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THE UNIVERSITY OF ALBERTA

THIAMIN IN DIETS FOR FEEDLOT CATTLE

by

© GERALD A. GRIGAT

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

IN

ANIMAL NUTRITON

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ABSTRACT

In the first experiment 30 steers were fed an all-concentrate barley based diet supplemented with 1.9 mg thiamin/kg of diet or a similar unsupplemented diet. Average daily gains (1.14 versus 0.92 kg/day) and carcass weights (281 versus 265 kg) were higher (P≤0.05) for the thiamin supplemented group. Eleven samples from nine animals exhibited more than a 15% increase in the activity of the erythrocyte transketolase enzyme (E.C.2.2.1.1) due to the addition of thiamin pyrophosphate (TPP effect). A trend (P≤0.10) for a negative relationship was found between 2 week weight changes of the steers and the TPP effect. A positive relationship (P≤0.01) was observed between the TPP effect and plasma glucose concentration. Plasma urea, magnesium and calcium concentrations and the TPP effect were not influenced by dietary treatment, but plasma urea concentrations were low enough to suggest that a protein deficiency was present.

In further research, two experiments were conducted to determine whether additional benefit above that obtained by dietary thiamin supplementation would be obtained by a weekly injection of a vitamin B-complex containing thiamin, and if supplemental magnesium would aggravate a potential thiamin deficiency. The first experiment involved 96 steers which were fed an all-concentrate control diet or diets supplemented with 0.19% magnesium, 6.25 mg thiamin/kg of diet, or both nutrients. No significant differences relating

to dietary treatments were obtained for performance measurements, carcass data or blood measurements with the exception of plasma magnesium which reflected dietary magnesium levels. The numbers of bloats which occurred were 9, 14, 5 and 9 ($P \leq 0.05$) for the control, magnesium, magnesium plus thiamin and thiamin supplemented diets, respectively. The second experiment was conducted as a 3 x 3 factorial design with 72 steers to determine the effects of three levels of magnesium supplementation (0, 0.19, and 0.75% magnesium oxide) in diets with and without dietary thiamin or with dietary thiamin plus weekly vitamin B-complex injections. Neither feedlot performance nor incidence of bloat were significantly influenced by thiamin treatments. Average daily gains (1.64, 1.48 and 1.26 kg/day) decreased ($P \leq 0.05$) as dietary magnesium levels increased. Feed daily dry matter intakes were decreased by 5 and 12% and feed conversion efficiencies by 4 and 16% due to the inclusion of 0.19 and 0.75% magnesium oxide respectively. A trend ($P \leq 0.10$) was obtained in the incidence of bloat across magnesium levels, with the final number of bloats being one, six and eight for the control, low and high magnesium levels, respectively.

A survey involving a total of 645 samples, collected from 331 cattle from six sources, was conducted to determine the possible incidence of thiamin deficiencies in feedlot cattle in Alberta. The TPP effect was used as the index of thiamin status. It was found that 2.7% of the samples collected had a TPP effect in excess of 15%. A trend (r=0.824; $P \leq 0.10$) existed for a negative relationship between mean TPP effects and dietary crude protein concentrations. Weaning stress did not result in TPP effects above 15%.

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INTRODUCTION

Prior to the 1950's it was generally accepted that microbial synthesis would, under most circumstances, meet the mature ruminant animal's requirements for the B-vitamins. During the early 1950's, the feeding of high starch diets based on the use of feed grains, came into common practice as a means of finishing cattle faster and more profitably. However, since the ruminant evolved as a foraging animal normally consuming materials high in fiber content, many problems have been encountered with the use of large amounts of rapidly fermentable materials in the diet. It became clear that re-evaluation of the ruminant's requirement for thiamin and possibly the B-vitamins in general was necessary when it was discovered in 1966 that polioencephalomalacia (also known as cerebrocortical necrosis), a disease which is precipitated by lactic acidosis resulting from the feeding of rapidly fermentable materials, was responsive to thiamin administration.

Although research has been conducted to determine the etiology of polioencephalomalacia, comparatively little is known about it. It has been concluded that the most likely cause of the disease is the presence of a thiaminase enzyme (E.C.2.5.1.2) in the rumen. It is further believed that this enzyme performs its function by substitution of the thiazole moiety of thiamin with a picolinium-like compound such as nicotinamide, histamine or histamine-like compounds, etc. (a finding which also places the availability of nicotinamide in question when the thiaminase enzyme is present). The resulting compounds may be effective antimetabolites of thiamin and it has been suggested that the production of such antimetabolites is probably the reason for the rapid onset of polioencephalomalacia. It has also been found that the presence of the thiaminase enzyme does not necessarily result in clinical thiamin deficiencies, although this does not preclude the possibility of subclinical deficiencies.

Limited research has been conducted to evaluate the significance of subclinical thiamin deficiencies in cattle. Feedlot trials designed to assess the possible benefit of thiamin supplementation of the diet or to the animal directly are very limited in number and the results are variable. General assessments of the thiamin status of feedlot cattle fed commercial feedlot diets are, to the author's knowledge, nonexistent. Interactions of thiamin with other dietary components such as magnesium, which are known to occur in monogastrics, also have not been investigated in the ruminant.

Three years prior to the initiation of this research it was found by other workers that the percentage increase in the activity of the erythrocyte transketolase enzyme (E.C.2.2.1.1) due to the *in vitro* addition of thiamin pyrophosphate (TPP effect), a technique used in assessing the thiamin status of humans, was also an effective means of assessing the thiamin status of ruminants. Therefore, it was felt that sufficient information existed to accurately determine whether subclinical deficiencies do commonly occur in feedlot cattle as a result of feeding large proportions of rapidly fermentable materials in the diet, and to examine the influence of possible thiamin deficiencies on animal production. Related work was also conducted to determine if any benefit could be derived from providing the animal directly with B-vitamin complex injections during the feeding period and to assess the significance of a possible dietary magnesium x thiamin interaction.

I. THIAMIN SUPPLEMENTATION OF AN ALL-CONCENTRATE DIET FOR FEEDLOT STEERS

A. ABSTRACT

Thirty beef type steers were fed an all-concentrate barley based diet supplemented with 1.9 mg thiamin/kg of diet or a similar unsupplemented diet. Average daily gains (1.14 kg/day vs 0.92 kg/day) and carcass weights (281 kg vs 265 kg) were higher ($P \leq 0.05$) for the supplemented group. Supplemental thiamin had no significant effect on other measures of performance or other carcass traits. Eleven samples from nine animals exhibited more than a 15% increase in the activity of the erythrocyte transketolase enzyme (E.C.2.2.1.1) in response to added thiamin pyrophosphate (TPP effect) which suggested that a marginal thiamin deficiency existed in some of these steers. Supplemental thiamin did not significantly reduce the TPP effect. A trend ($P \leq 0.10$) for a negative linear relationship was found between 2 week weight gains of the steers and the TPP effect. A positive relationship ($P \leq 0.01$) was found between the TPP effect and glucose concentrations. Plasma urea concentrations were not related to treatment but were low enough (4.52 mg urea N/dL) to suggest that the animals were deficient in dietary protein. Plasma magnesium and calcium concentrations were unrelated to treatment or animal performance, but plasma magnesium concentrations did increase ($P \leq 0.001$) in the first half of the trial. The

results indicated that some animals were marginally deficient in thiamin and that decreased production in cattle fed all-concentrate diets is possible due to thiamin inadequacies.

B. INTRODUCTION

It is generally considered that sufficient amounts of B-vitamins are synthesized in the rumen to meet mature ruminant animal's demands (National Academy of Sciences 1976). However, acute deficiencies of thiamin have been found in ruminants (Loew 1975), and there are indications that subclinical deficiencies may occur (Linklater *et al.* 1977; Brent 1976).

Loew (1975) described six theoretical situations in which ruminants could become deficient in thiamin. These included inadequate net microbial synthesis of thiamin, impaired absorption or utilization of thiamin, presence of thiamin antimetabolites, lack of sufficient apoenzyme, increased metabolic demand for thiamin and increased rate of thiamin excretion.

Buziassy and Tribe (1960) reported that intra-ruminal synthesis of thiamin in sheep was only 2.3 mg/day which is consistent with other calculations of 1 to 3.5 mg/day (Hungate 1966; Barr *et al.* 1971; Breves *et al.* 1980). Naga *et al.* (1975) were able to demonstrate that mature sheep fed a purified diet containing urea developed typical thiamin deficiency symptoms such as general nervousness, repeated teeth gnashing, muscle tremors and gradually increasing polyuria as well as impaired rumen motility after about 7 months. Based on their own estimates of the requirement for thiamin by sheep of 2 to 4 mg/day they also suggested that, if estimates of rumen synthesis are correct, sheep must necessarily develop a thiamin deficiency unless the vitamin is contained in the diet.

Some rumen bacteria have a specific requirement for thiamin (Hungate 1966). Hoeller *et al.* (1977) found that *in vitro* addition of thiamin to rumen contents of sheep fed a urea based purified diet resulted in increases of up to 123% in microbial protein production but only slight and inconsistent increases in production of volatile fatty acids.

Jensen *et al.* (1956) first described the deficiency disease, polioencephalomalacia, and Davies *et al.* (1965) found the disease to be responsive to thiamin administration. Edwin *et al.* (1968) proposed that polioencephalomalacia was caused by a thiaminase enzyme (E.C.2.5.1.2) and later demonstrated this to be the most likely cause (Edwin and Jackman 1970). Mella *et al.* (1976) were able to initiate polioencephalomalacia in as little as four days using a feeding system based on molasses and urea. In a review, Brent (1976) stated that the development of lactic acidosis due to the feeding of rapidly fermentable materials is conducive to the initiation of polioencephalomalacia. Brent also noted that he has observed no cases of the disease in the absence of lactic acidosis.

In a review by Loew (1975), polioencephalomalacia was said to be characterized by 'elevations of blood lactate, pyruvate and glucose concentrations...and by lowered lactate:pyruvate ratios.' Quaghabeur *et al.* (1974) found the method of Brin (1966) for determining the response of the erythrocyte transketolase enzyme (E.C.2.2.1.1) to added thiamin pyrophosphate (TPP effect) to be an effective means of identifying the disease condition.

Few feedlot trials have been conducted to determine what influence supplemental thiamin may have on animal performance although Loew (1975) indicates that thiamin deficiencies can be a serious problem when high carbohydrate diets are fed. Brethour (1972) found average daily gains of steers increased from 1.14 kg/day to 1.26 kg/day ($P \leq 0.05$) due to the addition of 1 g thiamin and 100 g sodium bicarbonate to a wheat based ration. A second trial using the same diet failed to produce significant results.

The purpose of this experiment was to provide additional information on the possible benefits of the addition of thiamin to feedlot diets. Erythrocyte transketolase activities and the TPP effect were also utilized to determine the incidence and severity of thiamin deficiencies.

C. MATERIALS AND METHODS

Thirty beef type steers from a previous silage trial were utilized for purposes of this experiment which was conducted at the feedlot facilities of the Ellerslie Research Station. The steers were provided with a good quality alfalfa-grass hay and a 50:50 mixture of trace mineralized salt and calcium phosphate, given an intramuscular injection containing 1000000 I.U., 150000 I.U. and 100 I.U. of vitamins A, D and E respectively, and implanted with Zeranol (Ralgro; International Minerals and Chemical Corp. Terre Haute, IN.) prior to this trial. These animals had previously been vaccinated for blackleg and malignant edema. A barley concentrate (1.8 kg/head/day) was introduced to the diet 6 days pretrial to ensure that the animals would be accustomed to grain.

For the experiment the steers were ranked according to initial weight and the ranks divided into five groups of six animals. The six animals within each group were then randomly allocated to one of six pens (4.3 x 10.2 m).

The feeding program involved beginning the animals at 1.6 kg concentrate (Table I.1) per head/day. The concentrate portion of the diet was then increased at a rate of 0.3 kg/head/day and the hay portion decreased about 0.6 kg/head/day for the first 10 days on trial until an all-concentrate feeding regime was achieved.

Feed intakes were recorded daily on a pen basis. Animal weights were taken on three consecutive days at the

Table I.1 Diet composition and analyses

	Thiamin	Control	SEM[1]
Ingredients (% air dry basis)			
Barley, dry rolled	98	98	
Calcium carbonate	1.1	1.1	
Trace mineralized salt	0.275	0.275	
Thiamin premix[2]	0.625	-----	
Control premix[3]	-----	0.625	
Analyses (dry matter basis)			
Dry matter (%)	84.3	84.4	0.19
Crude protein (%)	12.0	12.2	0.10
Thiamin (mg/kg)			
Actual	4.81	3.05	0.05
Calculated	4.93		
Ash (%)	3.66	3.66	0.02
Calcium (%)	0.44	0.48	0.002
Phosphorus (%)	0.43	0.43	0.01
Magnesium (%)	0.16	0.15	0.002

[1] Standard error of the mean is based upon 1 analysis on each of 10 samples for dry matter, 4 analyses per sample for crude protein, 6 analyses per sample for thiamin, 2 analyses per sample for ash and magnesium and 3 analyses per sample for calcium and phosphorus.

[2] Thiamin premix to supply 1.875 mg/kg of thiamin as thiamin·HCl and 6000 I.U., 275 I.U. and 90 I.U./kg of diet of vitamins A, D and E respectively.

[3] Control premix to supply 6000 I.U., 275 I.U. and 90 I.U./kg of diet of vitamins A, D and E respectively.

beginning and end of the trial and once every 2 weeks during the trial. Water was withheld for 16 h prior to weighing.

The animals (mean weight 361 kg) were divided into two groups across diets for purposes of blood sampling. One group from each diet was then sampled on alternate weeks. Samples were not collected on weeks 11 and 12.

All animals were slaughtered one day after termination of the 98 day feedlot trial. After overnight chilling the carcasses were graded by plant officials in the normal manner under the Agriculture Canada Beef Carcass Appraisal Service (Agriculture Canada 1972). Dressing percentages were calculated from warm carcass weights and final weights in the feedlot.

Analytical procedures

Crude protein was determined using the Kjeldahl method for nitrogen determination (Association of Official Agricultural Chemists 1980). Calcium and magnesium determinations on blood plasma samples were conducted according to the method of Young and Booth (1967) using a Pye Unicam SP2900 atomic absorption spectrophotometer. Sample preparation for calcium and magnesium determinations in feedstuffs was conducted according to Association of Official Agricultural Chemists (1980) with subsequent treatment in the same manner as for blood samples. Phosphorus was measured using the meta-vanadate method (Association of Official Agricultural Chemists 1980). Thiamin was determined according to Association of Official

Agricultural Chemists (1980) for dry or semi-dry materials containing no appreciable amounts of basic substances.

