HYPERALIMENTATION IN LAMBS: A MODEL FOR THE STUDY OF POLIOENCEPHALOMALACIA

by

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TABLE OF CONTENTS

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INTRODUCTION

In recent years much research has focused on maximizing the efficiency of ruminants. Breeding animals which gain faster, and processing grains to increase digestibility have helped to increase the productivity of ruminant finishing operations. However, progress through breeding is slow and no new breakthroughs in grain processing seem imminent. Further, it has been noted that increasing the digestibility of rations does not appreciably increase weight gains. Since chemostatic mechanism maintain intake at the same level of digestible calories, animals on processed rations adjust intake to fit energy needs and make more efficient gains by consuming less feed to produce the same gains. Therefore, it would seem logical to investigate the possibility of inducing ruminants to eat at higher levels than normal. It has been reported that increasing intake of any given feedlot ration results in higher energy intake and consequently, improves performance and increases efficiency of utilization (Theurer, 1970).

Little is known about the effects of hyperalimentation in the ruminant and the changes that will occur in rumenal end products as a result. Many factors must be considered in any discussion of feed intake control in ruminants. Not only must factors active in the normal regulation of intake be considered, but also complications which may arise from inducing ruminants to consume nutrients at levels above normal. In an attempt to describe these factors, this literature review will discuss the mechanisms for regulating feed intake and discuss the effect of hyperalimentation on thiamine metabolism.

LITERATURE REVIEW

Influence of Appetite, Palatability, Hunger and Satiety

The terms appetite (desire for food and drink) and palatability (state of being agreeable to the palate) have come to describe physiological functions other than those mentioned above (Conrad, 1966). Corbett (1961) used appetite to describe the rate of eating or its extent. Bruce and Kennedy (1951) used appetite to describe only the urge to eat resulting from psychological factors such as palatibility. Hunger is also the urge to eat but it develops from the deprivation of food of a general or specific type and is abolished by the ingestion of food. Satiety, on the other hand, is expressed by a lack of desire for food (Conrad, 1966). Blaxter et al. (1961) considered palatability to be the animal's subjective evaluation of its feed. Experiments with ruminants (Balch and Campling, 1962) indicate that without choice of feed, taste and other senses play a bigger role in perception and investigation of food than in determining the amount eaten. Palatability, therefore, can be said to be the result of an animal's analysis of a feed using its five senses.

Goatcher and Church (1970a) used a 2-choice preference test technique in studying taste responses of sheep to various sugars, sodium saccharin, ethyl alcohol, and various salts of volatile fatty acids. Tests with sugars and saccharin showed adequately fed sheep to be essentially indifferent to compounds evoking taste sensations, although some sheep showed pronounced preference for some sugars. While a positive preference was never manifested for ethyl alcohol, the tolerance for this chemical was remarkably high. With the exception of the sugars, the sheep accepted molar concentrations higher than for any of the other chemicals studied. Effectiveness of stimulation

with salts; sodium acetate (NaAc), sodium propionate (NaPr) and sodium butyrate (NaBu), was greater than with sugars. Smell may have been partly responsible for this. In another study, (Goatcher and Church, 1970b), sheep were found to be more sensitive to sour and bitter tasting substances than to sugars and salts.

Sense of smell has been shown to have only minor importance in influencing the injestion of food by stall fed sheep (Tribe, 1949). Sheep accustomed to the smell of feces even consumed feed contaminated with feces. Further evidence shows that sense of smell plays no role in selection of herbage species by sheep, but rather is important only in initial stimulation of appetite (Tribe and Gordon, 1949).

The Role of the Central Nervous System

Hunger, like appetite, is the urge to eat, but it is regulated by the hypothalamus (Bruce and Kennedy, 1951). Satiety is expressed as the lack of desire for food. Carlson (1916) recognized the existence of hunger centers in the medulla, diencephalon, and cerebral cortex, but because of the then popular gastric mechanism of feed intake regulation, few investigations into hypothalamic control were conducted before 1940.

Hetherington and Ranson (1940) discovered that obesity could be produced in rats by lesions confined to the hypothalamus. Anand and Brobeck (1951a,b) reported the existence of areas in the lateral hypothalamus of the rat at the same rostro-caudal plane as the ventro-medial nucleus, which when bilaterally destroyed, lead to complete inhibition of food intake. Hyperphagia could be obtained only if the lateral hypothalamus was left intact. After bilateral destruction of the lateral hypothalamus in these hyperphagic animals, the hyperphagia changed into complete aphagia. Anand et al. (1955) confirmed the

existence of similar areas in the cat and monkey. Destruction of these areas bilaterally lead to aphagia. Unilateral destruction of these areas did not produce aphagia. Hyperphagia was produced with lesions in the region of the ventro-medial nucleus, while no animal with caudal lesions gave this response.

It is clear from these observations that the hypothalamus of higher animals possesses mechanisms for both starting and stopping eating behavior. The more lateral area has been called a "feeding center" and the medial area a "satiety center" (Brobeck, 1956). The discovery that the hypothalamic mechanism is a dual one suggests that appetite and satiety may be two separate and distinct phenomena. If the region where lesion causes aphagia is an appetite mechanism, then its overactivity will cause food intake to increase, while underactivity in this region will cause intake to be reduced. Similarly, if the region where lesions cause hyperphagia is a satiety mechanism, then its underactivity will lead to overeating, while its overactivity will lead to decreased food intake. These predictions are in agreement with the results of Miller et al. (1950) who found that rats with hypothalamic hyperphagia show a decreased satiety, but no increase in appetite as measured by their willingness to work for food or eat food with an unpleasant taste.

There is also evidence that a central nervous regulatory mechanism exists in ruminants (Balch and Campling, 1962). Baile (1968) reported that electrolytic lesions made in the lateral area of the hypothalamus caused temporary aphagia lasting 4 to 12 days in three goats. These results suggest that the central nervous control of feed intake is similar in both ruminants and monogastric animals. In a fourth goat, bilateral lesions in the anterior hypothalamic area caused aphagia and hypodipsia lasting 17 days. During this time the goat had a rectal temperature of about 1.5^{o}C above normal. Lesions in the fourth goat apparently caused a malfunction in the heat loss center.

