAL II RUMEN VFA ANIMAL B2



Sample # 1 taken 2 hrs after concentrate mix presentation
 Sample # 2 taken 4 hrs after concentrate mix presentation
 Sample # 3 taken 9 hrs after concentrate mix presentation
 Sample # 4 taken 26 hrs after concentrate mix presentation
 Sample # 5 taken 30 hrs after concentrate mix presentation
 Sample # 6 taken 35 hrs after concentrate mix presentation
 Sample # 7 taken 50 hrs after concentrate mix presentation

Graph 13

TRIAL II RUMEN VFA ANIMAL R5



Sample # 1 taken 2 hrs after concentrate mix presentation
 Sample # 2 taken 4 hrs after concentrate mix presentation
 Sample # 3 taken 9 hrs after concentrate mix presentation
 Sample # 4 taken 26 hrs after concentrate mix presentation
 Sample # 5 taken 30 hrs after concentrate mix presentation
 Sample # 6 taken 35 hrs after concentrate mix presentation
 Sample # 7 taken 50 hrs after concentrate mix presentation

Table 4

RUMEN VFA (TOTAL)

	<u>Animal number</u>	<u>B6^a</u>	<u>B2^b</u>	<u>R5^c</u>
<u>Concentrate mix</u> <u>Presentation</u>	+ 2 hrs	35 ^d	34	34
	+ 4 hrs	38	19	33
	+ 9 hrs	20	65	28
	+ 26 hrs	110	75	86
	+ 30 hrs	106	63	80
	+ 35 hrs	105	59	65
	+ 50 hrs	23	47	82

a- B6 fasted 48 hours prior to concentrate mix presentation

b- B2 fasted 72 hours prior to concentrate mix presentation

c- R5 fasted 96 hours prior to concentrate mix presentation

d- Total rumen VFA (uM/ml rumen fluid)

Table 5

RUMEN THIAMIN VS STARVATION

	<u>Animal number</u> ---	<u>B6^a</u>	<u>B2^b</u>	<u>R5^c</u>
<u>Concentrate Mix Presentation</u>	+ 2 hrs	.31 ^d	.26	.40
	+ 4 hrs	.41	.46	.34
	+ 9 hrs	.33	.37	.25
	+ 26 hrs	.35	0	0
	+ 30 hrs	.05	0	.02
	+ 35 hrs	.07	0	.06
	+ 50 hrs	.02	0	.09

a- B6 fasted 48 hours prior to concentrate mix presentation

b- B2 fasted 72 hours prior to concentrate mix presentation

c- R5 fasted 96 hours prior to concentrate mix presentation

d- Rumen thiamin (ug/ml rumen fluid)

grams of gypsum. If the sulphate from gypsum was being reduced to sulphite in sufficient quantities, thiamin cleavage may have occurred, since rumen pH (Table 6) was close to the optimum described by Leichter and Joslyn (1969)

Rumen stasis had obviously occurred in these animals. It is conceivable that with reduced rumen motility, slowed passage of material from the rumen would cause gypsum accumulation. Sulphite could then be produced over a period of days, giving the low rumen thiamin seen in this trial. Although rumen thiamin remained near zero for 2 days, no signs of PEM were seen.

Table 6

RUMEN pH VS STARVATION

	<u>Animal number--B6^a</u>	<u>B2^b</u>	<u>R5^c</u>
<u>Concentrate mix</u>			
<u>Presentation</u>			
+ 2 hrs	5.43	5.60	6.00
+ 4 hrs	4.80	5.48	4.72
+ 9 hrs	4.90	5.60	5.40
+ 26 hrs	5.48	5.50	6.10
+ 30 hrs	5.40	5.80	5.80
+ 35 hrs	5.40	5.95	5.90
+ 50 hrs	5.58	6.20	5.30