Plasma glucose concentrations were measured using a glucose oxidase method (Worthington Biochemical Corp. Freehold, N.J.). Plasma urea was determined using the diacetyl-monoxamine method for the Technicon Autoanalyzer II (Method No. 4001FD4, Technicon Instruments Corp. Tarrytown, N.Y.). Blood L-lactate and pyruvate concentrations were measured using lactate dehydrogenase methods (Sigma Technical Bulletin No. 826-UV and No. 726-UV respectively, Sigma Chemical Co. St Louis, MO.). Assessments of erythrocyte transketolase activity (E.C. 2.2.1.1) and TPP effect were conducted by the procedure of Brin (1970). Activity of the transketolase enzyme was expressed as mg ribose metabolized/g hemoglobin in sample/h to allow for more accurate comparisons of enzyme activity between samples. Hemoglobin was determined using the procedure outlined in Sigma Technical Bulletin No. 525 (Sigma Chemical Co. St Louis, MO.).

Statistical analyses

A *t* test for unbalanced data was applied to carcass characteristics, feed intake, weight gain and feed conversion efficiency data according to the method of Steel and Torrie (1980) to examine differences between treatments.

Blood data was tested for homogenity between sampling groups across weeks. The data was subsequently subjected to repeated measures analysis using the method of Grizzle and

Allen (1969).

Covariate anaylsis, according to Steel and Torrie (1980) was utilized to determine if significant relationships existed between animal gains and blood parameters. This analysis was also used to determine if significant relationships existed between selected blood parameters.

D. RESULTS

Feedlot performance and carcass characteristics

During the first week of the experiment two animals on the thiamin diet exhibited symptoms characteristic of lactic acidosis. These animals were removed from the trial and subsequently recovered. No other obviously sick animals were observed during the course of the trial with the exception of two bloated animals on the control diet.

Thiamin supplemented animals had a higher ($P \leq 0.05$) average daily gain than unsupplemented animals (Table I.2). Initial separation in the animal weights occurred by week 4 (Fig. I.1) and continued throughout the course of the experiment. Final differences in average daily gains (0.22 kg/day) therefore seem to be attributable to a general improvement in performance following the 10 day introduction period onto the all-concentrate feeding regime. The improvement in performance of the thiamin fed animals was also reflected in heavier ($P \leq 0.05$) carcass weights (Table I.2). No other differences between treatments were noted for

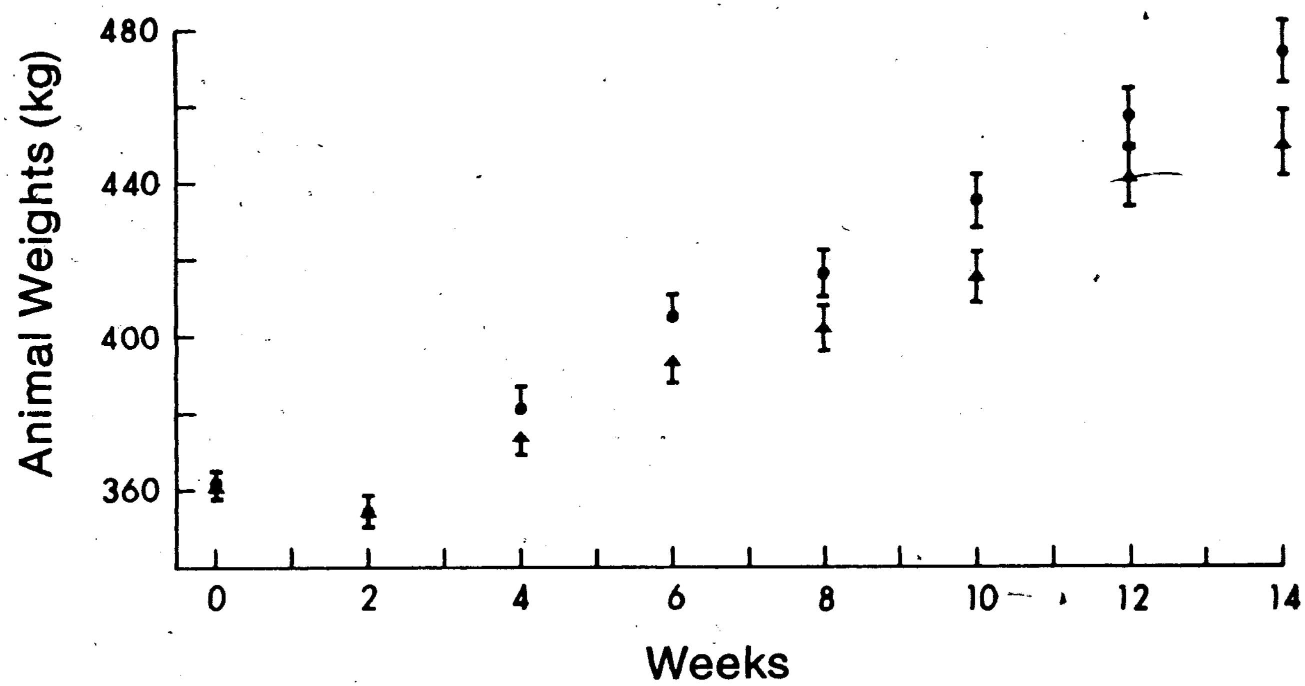

Figure I.1 Mean weights of steers during the feeding trial (● thiamin; ▲ control). Vertical bars are standard errors.

Table II.2 Feedlot performance and carcass characteristics

Item	Thiamin	Control	SEM[1]
Number of animals	13	15	
Days on trial	98	98	
Avg initial wt (kg)	362	360	3.40
Avg final wt (kg)	474	450	8.40
Avg daily gain (kg)	1.14	0.92	0.073 *
DM[2] consumed(kg/day)	7.03	6.57	0.293
Feed DM[2]/gain	6.32	7.20	0.598
Carcass wt (kg)	281	265	5.23 *
Dressing percentage	59.4	58.8	0.314
Rib eye area (cm[2])	69.1	66.8	1.89
Avg fat cover (cm)	1.28	1.24	0.071

[1] Standard error of the mean.

[2] DM=dry matter.

* Significant at ($P \leq 0.05$).

carcass characteristics, feed intakes, or feed conversion efficiencies.

Blood parameters

Although not significantly different, mean values for the TPP effect in this trial for steers fed the thiamin and control diet were 7.70 and 8.62% respectively (Table I.3). Higher ($P \leq 0.05$) values occurred during the first, ninth and tenth weeks than during other times (Fig. I.2). Individual TPP effects ranged from 1.96 to 25% with eleven samples (three from the thiamin supplemented diet and eight from the control diet) from nine animals having values greater than 15% at some point in the trial (Table I.3). Of the two animals on the thiamin diet removed due to severe lactic acidosis, neither exhibited TPP effects above 10%. The highest TPP effect (25%) was noted during the second week for a steer fed the control diet. The TPP effect in this steer decreased from 25 to 15.8 and 5.5% during weeks 2, 4 and 6 respectively, which demonstrates the transitory nature of elevated TPP effects observed in this experiment. Although no visible symptoms of polioencephalomalacia were apparent, the concentrations of plasma glucose (161 mg/dL) and blood pyruvate (2.7 mg/dL) for this animal during the second week were high enough to be considered abnormal and substantiate the occurrence of a thiamin deficiency. None of the other animals with TPP effects in the 15-20% range or the 20-25% range demonstrated plasma glucose or blood pyruvate concentrations high enough to substantiate a

Table I.3 Percentage of samples in each range of TPP effect[1] categorized by treatment and week

Parameter	Total number of samples	TPP Effect[1] (%) 0-5	5-10	10-15	15-20	20-25	Mean	SEM[2]
				Treatment				
Diet								
Thiamin	78	23.1	56.4	16.7	3.8	0.0	7.70	0.42
Control	90	16.7	55.6	18.9	5.6	3.3	8.62	0.39
				Time				
Week								
1	16	25.0	62.5	12.5	0.0	0.0	8.87	0.83
2	12	8.3	66.7	0.0	8.3	16.7	7.76	0.52
3	16	12.5	75.0	12.5	0.0	0.0	7.20	0.48
4	12	8.3	50.0	33.3	8.3	0.0	7.09	0.50
5	16	56.3	43.8	0.0	0.0	0.0	7.31	0.49
6	12	58.3	41.7	0.0	0.0	0.0	7.75	0.44
7	16	6.3	37.5	25.0	31.3	0.0	8.33	0.42
8	12	0.0	58.3	41.7	0.0	0.0	8.92	0.46
9	16	18.8	43.8	31.3	0.0	6.3	9.42	0.54
10	12	0.0	66.7	33.3	0.0	0.0	9.73	0.61
13	16	0.0	66.8	25.0	6.3	0.0	8.44	0.61
14	12	41.7	58.3	0.0	0.0	0.0	6.92	0.89
Mean		19.6	56.0	17.9	4.8	1.8		
Average two-week weight gains of steers (kg)[3]		15.4	12.9	13.2	3.1	7.3		

[1] Percentage increase in activity of the erythrocyte transketolase enzyme due to the addition of thiamin pyrophosphate.
[2] Standard error of the mean.
[3] The weight change over the two week period in which each blood sample was collected.

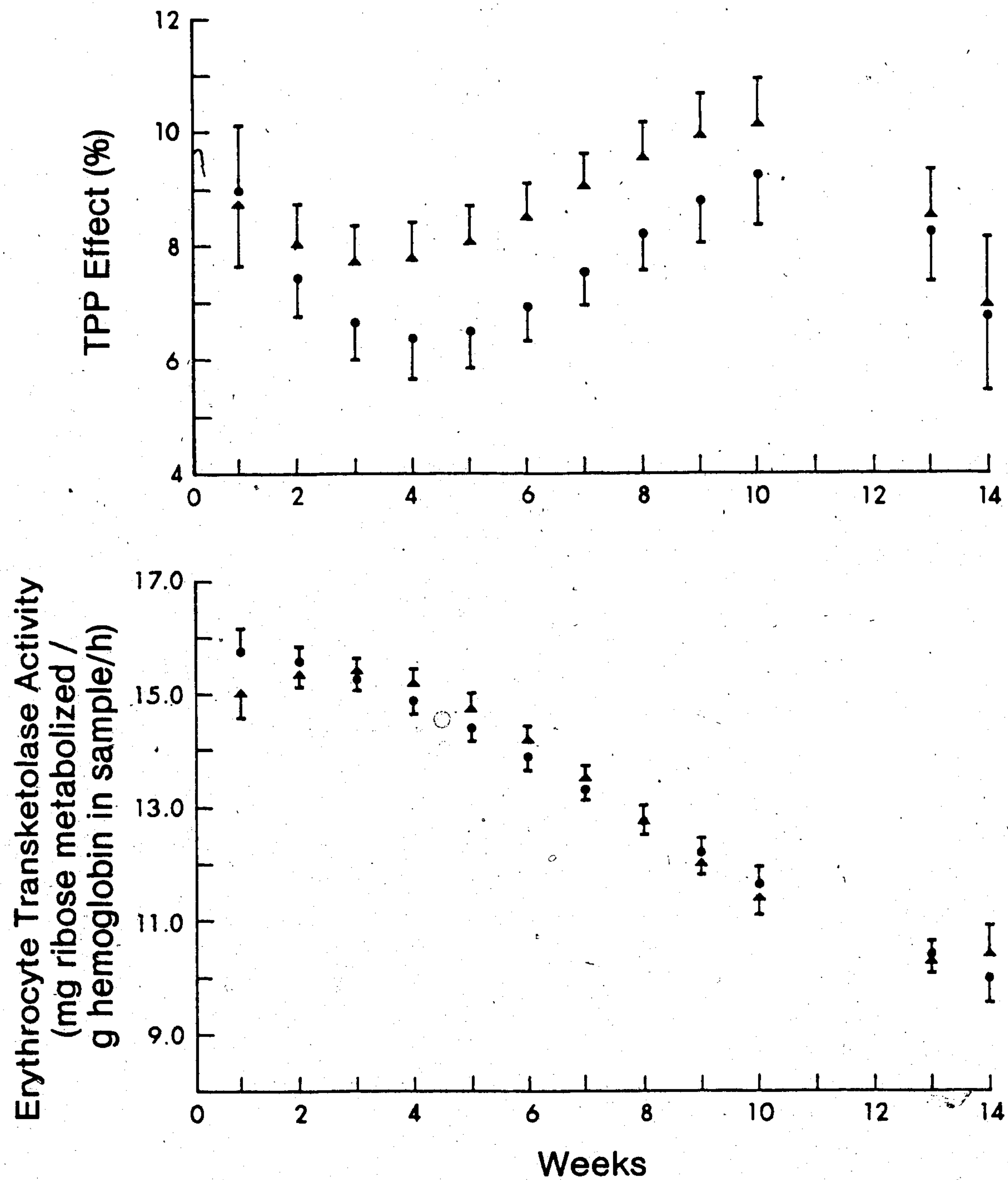

Figure I.2 Mean TPP effects and mean erythrocyte transketolase activities without added thiamin pyrophosphate throughout the trial (● thiamin; ▲ control). Vertical bars are standard errors.

thiamin deficiency.

A trend ($P \leq 0.10$) for an inverse relationship occurred between the TPP effect and two-week weight changes (Table I.4).

Erythrocyte transketolase activity was expressed on the basis of mg ribose metabolized/g hemoglobin in sample/h. This method of expression was considered to be superior to that of expressing activity on the basis of ug ribose metabolized/mL hemolyzed red blood cells (Brin 1966) because the latter procedure for sample preparation was often unrepeatable. Mean erythrocyte transketolase activities ranged from 8.75 to 19.04 mg ribose metabolized/g hemoglobin in sample/h (Fig. I.2). On the basis of ug ribose metabolized/mL hemolyzed red blood cell/h, activities ranged between 1375 and 1880, which is comparable to the range of 838 to 1784 reported by Quaghebeur *et al.* (1974).

No differences existed between treatments nor was there a treatment by week interaction for erthyrocyte transketolase activities (Fig. I.2). A time effect ($P \leq 0.001$) irrespective of treatments did exist with transketolase activities being 15.38 mg ribose metabolized/g hemoglobin in sample/h during the first week of sampling and decreasing to 10.25 mg ribose metabolized/g hemoglobin in sample/h by the final week of sampling. A highly significant ($P \leq 0.001$) negative relationship also existed between base transketolase activities and the TPP effect (Table I.4) which is consistent with the findings of Brin (1970).