Oropharyngeal Metering of Feed

After studing eating behavior in cattle, Balch (1958) considered it unlikely that oropharyngeal based mechanisms would limit feed intake in ruminants. Campling and Balch (1961) collected hay swallowed by cows as it passed through the cardia during the first three hours of a meal. The length of the eating period was almost doubled and the cows ate 177% of their pretrial intake. This evidence showed that the oral and pharyngeal areas play no great part in limiting either duration of feeding or amount consumed. It was also concluded that neither exhaustion of the salivary glands or musculature of the jaw was of very great importance in determining feed intake, although feeding rate did slow down at the end of the extended eating period. It seems, however, that if oropharyngeal metering played no part in intake, the cows would have continued to eat indefinitely. Duckworth and Shirlaw (1958) noted that the rate of eating, in bites per minute, declined during a meal of grass and suggested that the slackening in feeding rate of cows might reflect fatigue on the jaw muscles.

Thermostatic Regulation of Feed Intake

In terms of mode of action, control systems can be described as either short-term or long-term. Short-term regulation is concerned with the initiation and cessation of individual meals, while long-term regulation adjusts feed intake to match energy requirements over a period of days or weeks (Baumgardt, 1969). Short-term appetite control mechanisms require feedback signals which are produced during the course of a meal in order to regulate the size of that particular meal.

A prominent hypothesis regarding the regulation of feed intake assumes that the hypothalamus contains a thermosensitive area which responds to thermal

gradients. Strominger and Brobeck (1953) reported that the intake of food seems to a large extent to be regulated indirectly via the heat liberated in assimilating food, much as oxygen intake is regulated via carbon dioxide concentration and pH of the blood. They concluded the calorigenic effect of food determined the amount eaten. Andersson and Larsson (1961) found that cooling in the preoptic areas of the hypothalamus increased feed intake in goats. When other areas were warmed, eating stopped. While these results provide direct evidence for thermosensitive areas in the central nervous system, Andersson and Larsson (1961) cautioned that interpreting this as clear-out evidence of a thermostatic controlling mechanism is impeded by the unavailability of tools for experiments to measure very small changes in the temperature of the hypothalamus. Since these areas were cooled by 9°C or warmed by 8°C, the temperature was probably outside any normal temperature range for the hypothalamus.

Simkins et al. (1965b) noted a decrease in feed intake in cows receiving intraruminal infusions of acetate (neutralized acetic acid). It was concluded that perhaps the heat released during assimilation of the acetate was responsible for the depression in food intake.

At temperatures slightly above the so called "neutral" zone, rats may refuse food completely (Brobeck, 1948), while heat stress has also been observed to inhibit feeding in human infants (Cooke, 1952). Herrington (1940) showed that at these warmer temperatures, the metabolic rate of rodents is definitely higher than at slightly lower temperatures. Therefore, the reduced intake cannot be the result of a lessened need for energy.

The temperatures of goats maintained in metabolism stalls was monitored at several sites during oral, intraruminal, and false intraruminal feeding to

test the applicability of the thermostatic hypothesis of food intake regulation in ruminants (Dinius et al., 1970). Thermistor probes were placed in the hypothalamus, rectum, rumen, horn, ear and chest, and temperatures from these locations were measured every two minutes from 30 minutes before feeding to 90 minutes after feeding. Hypothalamic temperature increased at the time of feeding, irrespective of treatments, and also increased when ruminal fluid was sampled. A decrease in hypothalamic temperature was noted when the goats lay down. Thus, it appeared that hypothalamic temperature was related to activity rather than the amount of food consumed, the goats being more active during eating. No correlation between food consumption and temperature change could be established at any site. Also, there appeared to be no correlation between ruminal VFA concentration and body temperature.

Further evidence that food consumption is not normally a function of hypothalamic temperature was given by Baile et al. (1967c). Needle thermistors were implanted in the hypothalamus of goats in an effort to investigate the relationship between feeding behavior and body temperature changes. Goats tested were given the following treatments: water ad libitum (control), feeding period after 20 hours fasting, 1000 gm grain force fed through a rumen cannula, 250 ml of 1.0 M acetic acid injected intraruminally for two hours, and 2 ml/min. of 2.5 M sodium acetate injected intraruminally for two hours. Hypothalamic temperature decreased following eating, force feeding, and both acid and salt injections. Since hypothalamic temperature did not increase during a large meal or force feeding, it was concluded that short term satiety in ruminants is not normally a function of hypothalamic temperature. The fact that hypothalamic temperature did not increase following intraruminal injections of acetate indicates that feed intake depression produced by intraruminal infusion of

acetate is not a result of increased hypothalamic temperature. Further evidence against the thermostatic theory of food intake regulation is presented by Baile and Mayer (1968). They again showed that the temperature of the medial hypothalamus of goats did not increase as a result of eating or force feeding.

Chemostatic Regulation of Feed Intake (Glucostatic Theory)

Experimental work by Bulatao and Carlson (1924) produced gastric hunger contractions in dogs by hypoglycemia and abolished them by hyperglycemia. Later work using rats, mice, dogs and human subjects resulted in the proposal by Mayer (1953) of a "glucostatic mechanism" of feed intake regulation. Mayer (1953) found that temporary increases in blood glucose levels obtained by injecting glucose, fructose or small amounts of epinephrine, correspond to decreases in food intake. Injections which did not influence blood glucose had no effect on food intake. Mayer and Bates (1952) emphasized that the concept of a glucostatic scheme of food intake regulation does not point so much to glucose as a chemical messenger as it does to its role as the essential metabolite of the central nervous system tissue. It is possible that other metabolites may have an action on food intake similar to that of glucose. It emphasizes only the special position of glucose in the regulation scheme of glucose. Results by Kennedy (1952) using rats support the hypothesis of Mayer (1953), but because wide variations in the chemical composition of the diet used had no effect on caloric intake, he concluded that control of feed intake is dependent upon the whole complex of metabolites in the blood stream.