a- B6 fasted 48 hours prior to concentrate mix presentation

b- B2 fasted 72 hours prior to concentrate mix presentation

c- R5 fasted 96 hours prior to concentrate mix presentation

BIBLIOGRAPHY

- A.O.A.C. 1970. Official Methods of Analysis, 11th Ed.
Association of Official Agricultural Chemists. Washington
D.C.
- Barker, S.B. and W.H. Summerson. 1941. The colorimetric
determination of lactic acid in biological material. J.
J. Biol. Chem 138:535.
- Barrentine, B.F. and B.G. Ruffin. 1958. Salt and gypsum as
regulators of cottonseed meal intake. Miss. State Univ.
Ag. Exp. Sta information sheep #587
- Benevenga, N.J., R.L. Baldwin, M. Ronning and A.L. Black. 1967.
Pyruvate metabolism in thiamin deficient calves. J
J. Nutr. 91:63
- Broberg, G. 1960. Acute Overeating with Cereals in Ruminants.
Monogr. Lovisa.Nya. Tyckeri-Lovisa.
- Buziassy, C. and D.E. Tribe. 1960. The synthesis of vitamins
in the rumen of sheep. I. The effect of diet on the
synthesis of thiamin, riboflavin, and nicotinic acid.
Aust. J. Agric. Res. 11:989.
- Edwin, E.E. and R. Jackman. 1970. Thiaminase I in the develop-
ment of cerebro-cortical necrosis in sheep and cattle.
Nature. 228:772
- Edwin, E.E., R. Jackman and N. Hebert. 1975. An improved
procedure for the determination of thiamin. The
Analyst. 100:689.
- Edwin, E.E., G. Lewis and R. Allcroft. 1968a. Cerebrocortical
necrosis: A hypothesis for the possible role of thiaminase
in it's pathogenesis. Vet. Rec. 83:176.
- Edwin, E.E., J. B. Spence and A.J. Woods. 1968b. Thiaminases
and cerebrocortical necrosis. Vet. Rec. 83:417.
- Evans, C.W. . Thiaminases and their effects on animals.
- Freed, M. 1966. Methods of Vitamin Assay. (3rd Ed.) Inter-
science, New York, N.Y.
- Fujiwara, M. 1949. J. Jap. Biochem Soc. 21:200.

- Gyorgy, P. 1950. The Vitamins, Vol. I. Academic Press.
- Handbook of Chemistry and Physics. 1971-1972. 52nd Ed.
The Chemical Rubber C.
- Heinrich, C.P., D. Hornig and O. Wiss. 1973. Autoradiographic studies on thiamin distribution in normal and thiamin deficient rats. *Int. J. Vit. N.* 43:174.
- Henderickx, H. 1961. *Archs. Int. Physiol. Biochem.* 69:443.
- Herrick, J.B. 1971. Thiamin in treatment of bovine polioencephalomalacia. *Vet. Med.* 66:176.
- Hoeller, H.M., Fecke and K. Scholler. 1977. Permeability to thiamin of sheep rumen wall in vitro. *J. Anim. Sci.* 44:158.
- Jansen, B.C.P. 1936. *Recl. Trav. Chim. Pays-Bos Belg.* 55:1046.
- Jensen, R., L.A. Griner and O. R. Adams. 1956. Polioencephalomalacia of cattle and sheep. *J. Amer. Vet. Med. Assn.* 129:311.
- Kon, S.K. and J.W.G. Porter. 1954. The intestinal synthesis of vitamins in the ruminant. *Vitamins and Hormones.* 12:53.
- Leichter, J. and M.A. Joslyn. 1969. Kinetics of thiamin cleavage by sulphite. *Biochem. J.* 113:611.
- Lewis, G., S. Terlecki, L.M. Markson, R. Allcroft and J. E. Ford. 1966. Thiamin status of lambs in relation to cerebrocortical necrosis. *Proc. Nutr. Soc.* 26:xiii abs.
- Loew, F.M., R.H. Dunlop and R. G. Christian. 1970. Biochemical aspects of an outbreak of bovine polioencephalomalacia. *Can. Vet. J.* 11:57.
- Loew, F.M. and R. H. Dunlop. 1972. Induction of thiamin inadequacy and polioencephalomalacia in adult sheep with amprolium. *Am. J. Vet. Res.* 33:2195.
- Loew, F.M., O.M. Roberts and R.H. Dunlop. 1969. Developements in veterinary science. Polioencephalomalacia (Cerebrocortical necrosis). *Can. Vet. J.* 10:54.
- Lusby, K.S. and B.E. Brent. 1972. An experimental model for polioencephalomalacia. *J. Anim Sci.* 35:270.
- Markson, L.M., G. Lewis, S. Terlecki, E.E. Edwin and J.E. Ford. 1972. The aetiology of cerebrocortical necrosis: The effects of administering antimetabolites of thiamin to preruminant calves. *Br. Vet. J.* 10:54.