Table I.4 Covariate regression coefficients for two-week weight changes of steers, TPP effect[1] and plasma glucose on selected blood parameters

Covariates	Two week weight changes (kg)	TPP Effect[1] (%)	Plasma glucose (mg/dL)
Plasma urea (mg/dL)	-0.725 **		
TPP effect (%)	-0.565 *		
Transketolase activity (mg ribose metabolized/g hemoglobin/h)[2]	-0.696	-1.072 ****	
Plasma glucose (mg/dL)	-0.206 **	0.066 ***	
Plasma Mg (mg/dL)	0.958	-0.189	
Blood lactate/pyruvate	-0.034	0.021	0.142 **
Blood pyruvate (mg/dL)	0.855	0.431	10.42 ***

[1] Percentage increase in activity of the erythrocyte transketolase enzyme due to the addition of thiamin pyrophosphate.

[2] Erythrocyte transketolase activity measured without added thiamin pyrophosphate.

* $P \leq 0.10$; ** $P \leq 0.05$; *** $P \leq 0.01$; **** $P \leq 0.001$.

Plasma urea, glucose, magnesium, calcium and blood pyruvate concentrations, and the lactate to pyruvate ratios (Fig. I.3 and I.4) were not significantly influenced by dietary treatment or the treatment by week interaction. A week effect ($P \leq 0.001$) existed irrespective of treatments for blood pyruvate and the L-lactate to pyruvate ratios and plasma magnesium concentrations (Fig. I.3 and I.4). The mean plasma magnesium concentrations (Fig. I.4) increased ($P \leq 0.05$) from 1.79 mg/dL to 2.09 mg/dL by week 4 and remained relatively constant thereafter. No other significant time effects were noted for other blood constituents.

Plasma glucose demonstrated a highly significant ($P \leq 0.001$) positive relationship to the TPP effect (Table I.4). Negative relationships ($P \leq 0.05$) also existed for plasma urea and glucose with two-week weight changes (Table I.4).

E. DISCUSSION

Increases in average daily gain and carcass weight of steers were obtained by supplementation of an all-concentrate diet with thiamin under the conditions of this experiment (Table I.2). Brethour (1977) also found a significant increase in average daily gain of steers when a very high level of thiamin in combination with sodium bicarbonate was included in a diet composed of hard red winter wheat. It is not clear which of the ingredients in

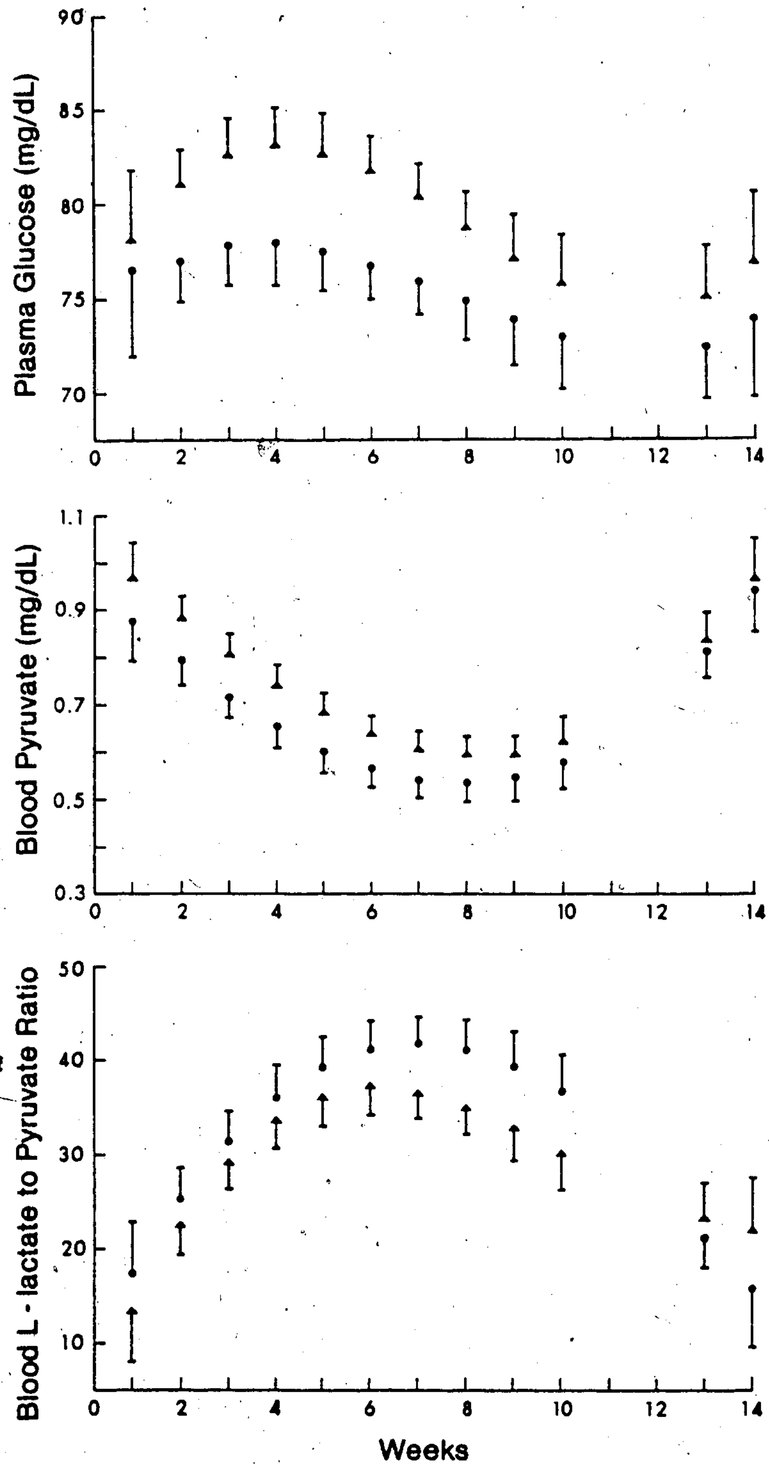

Figure I.3 Mean plasma glucose, blood pyruvate concentrations and L-lactate to pyruvate ratios throughout the trial (● thiamin; ▲ control). Vertical bars are standard errors.

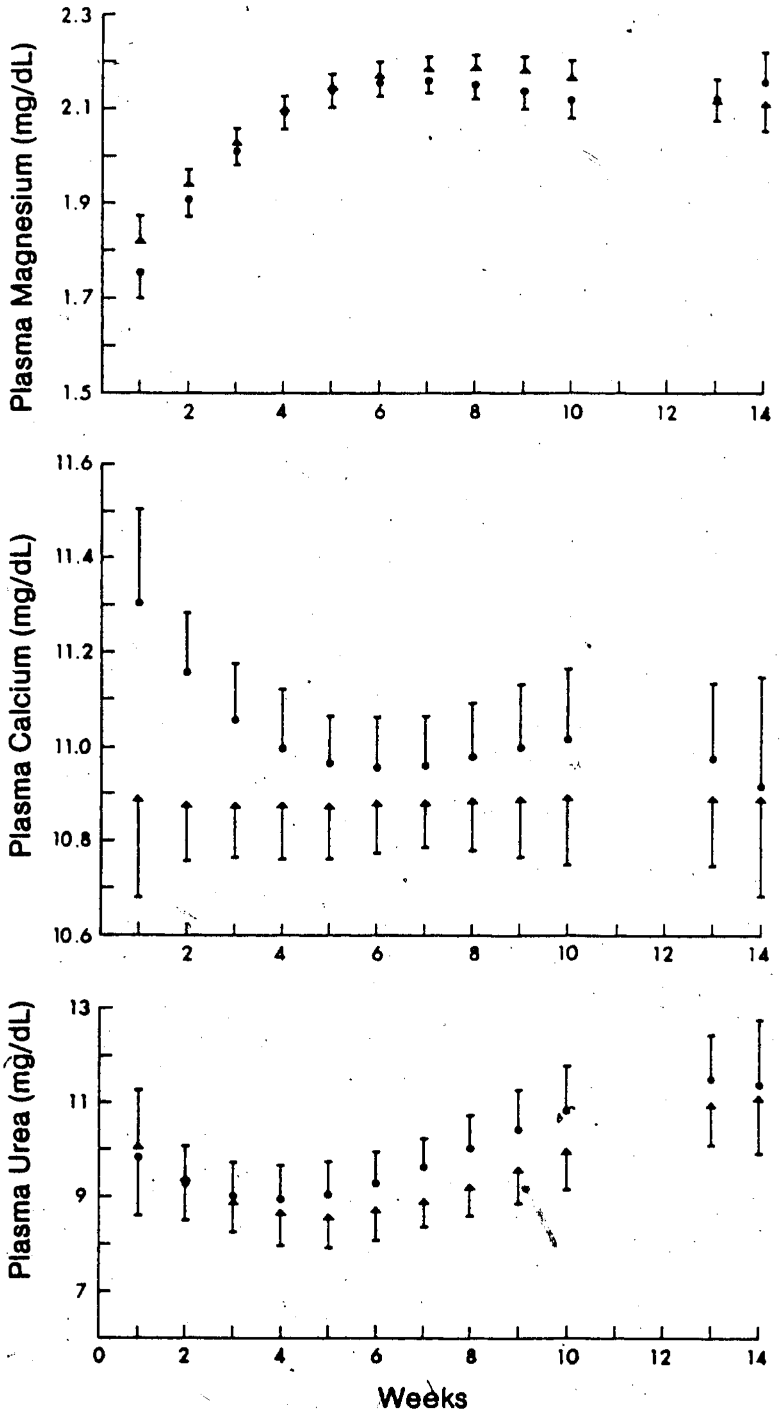

Figure I.4 Mean plasma magnesium, calcium and urea concentrations throughout the trial (● thiamin; ▲ control). Vertical bars are standard errors.

this combination was of value in improving animal performance.

Measurement of the TPP effect is accepted as the best method of determining the thiamin status of animals (Quaghebeur *et al.* 1974). Brin (1970) indicates that in humans TPP effects from 15 to 25 indicate a marginal deficiency and values of greater than 25% a severe deficiency. It is not clear whether these classifications may be applied to cattle although the results of Quaghebeur *et al.* (1974) suggest that a clinical deficiency may occur at values above the 25% level. In this trial the elevated plasma glucose (161 mg/dL) and blood pyruvate (2.7 mg/dL) levels found in the one animal with a TPP effect of 25% provides supporting evidence that thiamin deficiencies do occur when the TPP effect is in the range of 25% and that deficiencies of thiamin can exist without apparent symptoms being present.

Although 2 week weight changes are greatly influenced by gut fill and thus may not be closely related to animal health, a negative trend ($P \leq 0.10$; Table I.4) was observed between TPP effect and animal weight changes. In particular, there were indications that animals with TPP effects greater than 15% had lower 2 week weight gains than animals with TPP effects less than 15% (Table I.3). This provides further evidence that the relationships between TPP effects and health in humans, as outlined by Brin (1966), are similar to the relationships which might be expected with cattle.

The foregoing discussion indicates that measurement of the TPP effect is a suitable index of the thiamin status of cattle. When results of different experiments are compared, however, consideration must be given to the method by which the TPP effect is measured since literature values for TPP effects can be found to vary from 0 to 431% for cattle (Quaghebeur *et al.* 1974; Edwin *et al.* 1979; Loew *et al.* 1975). Quaghebeur *et al.* (1974) using the method of Brin (1966), which is based upon the rate of disappearance of ribose-5-phosphate or the appearance of glucose-6-phosphate, found that the TPP effect in apparently normal animals varied from 6.1 to 38%, and in polioencephalomalacia suspected animals the range was 9.1 to 59.5%. Edwin *et al.* (1979), using a modified method of Massod *et al.* (1971), which is based on the appearance of sedoheptulose-7-phosphate, found TPP effects of 2 to 114% (95% confidence interval) for apparently normal animals and TPP effects of 120 to 247% (95% confidence interval) for animals suspected of having polioencephalomalacia.

Supplementation of the diet with thiamin had no statistically significant effect in terms of lowering the TPP effects in this experiment in spite of the fact that dietary thiamin did have a positive influence on animal performance (Table I.2) and the magnitude of the TPP effect observed suggested that some animals were receiving inadequate dietary thiamin. The lack of a statistically significant influence of dietary thiamin on the TPP effect could have been because added dietary thiamin was broken down by thiaminase enzymes and thus did not reach the animals system. Brent (1976) suggested that high concentrate diets, and ensuing lactic acidosis which can occur when these diets are fed, is conducive to the development of the thiaminase enzyme and polioencephalomalacia. Siegmund (1979) suggested that supplemental dietary thiamin will be of little value in preventing polioencephalomalacia although Blood *et al.* (1979) recommended 5 mg thiamin/kg of diet for this purpose. Linklater *et al.* (1977) reported that in a study in which one or more sheep in a flock had succumbed to polioencephalomalacia, up to a third of the apparently normal sheep in the same flock were found to be excreting thiaminase on any one day, and that up to half the flock could be excreting thiaminase during an outbreak of polioencephalomalacia. Since Roberts and Boyd (1974) observed that fecal thiaminase activity reflected ruminal thiaminase activity, these results would suggest that it is quite possible for ruminal thiaminase to be present without any overt symptoms of polioencephalomalacia in the herd.

It is possible that animal performance was improved because the added thiamin influenced rumen fermentation which in turn influenced factors such as the supply of protein to the animal (Hoeller *et al.* 1977). In this regard, since all mean plasma concentrations were less than 11.5 mg urea/dL (5.4 mg urea N/dL) (Fig. I.4), there is reason to believe that the animals were deficient in dietary protein

(National Academy of Sciences 1978) even though the 12% crude protein level in the diet should have been adequate for steers of this size (National Academy of Sciences 1976).

The suggestion by Boyd and Walton (1977) that a thiamin deficiency might contribute to lactic acidosis seems inconsistent with the fact that the two animals removed from the experiment due to severe lactic acidosis were fed the thiamin supplemented diet. Also, these steers failed to exhibit TPP effects above 10% while on trial. Brent (1976) and Sapienza and Brent (1974) suggested, however, that lactic acidosis may precipitate the development of polioencephalomalacia rather than the converse.

It is not possible to provide a complete explanation as to why the TPP effect varied with time (Fig. I.2), although the observations of Brent (1976) and Sapienza and Brent (1974) that thiamin deficiencies in cattle are linked to the occurrence of lactic acidosis could explain the higher TPP effects during the initiation of animals onto the all-concentrate diet (weeks 1 and 2). It is not obvious why elevated TPP effects occurred again during weeks 9 and 10.