Marshall et al. (1955) demonstrated that mice injected with goldthioglucose develop lesions in the ventro-medial nucleus of the hypothalamus and thus become hyperphagic and obese. Mayer and Marshall (1956) further showed that these lesions will occur only if the moiety attached to the gold atom by the sulfur

bridge is glucose. Galactose, sorbitol, malic acid, glycerol or caproic acid caused no lesions when used in place of glucose. From these results it was concluded that the regulation of food intake in monogastrics is dependent upon ventro-medial glucoreceptors which can be destroyed by goldthioglucose.

Baile et al. (1970) injected goldthioglucose in the carotid artery of goats and sheep for 30 minutes while the opposite carotid artery was occluded. Even though this treatment caused gold concentrations of the venous blood to be 30 to 40 times that of concentrations which caused hypothalamic lesions in rats and mice, the sheep and goats developed no lesions. It was concluded that the glucostatic mechanism as described for monogastric animals would not be a useful satiety mechanism for ruminants. Further evidence by Dowden and Jacobson (1960) showed that glucose at the 25% level of maintenance had no effect on feed intake of cows when injected intravenously for a period of eight hours on three successive days.

Chemostatic Regulation of Feed Intake (Chemoreceptor Theory)

While glucose is the major source of energy in the monogastric animal, the adult ruminant receives its energy mainly from the volatile fatty acids; acetic, propionic and butyric acids (Jones et al., 1970). Therefore, it seems logical to assume that chemoreceptors sensitive to these and other metabolites may be operative in the control of feed intake.

Several major metabolites have been examined for their effect on appetite in cows (Dowden and Jacobson, 1960). Solutions were injected intravenously for a period of eight hours on three successive days. Acetic acid, propionic acid and sodium acetate reduced feed intake when administered at the level of 12.5% of maintenance requirements. Dowden and Jacobson (1960) claimed that these results showed evidence of chemoreceptor response to changes

in specific blood constituents. Rook et al. (1960) injected acetic, propionic and butyric acids continuously with water at a rate of 3500 kcal per day in cows. Intraruminal injections of acetic acid reduced feed intake significantly in these cows. Using sheep, Holder (1963) was unable to confirm the depressing effect of sodium acetate. Balch and Campling (1962) have criticized the experiment of Dowden and Jacobson (1960) on the grounds that the acid concentration in the peripheral blood undoubtedly exceeded the normal physiological limits. When considering the possible regulatory role of metabolites derived from fermentation in the rumen, Balch and Campling (1962) pointed out that although meal fermentation begins soon after the first mouthful is swallowed, its peak is likely to be reached only after the animal has already stopped eating. In the experiments of Balch and Rowland (1957), for example, the peak of fermentation with mixed diets was reached from 2 to 6 hours after feeding.

The effects of various intraruminal volatile fatty acid infusions on the voluntary consumption of pellets in two dairy cows was tested by Simkins et al. (1965b). Acetate infusion resulted in a highly significant (p .01) reduction in feed intake while propionate produced a smalled reduction (p .05). The infusion of butyrate depressed consumption, but the decrease was not significant at the 5% level of significance. Thus it appeared that acetate, propionate and butyrate can act as satiety signal compounds in the regulation of food intake in ruminants. If chemoreceptors are involved, then the receptors must be sensitive to all three of the volatile fatty acids investigated, since all three caused a reduction in feed intake.

The intraruminal infusion of acetic acid significantly decreased hay consumption of dairy cows (Montgomery et al., 1963). Propionic and butyric acids, as well as lactic acid, caused only a slight decrease in hay consumption.

These results indicate that one or more of the metabolites studied (either in the rumen or circulating blood) are important in controlling intake.

Baile and Mayer (1969) showed that a close relationship exists between rate of injection of acetate, propionate or a mixture of volatile fatty acids and the depression of feed intake in goats. Injection rates in the experiment were adjusted to nearly match the physiological rates of production following introduction of readily fermentable feedstuffs. It seemed likely that the feeding-depressant effect of the volatile fatty acids can play a significant role in the over-all regulation of energy balance since: (1) they are important energy sources to the ruminant, (2) their rates of production increase with feeding and (3) they are the first and most immediate products of the digestive process to be absorbed. Butyrate was totally ineffective as a depressant. The depressant effect of acetate on feed intake was also reported by Montgomery et al. (1963). The intraruminal infusion of acetic acid significantly decreased hay consumption in dairy cows while propionic and butyric acids caused little decrease in hay intake.

Baile and Mclaughlin (1970) presented data to show that acetate injected into the dorsal rumen is more effective in depressing feed intake of goats than acetate injected into the ventral rumen or reticulum. Acetate (0.75 and 0.65 moles per day) injected into the dorsal rumen or abomasum decreased feed intake up to 30% in goats during spontaneous meals. The fact that ventral reticular injections were ineffective in depressing feed intake was interpreted as evidence that receptors for acetate do not exist in the reticulum.

Simkins et al. (1965a) reported a significant (P .05) increase in rumen concentrations of acetic, propionic, butyric and valeric acids two hours after lactating cows were fed a grain and hay diet ad libitum once daily. Blood

acetate concentration was directly proportional to rumen acetate concentration. Satiation occured when blood and rumen volatile fatty acid concentrations were at a maximum. When heifers were fed a completely pelleted ration (60% corn and 40% alfalfa meal) ad libitum once daily for a 3-hour period, blood acetate and ketone concentrations increased (P .01) after feeding. Since it is doubtful that rumen distention or gastrointestinal tract fill limited intake of the pelleted ration, it is probable that a chemostatic or thermostatic mechanism is present.

Physical Capacity as a Regulatory Mechanism

Perhaps the most significant anatomical barrier to feed intake is the capacity of the body cavity. Balch and Campling (1962) cited a study in which the compartments of the stomach of the cow were separated at post mortem, and it was found that all compartments could be stretched appreciably with small increases in pressure. It appears that limits on the volume occupied by the compartments of the gastro-intestinal tract depend on the volume of the abdominal cavity. Warner (1961) made similar observations with the calf rumen.