- Morita, P.P., T. Kanaya and T. Minesita. 1969. *J. Vitam.* 15:16.
- Pearson, W.N. 1968. in P. Gyorgy and W.N. Pearson (Eds.)
The Vitamins XIII. 2nd Ed. Academic Press Inc., New York.
- Pippon, E.L. and A.L. Potter. 1975. Quantitating the recovery of thiamin (vitamin B₁) from decalco in the thiochrome method for the determination of thiamin. *J. Agric. Fi. Chem.* 23:523.
- Porter, J.W.G. 1960. in *Digestive Physiology and Nutrition of the Ruminant*. D. Lewis, ed., Butterworths.
- Rerat, A.H., H. LeBars and J. Malle. 1958a. Utilization d'une method de perfusion pour la mise en evidence de perfusion pour la mise en evidence de l'absorption des vitamines chez le mouton normalement alimente. *Compte. Rend. Acad. Sci.* 246:1920.
- Rerat, A.J., J. Malle and H. Lebars. 1958b. Mis en evidence chez le mouton de la permeabilite du rumen aux vitamines B et conditions de leur absorption a ce niveau. *Comp. Rend. Acad. Sci.* 246:2051.
- Sapienza, D.A. and B. E. Brent. 1972. Rumen thiaminase and polioencephalomalacia. *J. Anim. Sci.* 35:1134.
- Terlecki, S. and L.M. Markson. 1961. Cerebrocortical necrosis in cattle and sheep. *Vet. Rec.* 73:23.
- Upton, P.K., J.L. L'Estrange and D.M. McAleese. 1972. Effects of high intakes of dietary sulphate on voluntary feed intake and metabolism of sheep. *Irish J. Agric. Res.* 11:145.
- Wagon, K.A. 1960. Agricultural gypsum as a regulator for self-feeding cottonseed meal to cattle on the range. *J. Range Manage.* 13:279.
- Wooley, D.W. 1952. *A Study of Antimetabolites*. John Wiley and Sons, Inc. New York.

APPENDIX A

Thiochrome Procedure

- A. Principle- Thiochrome procedured depend upon the oxidation of thiamin to thiochrome, which fluoresces in ultraviolet light. Under standard conditions and in the absence of other fluorescing substances, the fluorescence is proportional to the thiochrome present.
- B. Reagents- If possible, reagents and solutions should avoid contact with rubber or cork, as these materials may contribute fluorescing substances which will interfere with the assay.
- 1) Anhydrous Sodium Sulphate, Granular.
 - 2) 1% Mercuric Chloride solution.
 - 3) 2.5 M Sodium Acetate solution. Dissolve 205 gm anhydrous $\text{NaC}_2\text{H}_3\text{O}_2$ in water and dilute to 1 liter.
 - 4) Isobutyl alcohol.
 - 5) Enzyme Solution. Prepare fresh daily, using Taka-diastase (3 gm) and Papain (3 gm) in 100 ml distilled water. Test enzymes to make sure they are thiamin free.
 - 6) 25% Potassuim Chloride solution. Dissolve 250 gm KCl in water and dilute to 1 liter. This reagent is stable.
 - 7) Acid 25% Potassium Chloride solution. Dilute 8.5 ml concentrated HCl to 1 liter with 25% KCl solution. This reagent is stable.
 - 8) Activated Decalso. The entire activation procedure can be carried out in a Buchner funnel. Cover Decalso with hot 3% Acetic Acid and allow the acid to remain in contact with Decalso for 10-15 minutes. Apply mild vacuum to drain off fluid. Repeat acid wash.. Repeat wash using hot 25% KCl solution. Wash once more with hot Acetic Acid solution. After draining wash solutions, rinse several times with hot distilled water.

- 9) 3% Acetic Acid solution. Dilute 30 ml acetic acid to 1 liter with distilled water.