Samples collected for determinations of blood glucose, pyruvate and L-lactate were obtained under stress conditions (ie. in a cattle squeeze) and thus caution must be used when drawing conclusions from these data. However, if used in context of substantiating a possible thiamin deficiency, in which unusually high plasma glucose and blood pyruvate concentrations and lowered L-lactate to pyruvate ratios are known to occur (Mella *et al.* 1974), these measurements may remain useful. The positive relationship ($P \le 0.001$; Table I.4) between plasma glucose concentrations and the TPP effect strongly indicated interference of glucose metabolism in some steers in this trial due to a thiamin deficiency. Blood pyruvate and the L-lactate to pyruvate ratio (Table I.4) failed to show a significant relationship to the TPP effect. The results of Quaghebeur *et al.* (1974) however, indicate that a relatively poor relationship exists between blood pyruvate and the TPP effect suggesting in turn that the measurement of blood pyruvate may be most useful only in confirming extreme cases of thiamin deficiency in cattle. The same qualification would then necessarily apply to the use of the L-lactate to pyruvate ratio.

Plasma magnesium concentrations were not significantly related to treatment (Fig. I.4), the TPP effect or 2 week weight changes (Table I.4). Yano and Kawishima (1977) observed that sheep fed a high concentrate diet, composed primarily of 90% flaked corn and 10% soybean meal, went into a negative magnesium balance when injected with 50 mg thiamin tetrahydrofurfuryl disulfide/head/day for 14 days but remained in a positive calcium balance. They observed no significant changes in plasma magnesium or calcium concentrations. Unfortunately magnesium balance was not measured in the current experiment and therefore these results cannot be confirmed by this work. The initial increase in plasma magnesium concentrations observed during the first 4 weeks of this experiment (Fig. I.4) could be due to an increased dietary intake of magnesium (Rowlands 1980) or to a reduction in forestomach pH which in turn resulted in increased availability of magnesium to the animal (Madsen *et al.* 1976).

The results of this trial indicated a negative relationship ($P \leq 0.05$) existed between plasma urea and 2 week weight changes (Table I.4). In a review by Rowland (1980) it is reported that a positive relationship exists between protein intake and plasma urea concentrations, but a negative relationship exists between energy intake and plasma urea concentrations. Therefore, as energy intake (ie. feed intake) decreases, weight gains also decrease and plasma urea concentrations may be expected to increase.

Although the results of this experiment indicated that thiamin supplementation of an all-concentrate diet did not significantly lower the TPP effect, a definite negative trend ($P \leq 0.10$) existed between the TPP effect and animal 2 week weight changes. This and the performance response obtained in the feedlot provide ample evidence that, in this trial, a lack of available thiamin resulted in a loss in animal production. It was suggested that supplemental thiamin may have influenced the amount of microbial protein synthesized in the rumen and thus animal performance indirectly since erythrocyte TPP effects were not significantly influenced by thiamin supplementation.

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II. THIAMIN AND MAGNESIUM SUPPLEMENTATION OF ALL-CONCENTRATE DIETS

A. ABSTRACT

Two experiments were conducted to determine whether; 1) the benefit of thiamin supplementation of feedlot diets as previously obtained could be substantiated, 2) additional benefit could be derived from an injection of thiamin with other B-vitamins, and 3) supplemental magnesium would aggravate a potential thiamin deficiency. In the first experiment 96 steers were fed one of four all-concentrate barley based diets: a control diet with no thiamin or magnesium supplementation, a magnesium diet supplemented with 0.19% magnesium oxide, a thiamin diet supplemented with 6.25 mg thiamin/kg diet and a magnesium-thiamin diet with both nutrients added. With the exception of plasma magnesium concentrations, which reflected ($P \leq 0.05$) feed magnesium concentrations, no significant treatment differences were obtained for rate of gain, feed conversion efficiencies or other performance or blood parameters measured in this experiment. The incidence, but not the severity, of bloat was significantly affected by diet with steers fed the magnesium diet beginning to bloat the earliest and having the highest incidence of bloat, steers fed the thiamin supplemented diet starting the latest and having the lowest incidence of bloat, and those fed the control and magnesium-thiamin diet intermediate in starting date and

incidence of bloat. A second 3 x 3 factorial experiment with 72 steers was conducted to examine the effects of three levels of magnesium supplementation (0, 0.19 and 0.75% magnesium oxide) in diets without dietary thiamin, with dietary thiamin, and with dietary thiamin plus a B-vitamin complex injection. Neither feedlot performance nor incidence of bloat was significantly influenced by thiamin treatment. Average daily gains (1.64, 1.48 and 1.26 kg/day) of the animals decreased ($P \leq 0.05$) as dietary magnesium levels increased. Feed daily dry matter intakes were decreased by 5 and 12% and feed efficiences by 4 and 16% with the low and high level of magnesium supplementation respectively. A trend ($P \leq 0.10$) was obtained across magnesium levels in the incidence of bloat with cumulative number of bloats during the experiment being one, six and eight for the control, low and high magnesium diets. The variable feed intake due to the low palatability of the magnesium oxide was felt to be the most likely reason for the depressed feedlot performance and the increased incidence of bloats in the second experiment, but did not adequately explain differences observed in the incidence of bloat in the first. It was concluded that supplemental thiamin may not be of value in improving the growth rate or feed conversions in cattle fed all-concentrate diets containing 13-16% crude protein, and that steers were adversely affected by supplemental magnesium. No evidence of a thiamin-magnesium interaction on growth rate or feed conversion was observed, but thiamin did lower the incidence of bloat in experiment 1 thereby offsetting the effects of magnesium.

B. INTRODUCTION

This author has previously found an improvement in average daily gains (0.92 versus 1.14 kg/day) and carcass weights (265 versus 281 kg) due to the addition of 1.9 mg thiamin/kg of a barley based diet (Chapter I). A negative trend also existed between the percentage increase in the activity of the erythrocyte transketolase (E.C.2.2.1.1) enzyme due to the addition of thiamin pyrophosphate (TPP effect) and animal gains, suggesting that animal weight gains may have been further improved by increasing the level of thiamin supplementation in the diet or by supplying the animal tissues directly with thiamin. Brethour (1972) found that average daily gains of steers increased from 1.14 to 1.26 kg/day due to the addition of 1 g thiamin and 100 g sodium bicarbonate to a wheat based ration, although it is not clear which of these ingredients was responsible for this increase. The second trial by these workers failed to produce a significant response in animal performance. In a review, Brent (1976) indicates that the development of lactic acidosis due to the feeding of rapidly fermentable materials is conducive to the initiation of polioencephalomalacia. Evidence therefore exists to question the thiamin adequacy of cattle on high concentrate diets.

Jensen *et al.* (1956) first described the deficiency disease, polioencephalomalacia, and Davies *et al.* (1965) found it to be responsive to thiamin administration. Edwin (1970) concluded that the most probable cause of the disease was the presence in the rumen of a thiaminase enzyme (E.C.2.5.1.2). The endproducts of the reaction catalyzed by the thiaminase enzyme were found to be the result of a substitution of the thiazole moeity in the thiamin molecule with a picolinium-like compound. It was also suggested that some of these endproducts may be metabolically competitive thiamin analogues. Fujita *et al.* (1954) found that histamine-like compounds acted as potent cosubstrates for the thiaminase enzyme. Roberts and Boyd (1974) found that nicotinic acid and many of its derivatives were also effective cosubstrates. It was also suggested by these workers that because of this, nicotinic acid deficiencies must also be suspected when a thiamin deficiency is present.

There is evidence which would indicate that a relationship exists between thiamin and dietary magnesium. Fujiwara and Kawishima (1976) demonstrated that in rats excess thiamin increased magnesium excretion and vice versa. Yano and Kawishima (1977) observed that sheep fed a high concentrate diet composed of 90% flaked corn and 10% soybean meal went into a negative magnesium balance when injected with 50 mg thiamin tetrahydrofurfuryl disulfide/day for 14 days.

The purposes of the following experiments were threefold. First it was desired to further substantiate, if possible, the beneficial effects of thiamin addition to all-concentrate diets. A higher level of dietary thiamin supplementation than was used in Chapter I since there were indications that additional supplementation may have been of value. The second objective was to determine if additional benefit could be derived from injecting thiamin and other B-vitamins directly into the animal. This route of administration has been demonstrated to be effective in the treatment of polioencephalomalacia (Edwin and Lewis 1971), it eliminated the possibility of the destruction of thiamin in the rumen by the thiaminase enzyme and also provided the animal with other B-vitamins such as nicotinamide. The final objective was to examine the possibility that supplementary dietary magnesium may influence the thiamin status of steers fed an all-concentrate diet.

C. MATERIALS AND METHODS

Experiment 1

Ninety-six beef type steers were purchased from a local livestock market for the purpose of this trial which was conducted at the feedlot facilities of the Ellerslie Research Station. Upon arrival the animals were given an intramuscular injection of 1000000 I.U., 150000 I.U. and 100 I.U. of vitamins A, D and E respectively, vaccinated for blackleg and malignant edema and implanted with Zeranol

(Ralgro; International Minerals and Chemical Corp.). The steers were also provided with a good quality alfalfa grass hay, trace mineralized salt and a 50:50 mixture of trace mineralized salt and calcium phosphate. Sixteen days pretrial, a barley-oat concentrate was introduced to the diet at a level of approximately 1.0 kg/head/day to ensure the animals were familiar with grain. The concentrate feeding rate was maintained at this level until initiation of the experiment.

The animals were ranked according to initial weights and the ranks divided into three groups of 32 animals. The 32 animals within each group were then randomly allocated to one of 32 pens.

Four treatments were used for this experiment including a control diet, a magnesium diet supplemented with 0.19% magnesium oxide, a thiamin diet supplemented with 6.25 mg thiamin/kg of diet, and a magnesium-thiamin diet with both nutrients added (Table II.1). The feeding program involved beginning the animals on a ration consisting of 3.3 kg/head/day of concentrate with a chopped alfalfa grass hay being offered at a rate of 6.7 kg/head/day. The concentrate was increased at a rate of 0.33 kg/head/day and the hay offering decreased at a rate of 0.67 kg/head/day for a period of 10 days to achieve an *ad libitum* all-concentrate feeding regime.

Feed intakes were recorded daily on a pen basis. Animal weights were measured on three consecutive days at the

Table II.1 Feed ingredients and analyses (experiment 1)

	Control	Magnesium	Thiamin	Magnesium and Thiamin	SEM[1]
Ingredients (% air dry basis)					
Barley, dry rolled	95	94.8	94.4	94.2	
Limestone (38% Ca)	1.0	1.0	1.0	1.0	
Trace mineralized salt	0.225	0.225	0.225	0.225	
Molasses (wet)	2.5	2.5	2.5	2.5	
Urea (45% N)	1.0	1.0	1.0	1.0	
Calcium sulfate (16% S)	0.05	0.05	0.05	0.05	
Vitamin premix[2]	0.225	0.225	0.225	0.225	
Magnesium oxide (54% Mg)	----	0.188	----	0.188	
Thiamin premix[3]	----	----	0.625	0.625	
Analyses (dry matter basis)					
Dry matter (%)	85.8	85.5	85.6	85.7	0.27
Crude protein (%)	16.6 *b*	15.7 *a*	16.3 *ab*	15.7 *a*	0.09
Thiamin (mg/kg)					
Actual	2.99 *a*	3.23 *b*	8.13 *d*	4.19 *c*	0.056
Calculated	----	----	10.06	10.06	
Ash (%)	3.83 *a*	3.94 *a*	3.79 *a*	4.23 *b*	0.057
Calcium (%)	0.54 *a*	0.54 *a*	0.58 *b*	0.65 *c*	0.002
Phosphorus (%)	0.34	0.34	0.33	0.35	0.006
Magnesium (%)	0.14 *a*	0.26 *b*	0.15 *a*	0.30 *c*	0.004
pH	6.5	8.1	6.6	7.9	

[1] Standard error of the mean is based upon 1 analysis on each of 7 samples for dry matter, 4 analyses per sample for crude protein, 6 analyses per sample for thiamin, 2 analyses per sample for ash and magnesium and 3 analyses per sample for calcium and phosphorus.
[2] To provide 5625 I.U., 928 I.U. and 22.5 I.U. per kg of diet of vitamins A, D, and E respectively.
[3] To provide 6.25 mg thiamin as thiamin HCl per kg of diet.
a-d Means in same row followed by different letters differ ($P \leq 0.05$).

beginning and end of the trial and once every 2 weeks during the trial. Water was withheld for 16 h prior to weighing.

A five point subjective bloat score was established to record the incidence and relative severity of bloat. A score of 1 was given for a mild bloat with only a slight distension of the left side. A score of 2 indicated mild bloat with obvious distention of the left side. A score of 3 indicated severe bloat with extreme distention of the left flank and signs of discomfort. A score of 4 was given animals with a severe bloat and obvious signs of distress. A score of 5 was applied to animals which died from bloat. Bloating animals were treated by insertion of a stomach tube to release contained gases and for the administration of 2-3 L mineral oil. Treated animals were returned to experimental pens.

Four pens from each of the four treatment groups were randomly selected for purposes of blood sampling. Samples were collected 3 days pretrial and at days 10, 24, 52 and 90 during the trial. Blood samples were also collected from all bloating animals.

All animals were slaughtered four days after termination of the feeding trial. After overnight chilling the carcasses were graded by plant officials in the normal manner under the Agriculture Canada Beef Carcass Appraisal Service (Agriculture Canada 1972). At slaughter, hearts, kidneys and livers were inspected for gross abnormalities and the frequency of abcessed livers was recorded. Dressing percentages were calculated from warm carcass weights and final weights in the feedlot.

Experiment 2

Seventy-two beef type steers obtained from commercial sources were utilized for purposes of this trial also conducted at the feedlot facilities of the Ellerslie Research Station. Upon arrival the animals were treated in the same fashion as for experiment 1. Ten days pretrial a barley concentrate was introduced to the diet at a level of approximately 1.0 kg/head/day to ensure that the animals were accustomed to grain.

The animals were ranked according to initial weight and the ranks divided into two groups of 36 animals. The 36 animals from each group were then randomly allocated to one of 36 pens (4.3 x 10.2 m).