Blaxter (1950) found that the amount of feed consumed, measured in terms of dry matter, increases with increasing concentration of net energy in the ration. He also concluded that criteria were not available at the time to explain the difference in voluntary intake of cows. Crampton et al. (1960) suggested that recurring hunger in ruminants is primarily determined by rumen load.

Collection and removal of swallowed food at the cardia through a fistula induced cows to eat more (Campling and Balch, 1961). On the other hand, large bladders of water placed in the rumen for 10 to 14 days reduced feed intake by 24.5 gm per kgm of water. In order to test whether the reduced

intake caused by water bladders placed in the rumen was due to bulk or to space added to the rumen, Warner et al. (1966) treated steers with water-filled balloons containing water equal to 9% of body weight and air-filled balloons of a capacity equal to the amount of water corresponding to 7% of the live weight of the animal. The balloons were inserted just prior to feeding. Barrel circumference measurements were also taken during the feeding period. From the results it was clear that adding inert bulk reduced feed consumption. Further, it made little difference whether the bulk was of a density of 1.0 and thus sank or of a density of much less than 1.0 and floated. The crucial factor was the one of occupying space rather than a specific stimulus to the ventral or dorsal surface of the rumen. An increase in barrel circumference due to balloon addition was apparent. Carr and Jackson (1967) found that mass added to the rumen at 2, 6 and 10% of metabolic body weight (body weight $^{0.75}$) did not affect voluntary intake of hay. However, removal of rumen contents at the 10% level did have a significant effect.

Campling et al. (1962) observed that voluntary intake of cows fed hay or straw was related to the rates of disappearance of digesta and resulted in a constant amount of rumen digesta just before a meal. In a subsequent experiment by Freer et al. (1962), the amount of digesta in the rumens of cows fed grass hay was almost constant after each meal. These results indicate that as digestibility increases, limits on intake are set by factors other than physical capacity. McCullough and Russel (1962) reported that any influence of digestibility on intake declines when digestibility is above 65%. Conrad et al. (1966) has suggested that the breaking point is at the dry matter level of 66%. Beyond this point, body weight $^{0.75}$ seems to be the most accurate indicator of feed intake (Baumgardt, 1969). When ruminants are fed

rations with energy values similar to those of non-ruminant rations, it is clear that ruminants possess the ability to regulate feed intake on an energy basis.

It has been noted, however, that several factors may influence energy regulation of feed intake. Energy intake is often less on very high energy rations than it is on rations that are moderately high in energy (Flatt et al., 1966). One explanation might be that rumen pH decreases to the point where normal fermentation is impaired. Freer and Campling (1963) reported a lower digestible energy intake from animals fed all-concentrate than from animals fed dry grass when the feeding period was limited to 5 hours. One hour after feeding rumen pH was below 5 in the animal fed all-concentrate.

Blaxter (1961) conducted a series of experiments with sheep in an attempt to place on a quantitative basis the theory that the voluntary food intake of ruminants increases with the quality of fodder they are given. It was shown that voluntary intake varied with a fractional power of body weight close to 0.734. It was found that voluntary intake of long fodders was related to the apparent digestibility of their energy, increasing rapidly as digestibility increased from 38% to 70% and thereafter more slowly. The feeding of concentrated feed resulted in a drop in the voluntary intake of fodder. Calculations showed that the dry matter level of gut contents was the same irrespective of the quality of feed given.

Ward and Kelly (1969) reported differences in ad libitum consumption of diets consisting of : 5.5:1, 2.2:1 and 1:1 weight ratios of alfalfa hay to concentrate mixtrue by lactating cows. Total feed consumption increased consistently with each increase of concentrate: hay ratio. One kilogram of hay replaced 0.43 to 0.45 kg. grain in feed capacity on the average ad libitum

fed cow. Their results support the conclusion that rumen fill limits feed intake in rations containing 40% or more forage.

In order to further investigate voluntary feed and energy intake of rations varying in digestibility and density, Dinius and Baumgardt (1970) diluted a concentrate mixture with each of four diluents. The basic concentrate mixture was diluted from 5 to 50% at 5% increments with (1) oak sawdust, (2) oak sawdust with 3% kaolin clay, (3) verxite and (4) as in (1) except that nitrogen was kept constant at 17.4% crude protein equivalent. Each ration was pelleted and fed to four sheep in metabolism stalls. Density of the rations was measured on a dry matter basis. They observed that dry matter intake ($gm./Wkg.^{0.75}$) increased as the digestible energy per gram increased to 2.5 kcal for ration sequences 1, 2 and 3. With rations having digestible energy values above 2.5 kcal/gm. dry matter intake decreased and digestible energy intake ($kcal/Wkg.^{0.75}$) remained static. Apparently, fill limited intake in the sheep when digestible energy intake was below 2.5 kcal/gm., whereas energy intake was regulated above this level.

Boling et al. (1967) fed steers a basal hay, corn and soybean meal diet diluted with 14.2%, 21.8% and 29.3% shredded polyethylene fibers respectively. A trend toward compensatory food intake to meet energy requirements was noted in each case, indicating that energy concentration of the diet is of importance in the regulation of food intake. Similar work by Welch (1967) reported the effects of intraruminally added indigestible polypropylene fibers on roughage intake by sheep. In all cases, hay intake was reduced by presence of polypropylene fibers in the rumen. In sheep fed fibers 7 cm. in length, intake returned to normal as the fibers were passed out through the feces. Animals fed 30 cm. fibers retained virtually all these fibers and did not regain their

their previous hay consumption levels. It was concluded that one factor in roughage intake may be the rate of passage of indigestible fibers from the rumen.