C. Procedure

I. Extraction and Dephosphorylation

- 1) Accurately measure 1 ml rumen fluid into a 25 ml Erlenmyer flask. Add 2.5 M Sodium Acetate to pH 4.5 to 5.0.
 - (a) This step assumes that rumen fluid has been acidified to ensure thiamin stability. If enzyme solution is added before pH is adjusted, acid in rumen fluid will denature enzymes.
- 2) Add 2 ml enzyme solution. Incubate at 45 to 50 degrees C for 2 hours, shaking constantly.
 - (a) If analysis can not be completed in one day, this is a convenient place to stop. Samples can be stored over-night in a refrigerator.

II. Purification

- 1) Add approximately 1.0 gram active Decalso to sample solution in Erlenmyer flask. Shake at 45 to 50 degrees C for 30 minutes and allow to sit for another 30 minutes.
- 2) Wash Decalso and sample solution from Erlenmyer flask into a sintered glass crucible. Use two 10 ml washes of hot distilled water. After each wash, draw filtrate through crucible by vacuum.
 - (a) Filtrate can be saved for further analyses for substances which will not adsorb onto Decalso.
- 3) Place sintered glass crucible into top of a Buchner funnel (43mm plate and 51 mm OD) using a rubber filter ring seal around bottom of filter. Using a one hole rubber stopper, insert stem of funnel into mouth of a 40 ml vacuum flask. Seal off side arm with rubber pipet bulb.

(This allows adding KCl solution to crucible without it flowing through the sinter.

- 4) Place 10 ml hot acid KCl on Decalso in sintered glass crucible, and allow KCl to sit on Decalso 5 minutes, then suck into flask by applying vacuum to side arm.

Replace rubber bulb and repeat KCl wash 3 times.

- (a) If apparatus is arranged properly, only a few drops of KCl solution will pass through filter before being stopped by pressure within vacuum flask.

- 5) After final KCl wash, draw off all liquid remaining in filter and funnel. Filtrate now contains thiamin.

- (a) Filtrate also contains pyrithiamin, which can be developed separate from thiamin if desired.
separate from thiamin if desired.

III.

III. Development of Thiochrome

- 1) Take a 5 ml aliquot of KCl + thiamin solution from vacuum flask and place in 50 ml test tube. Add 0.3 ml 1% HgCl_2 . Shake. Add 2 ml 30% NaOH. Shake. Add 7 ml isobutanol and shake 60 seconds.

- (a) More or less KCl + thiamin solution may be used in order to increase or decrease amount of thiochrome.

- 2) Read fluorescence in a spectrofluorometer (excitation = 370 nm, emission = 445 nm) and compare with standards containing thiamin levels within and above the range for the unknown, and treated identically to the unknown.
Blank is 95% ethanol (standards are in 95% ethanol) treated identically to standards.

THE EFFECTS OF GYPSUM ON RUMEN FERMENTATION
AND THIAMIN STATUS

by

TIMOTHY FRANK BROWN

B.S., Virginia Polytechnic Institute
and State University, 1976

-

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas
1979

Two trials were conducted to examine the effects of agricultural gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) in a concentrate mix on certain sheep rumen fermentation products. Trial I involved feeding gypsum as 0, 10, 15, 20, 25, and 30 per cent of the concentrate mix, allowing 21 days on each level before taking rumen fluid samples. Trial II involved starving animals for 48, 72, and 96 hours prior to allowing them access to concentrate containing 30 percent gypsum. Rumen pH, lactic acid, thiamin, and volatile fatty acids (total and molar ratios) were determined.

When fed on a daily basis, gypsum caused variable reduction in both concentrate and hay intake. Intake generally declined as percentage gypsum increased. Rumen pH, lactic acid, and thiamin were not affected by gypsum level. Rumen volatile fatty acids were affected only in absolute amount; ratios were not altered.

Animals receiving concentrate with 30 per cent gypsum following periods of starvation consumed approximately 700 grams of concentrate mix (200 grams gypsum). Rumen lactic acid reached high levels within 2 hours but returned to normal levels within 24 hours. Total volatile fatty acids increased on day 2 following feeding but returned to normal on day 3. Volatile fatty acid molar ratios were altered, but with no apparent pattern. Rumen thiamin was within normal range on day 1 but dropped to near zero on day 2 and day 3. No signs of thiamin deficiency appeared in either trial.

We had hypothesized that gypsum could cause polio-encephalomalacia (PEM) by destroying rumen thiamin. However, no signs of PEM developed. Reduced rumen thiamin in conjunction with lactic acidosis bears further investigation.