Nine treatments were used in this experiment including three levels of magnesium (Table II.2) and three thiamin treatments in a 3 x 3 factorial design. Magnesium treatments included supplementation with 0, 0.19 or 0.75% magnesium oxide. The thiamin treatments involved either the addition of no supplemental thiamin, the addition of 6.25 mg thiamin/kg of diet (Table II.2), or the addition of thiamin to the diet and a weekly injection of 2 mL of a B-vitamin complex preparation containing 200 mg thiamin·HCl, 4 mg riboflavin, 4 mg pyridoxine HCl, 200 mg nicotinamide, 20 mg pantothenate and 4 μg B_{12} (Dominion Multiple Vitamin B Complex, Dominion Veterinary Laboratories, Ltd. Winnipeg,

Table II.2 Feed ingredients and analyses (experiment 2)

	0% Magnesium oxide		0.19% Magnesium oxide		0.75% Magnesium oxide		
	Control	Thiamin	Control	Thiamin	Control	Thiamin	SEM[1]
Ingredients (% air dry basis)							
Barley, dry rolled	94.8	94.2	94.7	94.0	94.1	93.5	
Limestone (38% Ca)	1.0	1.0	1.0	1.0	1.0	1.0	
Trace mineralized salt	0.23	0.23	0.23	0.23	0.23	0.23	
Urea (45%)	1.0	1.0	1.0	1.0	1.0	1.0	
Molasses (wet)	2.5	2.5	2.5	2.5	2.5	2.5	
Calcium sulfate (16% S)	0.22	0.22	0.22	0.22	0.22	0.22	
Vitamin premix[2]	0.23	0.23	0.23	0.23	0.23	0.23	
Magnesium oxide (54% Mg)	----	----	0.19	0.19	0.75	0.75	
Thiamin premix[3]	----	0.63	----	0.63	----	0.63	
Analyses (dry matter basis)							
Dry matter (%)	86.9	[illegible]8	86.8	86.8	86.9	86.8	0.18
Crude protein (%)	14.3 *b*	14.2 *b*	13.2 *a*	14.0 *ab*	13.4 *a*	13.2 *a*	0.03
Thiamin mg/kg							
Actual	2.32 *a*	5.83 *e*	2.58 *b*	3.47 *d*	2.66 *b*	3.27 *c*	0.028
Calculated	----	8.52	---	8.78	----	8.86	
Ash (%)	3.65 *a*	3.80 *b*	3.64 *a*	4.11 *c*	4.46 *d*	4.45 *d*	0.036
Calcium (%)	0.48 *a*	0.55 *b*	0.47 *a*	0.56 *b*	0.56 *b*	0.60 *c*	0.007
Phosphorus (%)	0.37 *b*	0.36 *ab*	0.36 *ab*	0.35 *a*	0.37 *b*	0.36 *ab*	0.003
Magnesium (%)	0.14 *a*	0.14 *a*	0.29 *b*	0.31 *b*	0.51 *c*	0.44 *c*	0.004
pH	6.3	6.3	7.4	7.7	9.2	9.0	

[1] Standard error of the mean is based upon 1 analysis on each of 7 samples for dry matter, 4 analyses per sample for crude protein, 6 analyses per sample for thiamin, 2 analyses per sample for ash and magnesium and 3 analyses per sample for calcium and phosphorus.
[2] Vitamin premix to provide 5625 I.U., 928 I.U., and 22.5 I.U. per kg of diet vitamins A, D and E respectively.
[3] To provide 6.25 mg thiamin as thiamin HCl per kg of diet.
a-e Means is same row with different letters differ ($P \leq 0.05$).

Man.). The procedure for introduction of the animals onto the diets was the same as was used in experiment 1 Feed intakes were recorded daily on a pen basis. Weights were recorded on three consecutive days at the beginning and end of the trial and weekly during the trial. Water was withheld for 16 h prior to weighing. The subjective bloat score as described in experiment 1 was also used in this experiment to determine the incidence and relative severity of bloats. All animals were slaughtered on the final day of the feeding trial. Procedures for carcass evaluation were the same as used in experiment 1.

Analytical procedures

Crude protein was determined using the Kjeldahl method for nitrogen determination (Association of Official Agricultural Chemists 1980). Calcium and magnesium determinations on blood plasma samples were conducted according to the method of Young and Booth (1967) using a Pye Unicam SP2900 atomic absorption spectrophotometer. Sample preparation for calcium and magnesium determinations in feedstuffs was conducted according to Association of Official Agricultural Chemists (1980) with subsequent treatment in the same manner as for blood samples. Phosphorus was measured using the meta-vanadate method (Association of Official Agricultural Chemists 1980). Thiamin was determined according to Association of Official Agricultural Chemists (1980) for dry or semi-dry materials containing no appreciable amounts of basic substances.

Appropriate tests were conducted to ensure that added magnesium oxide had no influence on the detection of thiamin. Plasma glucose and urea were determined using the neo-cuproine and diacetyl-monoxamine methods respectively for the Technicon Autoanalyzer II (Method Nos. SE4-0002FF4 and 4001FD4 respectively, Technicon Instruments Corp. Tarrytown, N.Y.) Assessments of erythrocyte transketolase activity (E.C.2.2.1.1) and the TPP effect were conducted according to the procedure of Brin (1970). Activity of the enzyme was expressed as mg ribose metabolized/g hemoglobin in sample/h. Hemoglobin was determined using the procedure outlined in Sigma Technical Bulletin No. 525 (Sigma Chemical Co. St Louis, MO.).

Statistical analyses

Analysis of variance according to Steel and Torrie (1980) was applied to feedlot performance data and carcass characteristics to determine treatment differences. A Student-Neuman-Kuels test (Steel and Torrie 1980) was used for multiple comparison of means.

Blood data were subjected to a repeated measures analysis using the method of Grizzle and Allen (1969). Covariate analysis, following the method of Steel and Torrie (1980) was utilized to determine if significant relationships existed between animal gains and blood parameters. This analysis was also used to determine if significant relationships existed between selected blood parameters.

For the first trial where numerous bloats occurred, simple linear regressions between days on test and the cumulative number of bloats in each treatment were calculated. Predicted values for the number of bloats at 98 days were computed for each equation and 95% confidence intervals were calculated for each of the predicted values. Values not overlapping were considered to be significantly different from each other. This method of analysis was considered to be superior to the Chi-square analysis since time to initial bloat and the rate of occurrence of the bloats could be considered in the analysis. The number of bloats on the second trial were limited so this analysis was not possible thus a Chi-square analysis was utilized.

D. RESULTS

Experiment 1

Some destruction of thiamin occurred in the feeds of the magnesium-thiamin supplemented diet as indicated by comparing the actual and calculated values in the diet for the vitamin (Table II.1). Since all feed samples were held at -20° C after they were collected, and the thiamin was premixed only with barley and other vitamins, it was assumed that the majority of the thiamin loss occurred during the 5-10 day period which normally existed between mixing and feeding.

During the experiment, one animal fed the control diet died of prolonged chronic pneumonia and two animals given the magnesium supplemented diet were lost due to acute bloat. Bloated animals died on days 34 and 43 of the feeding trial.

No significant differences due to dietary treatments were obtained for feedlot performance or carcass characteristics (Table II.3). A response ($P \le 0.05$) was however obtained for the incidence of bloat due to dietary treatment (Fig. II.1). The magnesium supplemented steers began to bloat at day 33 and had the highest ($P \le 0.05$) incidence of bloat. The thiamin supplemented group started to bloat at day 72 and had the lowest ($P \le 0.05$) incidence of bloat (Fig. II.1). The control and magnesium-thiamin supplemented groups were intermediate in both day of first bloat and cumulative number of bloats during the trial and were not significantly different from each other but were different ($P \le 0.05$) from the magnesium and thiamin groups. Mean bloat scores with standard errors were 2.78±0.32, 2.73±0.33, 2.56±0.29 and 2.80±0.37 ($P > 0.05$) for the control, magnesium, magnesium-thiamin and thiamin diets respectively.

Mean TPP effects were 7.73, 7.16, 6.91 and 7.12% for the control, magnesium, magnesium-thiamin and thiamin diets respectively ($P > 0.05$). TPP effects varied from 0.0 to 17%. Bloated animals had a mean TPP effect of 6.73% with a range of 1.4 to 16%. TPP effects were not related to 2 week weight changes (Table II.4) but were influenced ($P \le 0.05$) by time with the highest values occurring at days -3, 10 and 90. A highly significant ($P \le 0.001$) negative relationship existed

Table II.3 Feedlot performance and carcass characteristics (experiment 1)

Item	Control	Magnesium	Thiamin	Magnesium and Thiamin	SEM[1]
Number of animals	23	22	24	24	
Days on trial	98	98	98	98	
Incidence of bloat	9	14	5	9	
Avg initial weight (kg)	340.4	340.2	339.8	339.0	1.00
Avg final weight (kg)	481.3	484.3	487.2	480.0	4.46
Avg daily gain (kg)	1.45	1.45	1.50	1.44	0.046
Feed DM[2] per day (kg)	8.26	8.18	8.41	8.24	0.166
Feed DM[2] per gain	5.76	5.70	5.62	5.73	0.221
Warm carcass weight (kg)	277.1	275.5	279.9	275.9	3.26
Dressing percentage (kg)	57.6	57.1	57.4	57.5	0.47
Area eye muscle (cm^2)	74.2	78.4	73.1	75.6	1.55
Avg fat cover (cm)	1.37	1.25	1.25	1.28	0.078
Incidence of abcessed livers	8	9	9	6	

[1] Standard error of the mean.
[2] DM=dry matter.

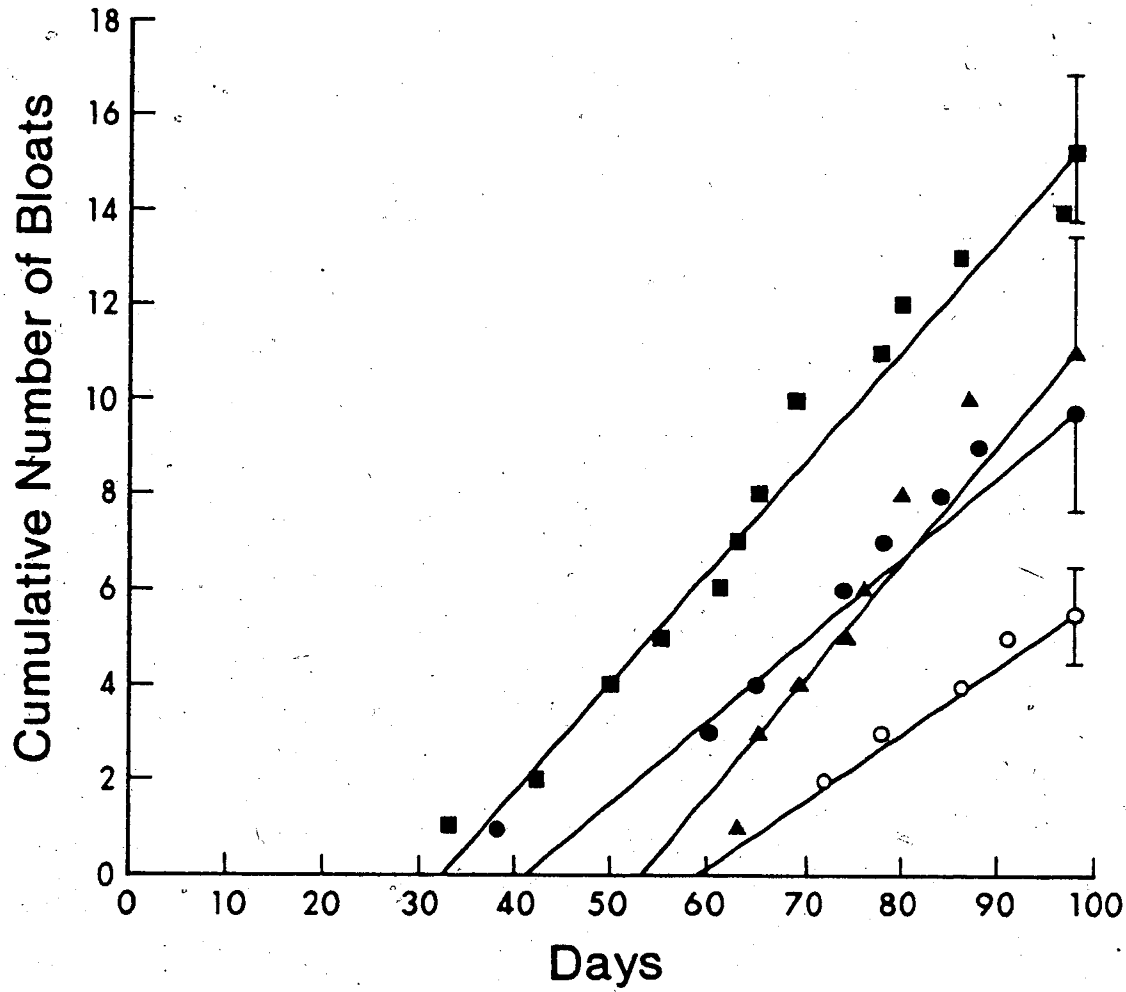

Figure II.1 Cumulative number of bloats during the feeding trial (experiment 1) (● control; ■ magnesium; ▲ magnesium-thiamin; ○ thiamin). Vertical bars are standard errors.

between the TPP effects and erythrocyte transketolase activity (Table II.4). No significant relationships existed between the TPP effects and plasma glucose, magnesium or urea concentrations.

Erythrocyte tranketolase activities varied from 7.68 to 17.84 mg ribose metabolized/g hemoglobin/h which is comparable to a range of 8.75 to 19.04 mg ribose metabolized/gm hemoglobin in sample/h reported in Chapter I. Erythrocyte transketolase activities were also significantly ($P \leq 0.001$) influenced by time with the highest activities occurring at 52 days (Fig. II.2).

Plasma glucose concentrations were not significantly influenced by treatment (Fig. II.3) and were unrelated to 2 week weight changes (Table II.4). Plasma urea concentrations were also not significantly influenced by treatments (Fig. II.3) but were related to 2 week weight changes ($P \leq 0.05$; Table II.4), and were influenced by time ($P \leq 0.001$).

Plasma magnesium concentrations were influenced ($P \leq 0.05$) by treatments over time (Fig. II.4). In the animals fed the magnesium and magnesium-thiamin diets, magnesium levels increased from 19.7 and 19.3 mg/dL, respectively, at day -3 to 23.5 and 24.0 mg/dL respectively by day 52 of the trial and remained relatively constant thereafter. Initial plasma magnesium concentrations for the control and thiamin supplemented animals were 20.1 and 19.9 mg/dL, respectively, and these levels remained relatively constant thoughout the experiment.