Relationship Between Rumen pH and Feed Intake

It is well known that the normal pH or rumen contents approaches neutrality just prior to a meal. As feed is ingested the pH drops due to the formation of organic acids from the fermentation of the feed. It has also been well established that the rumencontents of animals fed primarily on grain have distinctly lower pH than with roughage diets (Warner et al., 1966). Gordon et al. (1961) has shown that the voluntary intake of silage by ruminants is lower than that of hay made from the same crop. This low level of intake may result in low levels of production from animals fed only on silage. The reasons for the low intake of silage have not been clearly established. The bulk of ingesta within the rumen has been found to limit the intake of a wide range of dried forages (Balch and Campling, 1962), but the quantity of dry matter contained in the rumen is less for animals fed silage ad libitum than for those fed hay ad libitum (Thomas et al., 1961a). The hypothesis that the restricted intake of silage might be caused by the high content of organic acids present in silage was investigated by King (1943) who found that the addition of sodium bicarbonate to neutralize the acids in silage before feeding resulted in increases in consumption.

Mcleod et al. (1970) altered the free-acid content of grass silages by the addition of sodium bicarbonate to raise pH and the addition of lactic acid to lower pH. In each of four comparisons the addition of sodium bicarbonate to increase pH from about 4.0 to about 5.4 resulted in significant increases in intake of dry matter which ranged from 9.7 to 20.7%. Intake of

organic matter was consistently increased by this partial neutralization treatment. The addition of sodium as sodium chloride rather than sodium bicarbonate did not alter the intake of a highly acid silage. Addition of lactic acid to reduce the pH of a silage feed from 5.4 to 3.8 resulted in a decrease in dry matter intake of 22%. It was concluded that the acids produced during the normal silage fermentation can limit the intake of the silage. Warner et al. (1966) infused rumen fistulated steers with sufficient quantities of each of three acids (phosphoric, lactic or citric) to reduce rumen pH to 6.0 prior to the feeding of hay. In each case, the average air dry hay intake of the steers decreased significantly ($p < .01$). Balch (1968) has suggested the lowered rumen pH on diets limited in roughage may be due to decreased production of saliva such that volatile fatty acids produced are not properly neutralized.

A relationship between pH of the rumen and voluntary intake on concentrates by ruminants has been noted by several workers. Montgomery and Baumgardt (1965b) working with heifers fed pelleted rations, noted that as the percent concentrate was increased in the ration, daily dry matter consumption decreased, accompanied by decreased rumen pH and changes in molar proportions of VFA's. Similar results were reported by Cowsert and Montgomery (1969) with heifers fed pelleted isonitrogenous rations at different concentrate levels. Along with a decrease in pH, an inhibition of cellulose digestion has been observed when concentrate is added to a roughage ration (Montgomery and Baumgardt, 1965b). This tends to suggest that the addition of concentrate causes marked changes in the rumen microbial population. The shift toward soluble carbohydrate fermentation produces a lowered pH and thus inhibits the activity of cellulolytic organisms.

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THIAMINE AND RUMEN HYPERALIMENTATION

When studying feed intake with the intent of increasing intake above the amount normally consumed, the possibility of metabolic complications must not be overlooked. This is especially true of ruminants, in which changes in feed type and feed amounts can greatly alter the types of products from rumen fermentations. A thiamine deficiency or inhibition of thiamine metabolism by some antimetabolite is one such possible complication arising from high energy concentrate rations.

Until recently very little has been known about the occurance of thiamine deficiency in ruminants. Davies et al. (1965) first showed the involvement of thiamine deficiency in causing cerebrocortical necrosis (CCN), also known as polioencephalomalacia (PEM), in sheep and cattle.

Climical signs of this disorder usually include dullness and ataxia, leading to collapse and convulsive struggling. Death in a state of coma after an illness lasting from 2-6 days usually results (Terlecki and Markson, 1961). At post-mortem examination the only consistent lesions are in the brain, and in a high proportion of cases the lesions are visible to the naked eye, consisting of areas of yellowish discoloration in the cerebral cortex. Histological examination shows characteristic lesions of neuronal necrosis, and microglial phagocytic reaction. The disseminated lesions of the cerebral cortex have been the only constant finding in the disease (Terlecki and Markson, 1961; Spence et al., 1961).

Edwin et al. (1968) reported that the age of highest incidence for PEM in sheep is at age 3-6 months, while Jubb and Kennedy (1967) state that the prime age group is from weaning to 18 months. However, both indicate that the disease may occur at later ages when the rumen is fully functional and no

extraneous supply of the vitamin should be necessary. Several possible causes of PEM have been examined.

Failure of biosynthesis is unlikely since an absence of thiamine in ruminal contents of affected animals has not been observed (Lewis et al., 1966); nor is PEM associated with deficiencies of other B complex vitamins. Malabsorption from the alimentary tract is also unlikely since response of PEM to administration of thiamine by stomach tube has been shown to be as effective as parental administration (Lewis et al., 1966). A combination of physiological, clinical and enzymic criteria was used by Benevenga et al. (1966) to characterize the onset of thiamine deficiency in calves fed a semi-purified diet. Blood pyruvate and lactate levels, and urinary pyruvate excretion increased, and liver transketolase decreased significantly, concomitantly with the appearance of overt deficiency symptoms. Draper (1951) studied the effect of omitting thiamine from the diet of the pre-ruminant lamb. Here also a uniform pattern was seen, characterized by anorexia in 2-3 weeks and a fall in growth rate followed in the fourth to fifth week by convulsions including opisthotonus. The tetanic spasms were similar to manifestations of thiamine deficiency in other species, and disappeared when thiamine was given. In these studies, however, brains were not examined. Lewis et al. (1967) fed a thiamine-deficient diet to lambs from 48 hours of age. The animals reached a crisis in 18-30 days and were killed in extremis. A fall in urine thiamine and rise in blood pyruvate was noted. The brain showed no gross lesions but histological examinations revealed slight changes in the cortex and thalamus resembling those found in field cases of PEM.

Thiamine deficiency can be induced in animals not only be feeding low-thiamine diets but by giving thiamine antagonists. These compounds may be

structurally similar to thiamine and act as true antimetabolites by competing with thiamine in several of its co-enzyme functions. Pyrithiamine, oxythiamine and amprolium are three such examples which have been studied extensively. Administration of these compounds to experimental animals is followed by a fall in growth rate and the manifestation of a number of effects which are also seen in nutritional thiamine deficiency. These are true antimetabolites since the deficiency symptoms can be reversed by the administration of thiamine.