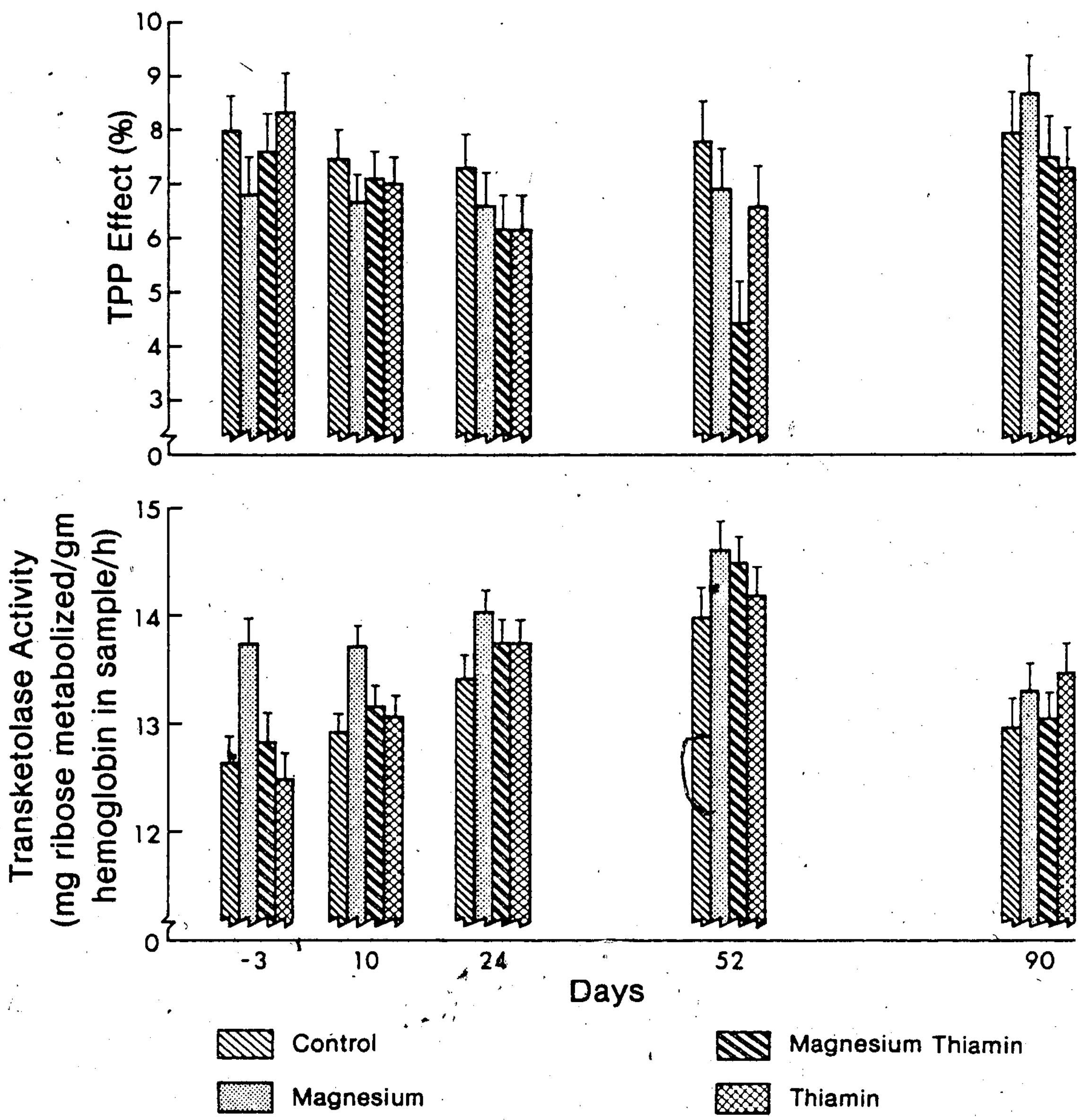

Figure II.2 Mean TPP effect and mean erythrocyte transketolase activities without added thiamin pyrophosphate throughout the feeding trial (experiment 1). Vertical bars are standard errors.

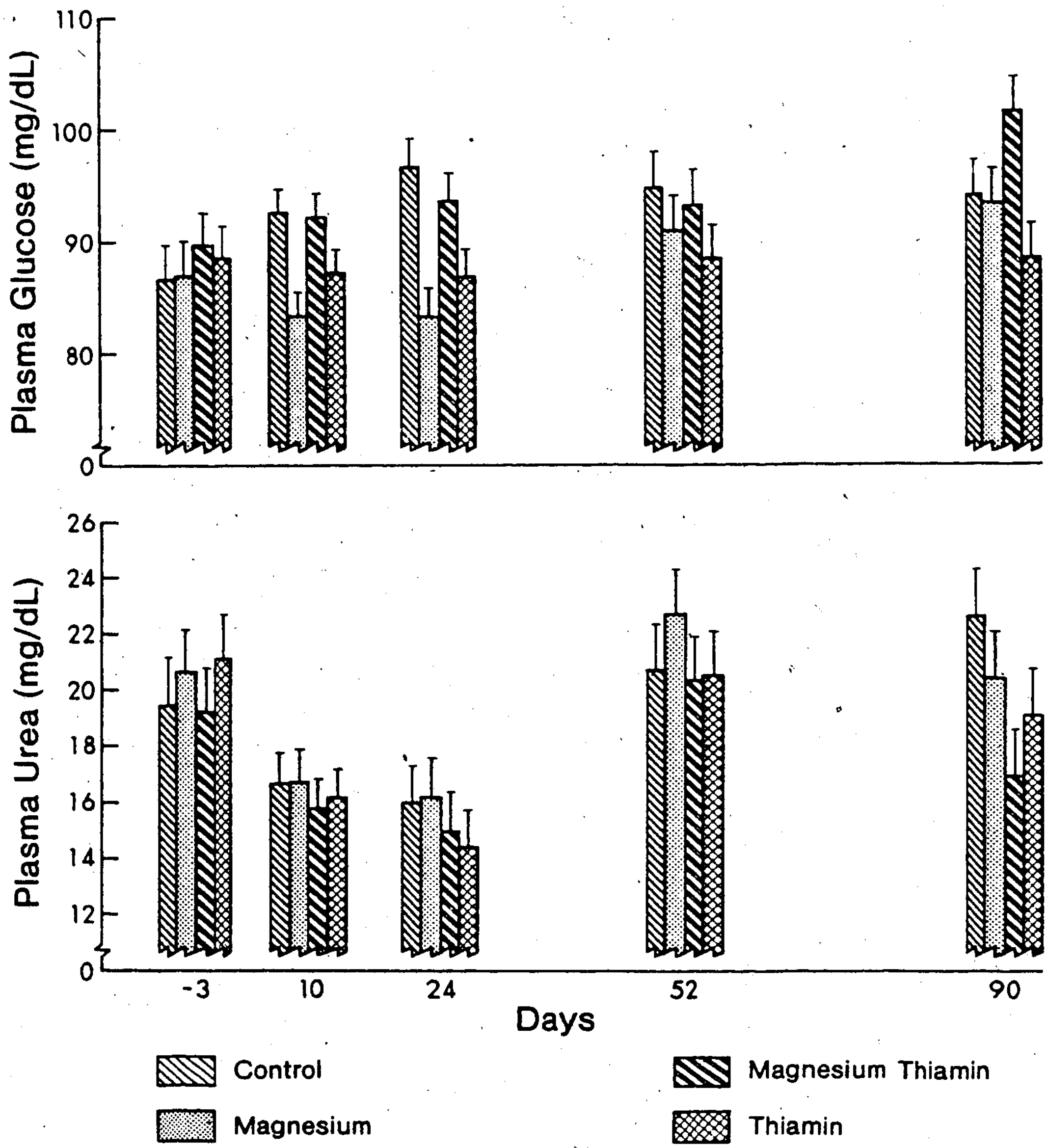

Figure II.3 Mean plasma glucose and urea concentrations throughout the trial (experiment 1). Vertical bars are standard errors.

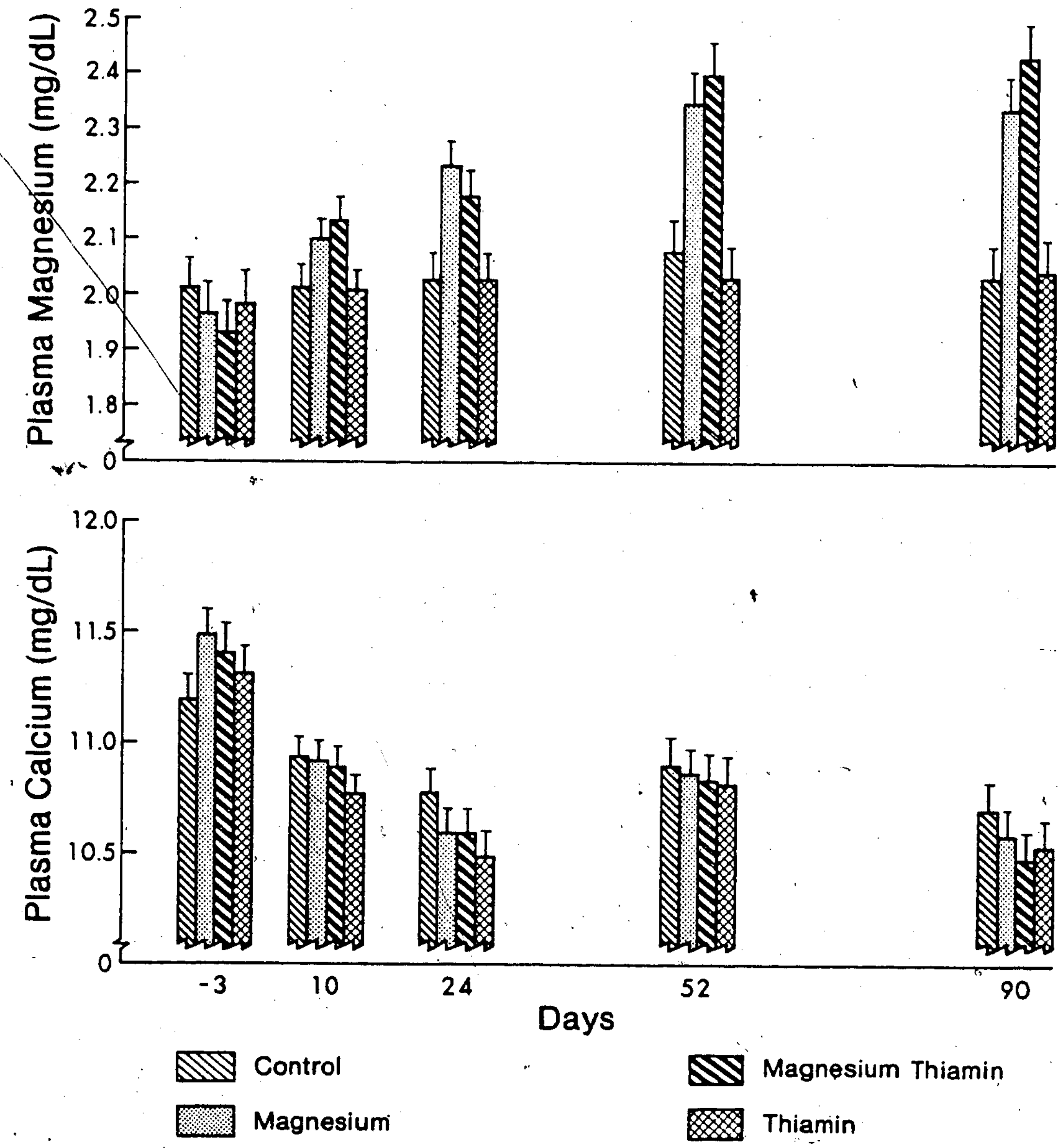

Figure II.4 Mean plasma magnesium and calcium concentrations throughout the trial (experiment 1). Vertical bars are standard errors.

Table II.4 Covariate regression coefficients for two week weight changes and the TPP effect[1] on selected blood parameters (experiment 1)

Covariates	Two week weight changes (kg)	TPP effect (%)
TPP effect[1] (%)	0.145	
Transketolase activity mg ribose metabolized/ g hemoglobin/h	-0.694	-0.867 *
Glucose (mg/dL)	0.026	0.021
Magnesium (mg/dL)	0.528	-0.025
Urea (mg/dL)	-0.667 *	0.043

[1] The percentage increase in activity of the erythrocyte transketolase due to the addition of thiamin pyrophosphate.

* Significant at ($P \leq 0.001$).

Plasma calcium was not significantly influenced by dietary treatments (Fig. II.4) and was unrelated to 2 week weight changes (Table II.4). A time effect ($P \leq 0.05$) was again noted with the highest values occurring in the pretrial samples (Fig. II.4).

Experiment 2

Loss of supplemental thiamin was again observed in this experiment and appeared to be greater on those diets containing supplemental magnesium (Table II.2).

Magnesium oxide supplemenation of the diet had a negative ($P \leq 0.05$) influence on average final weight, average daily gain, feed dry matter intake and carcass weight (Table II.5). Supplemental magnesium did not influence other carcass characteristics. Although only 15 bloats were observed during this experiment a positive trend ($P \leq 0.10$) existed between the frequency of bloats and magnesium levels (Table II.5).

Thiamin treatments had no significant effect on feedlot performance or carcass characteristics with the exception of average fat cover which inexplicably decreased when thiamin was added to the diet and no vitamin injections were given (Table II.5). No significant differences were observed in the occurrence of bloat across thiamin treatments.

No interactions between magnesium levels and thiamin treatments were detected for feedlot performance, carcass characteristics or incidence of bloat.

Table II.5 Feedlot performance and carcass characteristics (experiment 2)

Item	Magnesium oxide			Thiamin treatment			SEM[1]
	Control	0.19%	0.75%	Control	Fed	Fed and Injected	
Number of animals	24	24	24	24	24	24	
Days on trial	97	97	97	97	97	97	
Incidence of bloat	1	6	8 *	6	3	6	
Avg initial weight (kg)	361	362	362	363	361	361	1.4
Avg final weight (kg)	518 *a*	505 *a*	484 *b*	508	500	498	5.1
Avg daily gain (kg)	1.64 *a*	1.48 *b*	1.26 *c*	1.52	1.44	1.42	0.052
Dry matter consumed (kg/day)	9.63 *a*	9.14 *ab*	8.50 *b*	9.30	9.12	8.86	0.454
Feed dry matter/gain	5.92 *a*	6.18 *a*	6.88 *b*	6.18	6.48	6.32	0.328
Carcass weight (kg)	293 *b*	267 *a*	274 *a*	286	283	285	3.2
Dressing percentage	56.6	56.7	56.7	56.3	56.6	57.2	0.23
Area eye muscle(cm^2)	68.5	68.5	67.7	68.5	68.5	67.6	1.72
Avg fat cover (cm)	1.40	1.38	1.25	1.44 *a*	1.19 *b*	1.40 *a*	0.061
Incidence of abcessed livers	14	12	16	17	12	13	

[1] Standard error of the mean
* Significant at ($P \leq 0.10$).
a-c Means in same row followed by different letters differ ($P \leq 0.05$).

E. DISCUSSION

Feedlot performance and carcass characteristics

The discussion of the interrelationship between magnesium and thiamin is somewhat complicated by the fact that a breakdown of supplementary thiamin appeared to occur when magnesium oxide was added to the diet (Table II.1 and II.2). The Merck Index (1976) indicates that thiamin as thiamin·HCl is unstable at a pH above 5.5. Since the pH of the diets was increased when magnesium was added (Table II.1 and II.2), this probably accounts for the loss of supplemental thiamin. Thiamin contained in the grain itself appeared to be stable in the presence of magnesium oxide.

The all-concentrate diets fed in these experiments contained between 0.14 and 0.51% magnesium (Table II.1 and II.2). Recommended levels of dietary magnesium are given as 0.04 to 0.10% of dietary dry matter by the National Academy of Sciences (1976). The Agricultural Research Council (1980) suggests that 300 kg steers gaining at 1.5 kg/day require 5.2 g of magnesium/day and should be provided with an allowance of 8.9 g/day. This translates into 0.06 to 0.10% magnesium in the dietary dry matter at an intake of 9 kg of diet/day. All of the diets in both experiments thus contained more than the recommended level of magnesium (Table II.1 and II.2).

Supplemental magnesium oxide had no influence on growth rate or feed intake of the steers in experiment 1 (Table II.3), whereas there was a negative response to increasing magnesium levels in average final weight, average daily gain, feed dry matter intake, feed conversion efficiency and carcass weight in the second experiment (Table II.3). Depressed performance observed in experiment 2 with increasing magnesium levels can be explained by reduced feed intake (Table II.5). Sorting of magnesium oxide from the daily ration was apparent in experiment 2, particularly at the higher magnesium level and may have caused feed refusal. Sorting of the ration was not observed in experiment 1. Higher dietary levels of crude protein and less calcium sulfate were added to the diet in experiment 1 (Table II.1 and II.2) but it is not clear why this would cause differing responses of the steers to the added magnesium. Gentry *et al.* (1978) reported that in bull calves the inclusion of 1.34% magnesium as magnesium oxide resulted in feed refusal, reduced dry matter intake and depressed growth rates. However, Erdman *et al.* (1978) found no decreases in feed intake on a 40% corn silage, 60% concentrate diet fed to lactating dairy cows when magnesium oxide was included in the diet at a level of 0.8%.

The inclusion of magnesium in the diets of these steers caused an elevation ($P \leq 0.05$) of their plasma magnesium concentrations. This is consistent with the suggestion by Rowlands (1980) that plasma magnesium concentrations closely reflect the amount of magnesium available in the diet.

The addition of magnesium oxide to the diets caused an increase in the number of bloats in both experiments (Fig 1;

Table II.5). This relationship has also been observed with legume bloat. Smith and Wood (1962) observed that spraying alfalfa with calcium and magnesium salts increased the severity of bloat and that chelating agents reduced the severity. Stifel *et al.* (1968) found a positive correlation between bloat and leaf magnesium concentrations and a negative correlation with calcium concentrations. However, Miltimore (1970) found a negative correlation between bloat and leaf magnesium and a positive correlation with leaf calcium concentrations. It is suggested by Laby (1975) that the reason for the relationship between divalent cations and the incidence of legume bloat relates to the stable soaps produced with mono- and di-glycerides, particularly with calcium. Similar results were not found in the literature for concentrate diets, however, and since it is known that variations in feed consumption increase the incidence of bloat (Milligan 1973), the palatability problems encountered with magnesium oxide may have been the more likely cause of the increased incidence of bloat on the magnesium supplemented diet.

In the first experiment, thiamin had a beneficial effect in terms of lowering the incidence of bloat, but this effect was not seen in the second experiment. Naga *et al.* (1975) found that uncomplicated thiamin deficiencies in sheep were characterized by inappetence and a reduction in the intensity and pressure changes normally associated with contractions of the ventral sac of the rumen. There is some reason to speculate that a thiamin deficiency could increase the occurrence of bloat since large volumes of gases are being produced in the rumen and therefore weaker stomach contractions could result in gas accumulations. Furthermore, there is reason to believe that a potential thiamin deficiency could have been aggravated by excess magnesium. Fujiwara *et al.* (1976) reported that in rats, excess thiamin accelerated magnesium excretion in the urine and vice versa. Yano and Kawishima (1977) demonstrated that in sheep, the injection of 50 mg thiamin tetrahydrofurfuryl disulfide/head/day for 14 days resulted in increased magnesium excretion and a negative magnesium balance. Blood analyses for the first experiment, however, demonstrated that TPP effects were not significantly influenced by dietary treatment. Also the fact that in experiment 2, thiamin, whether supplemented in the diet or given by injection as well, did not decrease the incidence of bloats suggests that the tendency to bloat cannot be explained simply by the interaction between magnesium and thiamin as described by Fujiwara *et al.* (1977).

No significant differences were observed for feedlot performance or carcass characteristics across thiamin treatments in either experiment (Table II.3 and II.5). This occurred even though a higher level of dietary thiamin was used than in the previous trial in which a response in animal performance due to the supplementation of thiamin was obtained. Brethour (1972) also found that average daily gains of steers were increased significantly due to the addition of 1 g thiamin and 100 g sodium bicarbonate to a wheat based ration, whereas a second trial failed to produce similar results. No response in animal performance was obtained in the second trial of this experiment due to the injection of a B-vitamin complex containing thiamin. Edwin *et al.* (1976) found that the injection of 500 mg thiamin three times weekly lowered the TPP effect of 6 month old calves to 0 in 6 weeks and, although the amount of thiamin injected in this experiment was only 13% of that used by Edwin *et al.* (1976) and the animals were larger, the results do indicate the lack of a thiamin responsive condition in these animals. Plasma urea levels in this experiment averaged 18.5 mg urea/dL (8.63 mg urea N/dL) which suggests that the steers were receiving adequate crude protein (National Academy of Sciences 1978), whereas there was evidence of a protein deficiency during the experiment reported in Chapter I.

Dietary treatments had no influence on TPP effects in experiment 1 (Fig. II.2). Since initial samples were collected 3 days prior to the initiation of the steers on the various diets, the TPP effects reported for this day may represent typical values for hay diets. The decrease in TPP effects at 28 and 52 days and the subsequent increase at 90 days is as yet unexplained. When the TPP effects were classified into the ranges 0-5, 5-10, 10-15, and 15-20%, corresponding 2 week weight changes were 14.5, 16.3, 16.7

and 11.8 kg respectively ($P>0.05$). In Chapter I, this author found corresponding 2 week weight changes to be 15.4, 12.9, 13.2 and 3.1 kg, respectively, with a gain of 7.3 kg per 2 weeks being obtained for steers with a TPP effect of between 20-25%. This leads to the conclusion that no marked relationship exists between TPP effects and weight changes up to a TPP effect of at least 15%. Above this one might expect to see a reduction in animal performance as the magnitude of the thiamin deficiency, as measured by the TPP effects, increases.

The increase in erythrocyte transketolase activities to day 52 and the subsequent decrease in activities is inconsistent with the findings obtained in Chapter I (Fig. II.2). No explanation of these results is possible at this time.

Plasma glucose was observed to vary with time ($P\leq0.05$; Fig. II.3). However, since these samples were collected under stress conditions, caution must be used in drawing conclusions from this data. No unusually high plasma glucose concentrations were detected which would have been indicative of a thiamin deficiency (Loew 1975). Plasma glucose was also unrelated to TPP effects (Table II.4) indicating glucose metabolism was not being influenced by a thiamin deficiency.

A relationship ($P\leq0.05$) existed between plasma urea and 2 week weight changes (Table II.5). In a review by Rowlands (1980), it was reported that a positive relationship exists between protein intake and plasma urea concentrations, but a negative relationship exists between energy intake and plasma urea concentrations. Similar results were reported in Chapter I.

It can be concluded from these experiments that thiamin supplementation of all-concentrate diets does not consistently give a response in terms of improving animal performance. The injection of a B-vitamin complex preparation containing thiamin and nicotinamide did not result in an improvement in animal performance. Magnesium supplementation resulted in an increase in the incidence of feedlot bloat in both experiments and reduced performance in the second. There were also indications that an interaction existed between magnesium and thiamin in the first experiment but not in the second. Further investigations into factors which may influence the response of cattle to added thiamin are underway.

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III. A SURVEY OF THE THIAMIN STATUS OF GROWING AND FATTENING CATTLE IN ALBERTA FEEDLOTS

A. ABSTRACT

A survey involving a total of 645 samples collected from 331 steers at 3 locations within Alberta, was conducted to determine the incidence of thiamin deficiencies in growing and fattening cattle in Alberta. The percentage increase in the activity of the erythrocyte transketolase enzyme (E.C.2.2.1.1) due to the addition of thiamin pyrophosphate (TPP effect) *in vitro* was used as the index of thiamin status. It was found that 2.7% of the samples taken had a TPP effect in excess of 15% and thus these animals were expected to be marginally deficient in thiamin. A trend ($r=0.824$; $P \leq 0.10$) existed for a negative linear relationship between mean TPP effects and mean dietary crude protein concentrations suggesting that nitrogen availability may be influenced by, or may be influencing, the thiamin adequacy of cattle on high concentrate diets. Weaning stress did not result in TPP effects over 15%. It was concluded that a small and variable proportion of the animals on high grain feedlot type diets may be experiencing a loss in productivity due to a thiamin inadequacy.

B. INTRODUCTION

Polioencephalomalacia, a thiamin responsive disease, has been observed in feedlot cattle (Brent 1976; Blood *et al.* 1979). It is not known, however, whether subclinical deficiencies, which could limit animal production and possibly cause health problems in feedlot cattle, occur to any significant extent.

This author has previously found a significant improvement in average daily gains and final carcass weights due to the addition of 1.9 mg thiamin/kg of a barley based diet (Chapter I). A negative trend existed between the percent increase in the activity of the erythrocyte transketolase enzyme (E.C.2.2.1.1) due to the addition of thiamin pyrophosphate (TPP effect) and 2 week weight changes. However, second and third feeding trials (Chapter II) conducted to substantiate these findings, failed to detect significant differences between thiamin treatments in average daily gains or final carcass weights of the steers. However, the addition of 6.25 mg thiamin/kg of a barley based diet reduced the incidence of bloat in the second feeding trial even though it was observed that TPP effects only ranged from 0-17% as compared to 1.96 to 25% found in Chapter I. Brethour *et al.* (1971) also found variable results with the addition of 1 g thiamin and 100 g sodium bicarbonate to a wheat based ration for steers.

The purpose of this survey was to determine, using the TPP effect as an index of thiamin status, the incidence of subclinical thiamin deficiencies in three Alberta feedlots.

C. METHODS AND MATERIALS

In total, 645 samples involving 331 animals from six sources were collected for this survey. Included in this is sample data from Chapters I and II. which was utilized to broaden the information base. The first source of data (Chapter I) represents a total of 168 samples collected from 28 steers (at an average initial weight of 361 kg) throughout a feeding trial when an all-concentrate barley based diet was fed (Table III.1). Ninety of these samples were collected from steers fed a diet without supplemental thiamin and 78 were collected from animals supplemented with 1.9 mg thiamin/kg of diet. The second data source (Chapter II) involved a total of 238 samples collected from 96 steers (at an average initial weight of 340 kg) throughout a feeding trial in which all-concentrate barley based diets were given (Table III.1). Diets used included an unsupplemented diet, a diet supplemented with 0.19% magnesium as magnesium oxide, a diet supplemented with 6.25 mg thiamin/kg of diet and one diet with both supplemental magnesium and thiamin. In both experiments reported previously data within each source was grouped and each experiment treated as a single source of information since no significant differences were obtained for the TPP effect across treatments. Feed analyses data (Table III.1) represents the mean across treatments within individual

Table III.1 Diet compositions and analyses

	Chapter I	Chapter II	Kinsella (fed)[1]	Ellerslie	Brooks
Ingredients (% air dry basis)					
Barley	98	93.5-94.8	63.8	87.2-90.2	56.3
Oats			21.2		
Dehydrated alfalfa pellets			10		
Canola meal				5	
Distillers dried grains				0-3.0	
Beet tailings					38.[illegible]
Molasses (wet)		2.5		2.5	1.[illegible]
Urea		1.0			
Vitamin/mineral premixes[2]	0.905	0.46-0.86	5	0.50	0.[illegible]
Calcium carbonate (38% Ca)	1.1	1.0		1.1	0.[illegible]
Sodium bicarbonate				0.75	
Magnesium oxide (54% Mg)		0-0.75			[illegible]02
Calcium sulfate (16% S)		0.22			
Analysis (dry matter basis)					
Dry matter (%)	84.4	85.7	90.7	86.1	[illegible]
Crude protein (%)	12.1	16.1	14.6	13.8	[illegible]2.4
Calcium (%)	0.46	0.58	0.56	0.59	0.53
Phosphorous (%)	0.43	0.34	0.47	0.44	0.38

[1] Samples collected at Kinsella from 34 bull calves on full feed.
[2] Vitamin/mineral premixes contained adequate amounts of vitamins A and D based on levels suggested by NRC (1976) and either trace mineralized or cobalt iodized salt.

experiments.

The third source of data was obtained from blood samples collected from 45 newly weaned bull calves at the University of Alberta Kinsella Ranch (Kinsella, weaned). These animals had previously been on pasture with cows, and, at the time of sampling, had been separated from the cows for only 24-48 h. Collection of representative samples of pasture forages was not possible and these animals had not been creep-fed. For the 24-48 h period post-weaning, the calves were offered a long hay diet which was not sampled since it was felt it would not be representative of the feed they had been consuming. Samples were collected from 34 of these animals 35 days after the initial sampling when full grain feeding was being acheived (Kinsell, fed) to provide the fourth source of data.

The fifth source of data was from an experiment conducted at the Ellerslie Research Station (Mathison 1981) involving the use of distillers dried grains in an all-concentrate barley based diet in which the barley was either rolled or ground (Table III.1). Samples were collected from 60 steers (at an average initial weight of 355 kg) 2 weeks after initiation of the feeding trial. This was again treated as a single source of information and thus diets indicate the range of feed ingredients used (Table III.1).

Final samples were collected at a commercial feedlot in Brooks, Alberta from 100 steers (at an average initial weight of 269 kg) 6 days following the achievement of full feed (Table III.1).

Nutrients in the feeds from the last four sources were determined by procedures outlined in Association of Official Agricultural Chemists (1975). The TPP effect, using the method of Brin (1970), was utilized to determine the incidence and severity of thiamin deficiencies. Data were analyzed initially using the method of Harvey (1960) for least squares analysis with unequal subclass sizes. Data was then further subjected to a Chi-square analysis of association (Steel and Torrie 1980) to determine if association existed between the source of sample collection and the frequency of TPP effects observed in the classifications 0-5, 5-10, 10-15, 15-20 and 20-25%. Regression analysis according to Steel and Torrie (1980) was used to determine the relationship between mean crude protein concentrations and mean TPP effects.