When pyrithiamine was given to animals, thiamine pyrophosphate (TPP) was reduced in the tissues even though the diet may have contained free thiamine. The rate of disappearance of TPP was greater and the survival time markedly shortened when the animals (rats, mice and pigeons) were kept on thiamine-deficient diets (DeCarlo et al., 1958). The nature of the diet was also shown to have a profound effect on the rate of TPP depletion. A high carbohydrate diet was similar to a thiamine deficiency in this respect. Pyrithiamine has also been shown to displace thiamine from isolated nerve tissue. Cooper (1968) suggested that this action might result in interference with ion transport and consequently with nerve conduction.

The antithiamine potency of oxythiamine is not as great as that of pyrithiamine (Steyn-Parve, 1967). Urinary excretion of thiamine is increased to the same extent and the effect upon the brain is not so profound. However, high doses of oxythiamine can be toxic. Yonezawa and Iwanami (1966) have studied thiamine deficiency in nervous tissue using tissue culture techniques. The application of oxythiamine or pyrithiamine produced degenerative changes, either acute or chronic, depending on the concentration of the antimetabolites.

Amprolium is a much milder antagonist of thiamine than pyrithiamine or oxythiamine (Rogers, 1962). Pill et al. (1966) gave pyrithiamine in twice

daily injections of 10 mg over a period of 9 days to a pre-ruminant calf fed on a thiamine-low diet. After this treatment, amprolium was given orally for a period of 10 days. A condition indistinguishable from CCN was produced. Markson et al. (1966) produced CCN in four pre-ruminant calves by administering amprolium in a diet of calves milk.

After examining the various factors in the etiology of CCN, Edwin et al. (1968) suggested that a thiaminase might be involved. They showed that an antithiamine compound similar to amprolium might be formed from thiamine and the alpha-picoline in the alimentary tract of animals affected with CCN. The ruminal contents of calves and sheep affected with CCN have been examined and found to contain significant amounts of thiaminase (Edwin et al., 1968). Its presence was also demonstrated in intestinal contents and unvoided feces. Edwin and Lewis (1970) also reported that fecal samples from some in-contact animals were shown to contain thiaminase.

Dixon and Webb (1964) divide thiaminase into two types, thiaminase I and thiaminase II. Thiaminase I is found in freshwater fish, some invertebrates and in some plants. Thiaminase II is of bacterial origin and thus would be the logical suspect in cases of cerebrocortical necrosis in ruminants, especially ruminants being fed purified or semi-purified diets.

Edwin and Lewis (1970) concluded that CCN must be regarded as a particular form of thiamine deficiency, in which the ruminal supply of thiamine is cut off by the action of a thiaminase. The compound formed from the pyrimidine part of the thiamine molecule and the activating amine may have potent antithiamine activity which would further complicate the situation. This is probably the reason for the early involvement of cerebral functions in the disease, which does not occur in frank thiamine deficiency. Edwin and Lewis

(1970) further report that although this source of high concentrations of thiaminase in the rumen is unknown, there is increasing evidence that it is of microbial origin.

Edwin and Jackman (1970) showed the thiaminase in rumenal fluid from CCN cases to be of type I and to need nicotinic acid or nicotinamide as a specific activator. As a model for thiaminase I activity, thiamine and nicotinic acid were incubated with a culture medium of Bacillus thiaminolyticus, a compound resembling pyrithiamine and amprolium was produced. This data is in disagreement with Dixon and Webb (1964) who report that thiaminase I is of plant and animal origin.

INTRODUCTION TO RESEARCH

Since the rate or level of feed intake has such an important bearing upon the economics of livestock feeding, it has been the subject of much interest and research. Animals which voluntarily consume greater quantities of feed generally show an advantage in rate of gain over those on lower intake (Lofgreen and Garrett, 1968).

The mechanisms controlling voluntary intake in monogastric animals are fairly well understood. However, the question of controlling mechanisms in the ruminant animal have not been satisfactorily explained or described, and neither have the consequences of hyperalimentation.

As with other research, the first objective in studying appetite control and regulation is the control and standardization of all variables except those being studied. In this respect, the replacement of conventional feeding by a constant ruminal infusion of a complete ration would control practically all of the metabolic variation arising from the "meal eating" nature of the animals. The development of a complete liquid ration suitable for constant infusion and procedures for its introduction would be an invaluable tool for further studies on nutrient requirements and research into the mechanisms regulating appetite and satiety in the ruminant animal.

The present research is a continuation of work by Parks (1970) in an effort to develop a complete liquid diet with the ultimate objective of determining the metabolic capabilities of animals for increased feed intake, and assess the adequacy of the diet through measurement of rumen end products and blood components.

EXPERIMENTAL PROCEDURES

Five trials, four of which involved nitrogen balance and metabolism studies, were conducted to evaluate a liquid ration suitable for use in studying feed intake control and regulation. Fistulated wethers were placed in metabolism crates for 1-3 week preliminary periods before trials began in order to adjust to the crates and preliminary rations. Metabolism crates were constructed with sliding sides to allow access to the fistula and to facilitate the taking of blood samples. The left side was constructed of sheet metal to prevent damage to the canula. Animals were tethered in the crate by use of a chain attached to the neck. Urine was collected via a full length pan that drained into a collection vessel. Twenty ml of 36 N H_2SO_4 was added to the collection vessel to prevent ammonia volatilization. Five cm duct tape was secured with back tag cement around the perimeter of a 15 cm square surrounding the anus. Sheep were closely shorn before application and the wool around the anus washed with ether. Feces was collected by taping a plastic bag to this square with additional duct tape.

After the preliminary adaptation period, the animals were adjusted to a constant intake of a conventional ration (table 2) and nitrogen balance and digestibility studies were conducted (trials 1, 2, 3, and 5). Water was provided ad libitum.