D. RESULTS AND DISCUSSION

All mean TPP effects were below the 15% level (Table III.2) which has been previously concluded to be the level at which reductions in average daily gains might be expected to occur due to a thiamin deficiency (Chapter II). Individual TPP effects ranged from 0 to 25% with TPP effects above 15% being observed in data as reported previously (Chapters I and II) and in samples collected at the commercial feedlot in Brooks but not in the other three

Table III.2 Mean TPP effects[1] by data source

Source	Number of Samples	Number of Cattle	Mean TPP effect[1] (%)	SEM[2]
Chapter I	168	28	8.19 *bc*	0.238
Chapter II	238	96	7.13 *a*	0.200
Kinsella (weaned)[3]	45	45	6.72 *a*	0.460
Kinsella (fed)[4]	34	34	6.89 *a*	0.529
Ellerslie	60	60	7.36 *ab*	0.398
Brooks	100	100	8.83 *c*	0.309

[1] Percent increase in activity of erythrocyte tranketolase enzyme due to the addition of thiamin pyrophosphate.

[2] SEM=standard error of the mean.

[3] Samples collected at the University of Alberta Kinsella Ranch from newly weaned bulls.

[4] Samples collected at the University of Alberta Kinsella Ranch from 34 of the same bulls once on full feed.

a-c Means in same column followed by different letters differ ($P \leq 0.05$)

sampling groups. Overall only 2.2 and 0.5% of the samples collected fell into the 15-20 and 20-25% ranges for TPP effects (Table III.3). Individual Chi-square values indicate that, in relation to the overall data, a disproportionate number of samples were observed in the 15-20 and 20-25% TPP effect ranges for data from Chapter I. A disproportionately low number of samples were observed in the 0-5% TPP effect range and a disproportionately large number (4% of the location total) were observed in the 15-20% range for samples collected at Brooks.

The mean TPP effect was the lowest for the newly weaned bull calves (Table III.2) and no TPP effects above 15% were observed for this group suggesting that this class of animal, when given a hay diet, would be less likely to be deficient in thiamin than cattle given typical feedlot diets. The low TPP effects observed in these calves is probably not due to age since Edwin and Lewis (1971) and Jensen and Mackey (1979) state that the younger ruminating animal is particularly vulnerable to a thiamin deficiency. The results are also of interest since they suggest that a thiamin deficiency may not be contributing to the increased incidence of disease and weight loss commonly observed in nutritionally stressed calves at weaning (Marlowe 1977).

There were indications that some of the cattle given feedlot diets at Brooks and Ellerslie as reported in Chapter I were deficient in thiamin (Table III.3). Symptoms typical of polioencephalomalacia have been observed and successfully

Table III.3 TPP effect[1] of blood samples categorized by the range in TPP effect and source of samples

Location		TPP effect[1] (%) 0-5	5-10	10-15	15-20	20-25	Location Total
Chapter I	Number observed	33	95	29	8	3	168
	Percent of location total	19.6	56.6	17.3	4.8	1.8	
	Number expected	29.7	106.8	27.1	3.6	0.8	
	Chi-square	0.37	1.30	0.13	5.38	6.05	
Chapter II	Number observed	46	158	32	2	0	238
	Percent of location total	19.3	66.4	13.4	0.8	0.0	
	Number expected	41.1	151.3	38.4	5.2	1.1	
	Chi-square	0.36	0.30	1.07	1.97	1.10	
Kinsella (weaned)[2]	Number observed	9	32	4	0	0	45
	Percent of location total	20.0	71.1	8.9	0.0	0.0	
	Number expected	8.0	28.6	7.3	1.0	0.2	
	Chi-square	0.13	0.40	1.49	1.00	0.20	
Kinsella (fed)[3]	Number observed	10	20	4	0	0	34
	Percent of location total	29.4	58.8	11.8	0.0	0.0	
	Number expected	6.0	21.6	5.5	0.70	0.2	
	Chi-square	2.67	0.12	0.41	0.70	0.20	
Ellerslie	Number observed	8	45	7	0	0	60
	Percent of location total	13.3	75.0	11.7	0.0	0.0	
	Number expected	10.6	38.1	9.7	1.3	0.3	
	Chi-square	0.64	1.25	0.75	1.30	0.30	
Brooks	Number observed	8	60	28	4	0	100
	Percent of location total	8.0	60.0	28.0	4.0	0.0	
	Number expected	17.7	63.6	16.1	2.2	0.5	
	Chi-square	5.32	0.20	8.80	1.47	0.50	
Total sample		114	410	104	14	3	645
Percent of total		17.7	63.6	16.1	2.2	0.5	

[1] Percent increase in activity of erythrocyte tranketolase enzyme due to the addition of thiamin pyrophosphate.
[2] Samples collected at the University of Alberta Kinsella Ranch from newly weaned bull calves.
[3] Samples collected at the University of Alberta Kinsella Ranch from 34 of the same bulls once on full feed.
Raw Chi-square = 45.77 ($P \leq 0.0009$).

treated with thiamin injections at the commercial feedlot in Brooks, however, polioencephalomalacia has not been diagnosed at the Ellerslie Research Station. Linklater *et al.* (1977) found that up to a third of the sheep from an apparently normal herd on pasture, in previous contact with polioencephalomalacia, could be excreting fecal thiaminase at any one time. Similar results are reported by Roberts and Boyd (1974). Results presented here suggest that obvious symptoms of polioencephalomalacia in the herd are required for elevated TPP effects, indicative of a thiamin deficiency to be present in a herd of cattle on high concentrate diets.

The occurrence of thiamin deficiencies in these animals, as measured by the TPP effect, could not be related to any ingredient(s) specific to the diets used at Ellerslie (Chapters I and II) and the commercial feedlot in Brooks. Similar results were encountered in a survey conducted by Spence *et al.* (1961) where it was concluded that no common dietary ingredient(s) or management practices could be related to the occurrence of polioencephalomalacia. Mella *et al.* (1976), however, described a diet composed of sugar cane molasses containing 0.67% urea and a mineral mix given *ad libitum* with 400 g/head/day fish meal which could be used to initiate polioencephalomalacia in a little as 4 days.

In this survey, a trend ($r=0.824$; $P\leq0.10$) for a negative relationship was observed between dietary crude protein concentrations and the mean TPP effects (Fig. III.1). Hoeller *et al.* (1977) found that the *in vitro*

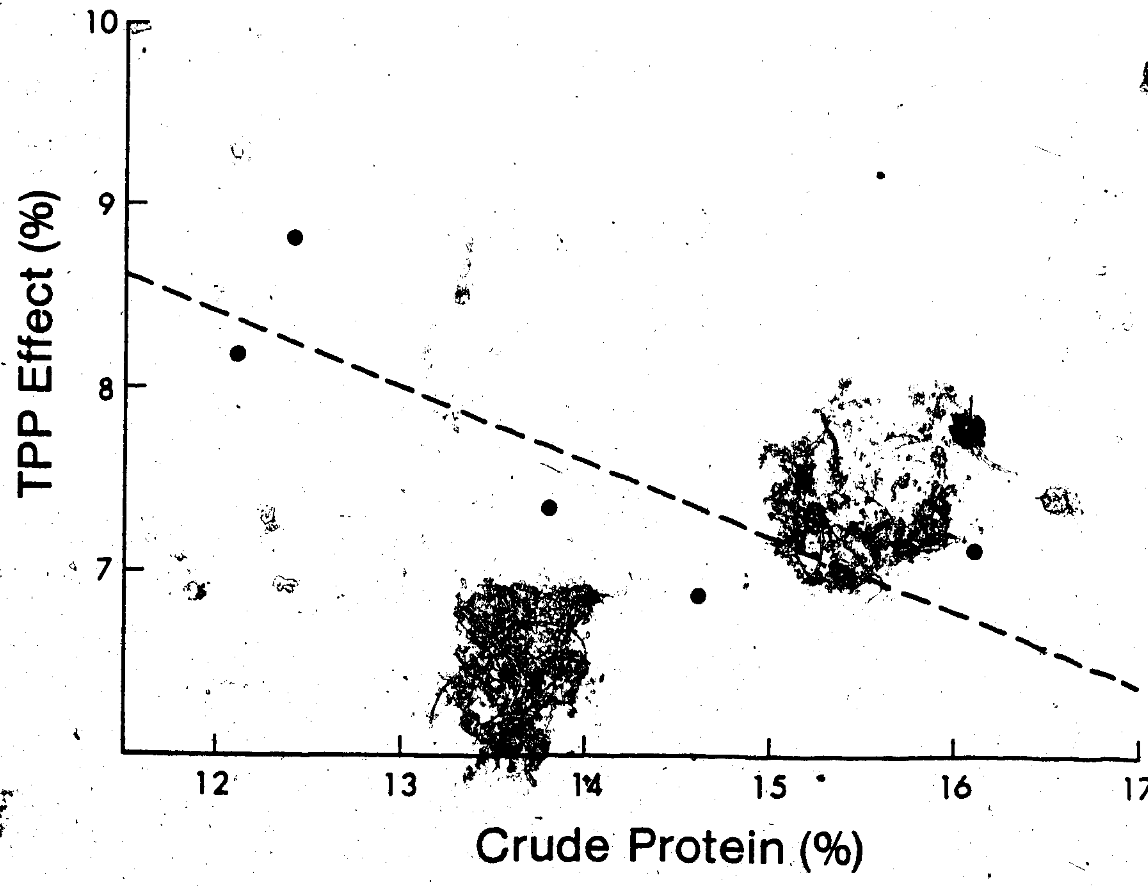

Figure III.1 Mean TPP effect versus mean crude protein concentration. (r=0.824; P≤0.10). Y=(-0.406 x X) + 13.27.

addition of thiamin to the rumen contents of sheep fed a protein free diet containing cellulose, starch, saccharose, urea and corn oil resulted in an increase in microbial protein production of up to 123%. Feeding a variety of nitrogen sources in diets with varying nitrogen and thiamin concentrations, Buziassy and Tribe (1960) found a positive relationship between thiamin concentrations in the rumen contents of sheep and the nitrogen content of the diet.

No significant relationships could be established between mean TPP effects and feed calcium or phosphorus. It is of interest that polioencephalomalacia has been associated with low levels of cobalt in forage (Siegmund 1979).

It may be concluded from this survey that newly weaned calves fed a hay diet are not as subject to a thiamin deficiency as cattle which are receiving high concentrate diets. Evidence existed to suggest that a variable proportion of the animals on feedlot diets containing large amounts of barley, may be experiencing a loss in productivity due to a thiamin inadequacy. Evidence also existed for a negative relationship ($P \leq 0.10$) between the crude protein content of the feedlot diets and the mean TPP effect indicating that nitrogen availability may be influenced by, or is influencing, the thiamin adequacy of cattle on high concentrate diets.

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GENERAL SUMMARY AND CONCLUSIONS

The major conclusion which can be drawn from this research is that subclinical thiamin deficiencies can occur in cattle fed high concentrate, barley based diets. This conclusion is based primarily on the observations made in Chapter 1 that elevated TPP effects indicative of a thiamin deficiency did occur, that the animals which were supplemented with thiamin had higher ($P \leq 0.05$) average daily gains and carcass weights than control animals and that a negative relationship ($P \leq 0.10$) existed between TPP effects and 2 week weight changes. Animals with a TPP effect above 15% had a mean 2 week weight change of 4.2 kg as compared to 13.5 kg for animals with a TPP effect below 15%. This strongly suggested that TPP effects above 15% are indicative of a marginal thiamin deficiency.

Although dietary thiamin supplementation did result in improved animal performance in Chapter [illegible] similar results were not observed in response to dietary supplementation with thiamin or the injection of thiamin and other B-vitamins in experiments reported in Chapter 2. The reason for this was not clear but these results served to illustrate the variability of the response to thiamin supplementaion. Results obtained in the first experiment of Chapter 2 also did not provide substantiating evidence for the negative relationship between the TPP effect and animal weight changes. The results were not inconsistant with those of Chapter 1, however, since only 2 of the 238 samples collected had a TPP effect over 15%. Also the mean 2 week weight gain of these two animals was 11.8 kg as compared to a mean of 16.0 kg for animals with TPP effects below 15%.

Magnesium supplementation had no significant influence on the TPP effects observed in either the first or second experiment reported in Chapter 2. The reason for the significant increase in the incidence of bloat with increasing levels of supplemental magnesium observed in the first experiment, and the trend ($P \leq 0.10$) for an increased incidence of bloat in the second experiment, is not clear. An apparent benefit of supplemental thiamin in reducing the incidence of bloat in magnesium-fed steers occurred in the first experiment of Chapter 2 and this suggested a possible interaction between these two nutrients but this possibility was not substantiated by the results of a second experiment.

In Chapter 3, using the TPP effect as an index of the thiamin status of feedlot cattle, it was observed in a survey of six groups of cattle that a low and variable proportion (0-4%) of cattle on feedlot diets may be experiencing a marginal thiamin deficiency at some time during the feeding period. Across all experiments 2.7% of the animals were believed to have this problem. The negative trend ($P \leq 0.10$) observed between mean TPP effects and mean crude protein concentrations of the diets observed in Chapter 3 deserves closer attention, particularly since all crude protein levels used would be considered adequate according to current feeding standards. Such a relationship may explain the variability of animal response to supplemental thiamin observed in the first three experiments, and may also explain similar variability found in the literature.

On the basis of this research the routine practice of supplementing feedlot diets with thiamin as a method of preventing the occurrence of subclinical thiamin deficiencies cannot be recommended at this time. This is because of the variable performance response obtained with supplemental thiamin, because dietary thiamin concentrations were not significantly related to the TPP effects in the animal in these experiments and because thiaminase enzymes in the rumen may simply degrade added dietary thiamin. Additional work is required to establish whether a repeatable relationship does exist between the occurrence of marginal thiamin deficiencies and the crude protein concentration in the diet. If such a relationship holds, dietary supplementation of thiamin may be of significant value in diets containing under 12.5% crude protein, particularly since the current cost of supplementing thiamin at a rate of 5 mg/kg of diet is only 0.32¢/day if an average feed intake of 9 kg/head/day is assumed.

Literature on the thiamin status of ruminants would tend to suggest that as the proportion of rapidly fermentable material in the diet is increased the proportion of animals experiencing clinical thiamin deficiencies will tend to increase. Subclinical thiamin deficiencies may become of greater importance in the future, then, if the use of byproducts from the sugar refining industry (which may contain a significant amount of soluble sugar) increases, or if the proportion of roughage normally included in feedlot diets decreases.