Following nitrogen balance on preliminary diets the animals were placed on a complete liquid diet (table 1). The liquid diet was prepared fresh daily, and brought up to the desired volume with water in a plastic pipette washer. Stock mineral solutions were made up in 5-day supplies using distilled water. The ration was kept in suspension by use of a variable speed continuous

TABLE 1. LIQUID RATION COMPOSITION
Trials I and II

SOURCE	AMOUNTS[1]	
Corn starch	460.000	(230.000)[5]
Cane sugar	230.000	(115.000)[5]
Casein	50.000	
Urea	5.000	
$MnSO_4 \cdot H_2O$	137.070[2]	
$K_2CO_3 \cdot 1\frac{1}{2}H_2O$	37.396	
$ZnSO_4 \cdot 7H_2O$	391.304[2]	
$Na_2MoO_4 \cdot 2H_2O$	6.944[2]	
$CaCl_2$	9.138	
Na_2HPO_4	11.916	
$MnSO_4 \cdot 7H_2O$	10.761	
$MgCl_2 \cdot 6H_2O$	4.068	
NaCl	10.000	
$CoCl_2 \cdot 6H_2O$	0.806[2]	
$CuCl_2$	33.884[2]	
KI	0.980[2]	
$CrK(SO_4)_2 \cdot 12H_2O$	1.440[2]	
$FeCl_2 \cdot 4H_2O$	1.100	
Vitamin A[3]	2200.000[4]	
Vitamin D[3]	550.000[4]	
Vitamin E[3]	11.000[4]	

1/ Expressed in grams per day unless otherwise indicated
2/ mgs per day
3/ Water-dispersable
4/ Expressed as international units per day
5/ Concentration used after day 9 of infusion on trial I and as base concentration in trial II

TABLE 1a

DAILY REQUIREMENTS

Nutrient	Requirement[3]	Supplied
Mn	70.00[2]	77.06[2]
K	17.70	17.70
Zn	90.90[2]	88.95[2]
Mo	2.80[2]	2.75[2]
Ca	3.30	3.30
P	2.60	2.60
Mg	1.54	1.55
Na	3.95	7.79
Cl	6.07	13.33
Co	0.02[2]	0.02[2]
Cu	16.00[2]	16.00[2]
I	0.75[2]	0.75[2]
Cr	0.15[2]	0.15[2]
Fe	0.22	0.22
S	1.50	1.40
Vitamin A	2200 IU per day	2200 IU
Vitamin D	550 IU per day	550 IU
Vitamin E		11 IU

1/ Expressed in gm per day unless otherwise indicated
2/ Mg per day
3/ Values taken from N.R.C. and a purified diet (Sidhu et al., 1969)

duty stirring motor[1] and 3-blade stainless steel propeller. One to two gm poloxaline[2] was added to the ration to prevent foaming in the pipette washers. Constant 24 hour infusion of the diet was obtained by use of a variable speed peristaltic pump[3] using approximately 300 cm of Silastic[4] tubing (0.8mmID X 4.0mmOD). Water and conventional ration were removed when infusion was started.

Blood samples were obtained by juglar puncture using an 18 gauge needle. Twenty ml of blood were taken, of which approximately 3 ml were heparinized for hematocrit (HCt) and hemoglobin (Hb) determinations. The remainder was allowed to clot and the serum collected for mineral, serum protein and serum urea determinations. Hematocrit was determined by use of a Clay Adams micro hematocrit centrifuge. Hemoglobin was determined by colorimetric analysis described by Crosby et al. (1964). Serum protein was determined by differential spectrophotometry using the method of Waddell and Hill (1956). Sodium and potassium were determined by flame emission spectrophotometry and calcium, magnesium, zinc and copper by atomic absorption spectrophotometry (Ullery et al., 1967). Phosphorus was determined by the phosphomolybdate blue technique (Varley, 1960). Urea was measured by the p-dimethylaminobenzaldehyde method (Brown, 1959). Lactic acid was determined by a modification of the Barker and Summerson method in which samples were suitably diluted and read in test tubes.

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[1] Laboratory stirrer, GT21-18, G. K. Heller Corp., Las Vegas, Nev.

[2] Polyoxypropylene block polymer, Smith Kline and French Laboratories, No. 18667, Philadelphia, Pa.

[3] Cole-Parmer, pump model no. 7545-15, pump head no. 7013, 7425 North Oak Park Avenue, Chicago, Illinois 60648

[4] Silastic tubing, Cole-Parmer, 7425 North Oak Park Avenue, Chicago, Illinois 60648

Daily fecal samples were taken during N balance studies by removal and replacement of the plastic collection bag. Daily urine samples were also taken. Feed samples during the conventional feeding phase of the trials were taken once daily. Feed and fecal samples were analyzed for nitrogen by the Kjeldahl method (AOAC, 1955) after drying, grinding and blending. Two gm feed samples and 2 to 4 gm fecal samples were digested. Urine samples (2 ml) were also analyzed by the Kjeldahl method. Approximately 10% of the daily urine and feces were refrigerated or frozen respectively for other possible analyses. Gross energy of the feed and feces was determined by oxygen bomb calorimetry.

Rumen canulas were prepared by a modification of the method of Yarns and Putnam (1960). A hard plastic tube was placed in the neck of the canula and secured with a hose clamp. A rubber stopper with holes for the infusion and sampling tubes was fitted tightly into the hard plastic tube and secured with duct tape. The sampling tube consisted of a 9.5 mm ID plastic tube with several 3.18 mm holes along the internal end, which was corked. The external end was closed with a screw-type pinch clamp except when samples were being withdrawn. Rumen fluid samples were taken by fitting a drench gun to the sampling tube and aspirating.

Samples of rumen fluid were taken for pH determination and aliquots were frozen after addition of 0.5 ml conc. H_2SO_4 for volatile fatty acid analysis. Volatile fatty acids were separated on a 183.0 cm X 3.18 mm stainless steel column packed with 60-80 mesh Chromosorb 101[1], using a flash vaporization inlet, hydrogen flame detection, and an oven temperature of 190° C (isothermal). The carrier gas was nitrogen.

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[1]Chromosorb 101, Johns-Manville, 22 E. 40st, New York, New York 10016

Heart rate and electrocardiogram were monitored using a physiograph,[1] EKG-EMG-EEG transmitter,[2] and biotelemetry receiver.[3] Rectal temperature was measured with a scanning telethermometer[4] and rectal probes.[5] Temperature was recorded on a null balance potent 10 metric recorder.

Animals were weighed once weekly with corrections made for rumen liquid volume. Rumen liquid volume was estimated by injecting 10 ml of 10% $LiSO_4$ into the rumen and measuring the rate of disappearance of lithium. Least squares analysis of log lithium concentration versus time was carried out and the resulting regression equation used to calculate lithium concentration at time zero. This figure was used for the calculation of rumen liquid volume. Rumen fluid samples were withdrawn at 1, 3 and 6 hours after injection. Lithium was determined by flame emission spectrophotometry.

TRIAL I

A 47 kg wether was placed in a metabolism crate and fed 800 gm per day of a concentrate ration (composition shown in table 2). A seven day nitrogen balance study was conducted. Following this trial, infusion of the liquid diet (table 1) was begun at the rate of 6 liters per day. Nitrogen balance was computed at 7-day intervals beginning one week after the start of infusion. Rumen

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[1] Physiograph, desk model type DMP-4A, E and M Instrument Co. Inc., Houston, Texas

[2] EKG-EMG-EEG Transmitter, FM-1100-E2, E and M Instrument Co. Inc., Houston, Texas

[3] Biotelemetry Receiver, Model FM-1100-7, E and M Instrument Co. Inc., Houston, Texas

[4] Scanning tele-thermometer, YSI Model 47, Yellow Springs Instrument Co.

[5] General purpose thermistor probe, Model 404, Yellow Springs Instrument Co.

samples were obtained periodically for monitoring pH and also for microscopic examination. Sodium bicarbonate was given via the sampling tube when the pH of the rumen contents fell below 5.0. After a total of 30 days on the liquid diet, the animal developed an infected fistula and was taken to Dykstra Veterinary Hospital for examination. On recommendation of the examining veterinarian, the animal was sacrificed and a necropsy performed.

TRIAL II

A 50.2 kg wether was fed 800 gm of the same concentrate ration used in trial 1 in a metabolism crate. After the adjustment period, a nitrogen balance study was conducted for one week. At the conclusion of this study, the liquid diet was infused at the rate of 4 liters per day. Rumen samples were taken for pH and VFA determinations. Blood samples were taken for hemoglobin (Hb), hematocrit (HCt), mineral, serum protein and serum urea determinations. Nitrogen and energy balances were computed weekly during both conventional and liquid phases of the trial. On the 15th day of infusion, the starch and sugar were increased to raise the total energy of the ration to 110% of the original level shown in table 1. On the 17th day of infusion, the animal developed a temperature of 40.9^{o}C and showed rapid, labored breathing. The animal was taken to Dykstra Veterinary Hospital. No cause for the increased temperature or respiratory distress could be found and after a period of about four hours, the animal's condition was much improved. At this point the infusion was continued. On the 27th day of infusion, the starch and sugar were again increased to bring the total energy in the ration to 135% of the original ration (table 1). On the 28th day the animal again showed an elevated temperature and increased respiratory distress with the temperature reaching 41.1^{o}C. After

TABLE 2

Conventional Rations

TRIAL	MILO[1]	UREA[1]
I & II	800	8
III, IV, V	560	5

1/ Expressed in gm per day

29 days on the liquid ration, the animal died and was taken to Dykstra Veterinary Hospital for necropsy.

TRIAL III

Two growing fistulated wether lambs weighing 36.6 kg and 38.7 kg respectively were adapted to an all-concentrate ration (table 2) in metabolism crates for a period of two weeks. A seven day N and energy balance trial was then conducted on the grain-urea ration. Following this trial, the lambs were infused at the rate of 4 liters per day of the base level ration shown on table 3, using metabolic body size (wt 0.75) to calculate the energy necessary for maintenance. Rumen fluid was sampled daily for pH and aliquots frozen for VFA determination. Blood samples were taken for Hb, HCt, serum protein, serum minerals, and serum urea. Respiratory rate was counted periodically. On the eighth day of infusion the energy level was increased to 125% of maintenance (table 3). On the ninth day both lambs showed increased temperature and very apparent respiratory distress. Both died within an eight hour period on the tenth day of infusion. The animals were removed to Dykstra Veterinary Hospital for necropsy.

TRIAL IV

Two fistulated wether lambs weighing 35.0 and 37.0 kg respectively were adapted to a sorghum grain diet (table 2) for a period of two weeks in open pens and then placed in metabolism crates. Both crates were in an environmentally controlled room with temperature held at 22.5°C. After two days of adjustment to the crates, both animals were infused with the same maintenance diet used in trial III. Respiration rate, heart rate, electrocardiogram and rectal temperature were recorded. Urine and fecal samples were not taken.

TABLE 3

ENERGY LEVELS

Trials III, IV, V and VI[1]

LEVEL	STARCH[2]	SUGAR[2]	GROSS ENERGY[3] (Calculated)
Maintenance (Base)	375	185	2.444
125% Base	482	227	3.057
150% Base	588	289	3.666
175% Base	695	341	4.277

1/ Mineral concentrations same as on Table 1
2/ Expressed as gms per day
3/ Expressed as megacalories per day

Rumen fluid was withdrawn periodically to monitor pH. On the second day of infusion, energy was increased by 25% as in trial III by increasing the amounts of sugar and starch (table 3). On the fourth day of infusion one lamb developed a temperature of 41.1°C, respiratory rate of 38, and heart rate of 188. Thiamine Hydrochloride (200 mg) was immediately injected intravenously via the juglar vein and another two cc was injected intramuscularly. Heart rate, ECG, respiratory rate and temperature improved within two hours after medication. However the animal's condition worsened again after four hours and the animal died shortly afterward. The animal was removed to Dykstra Veterinary Hospital for necropsy and the brain removed for histological studies.

On the 5th day of infusion (four days at the 125% base level) the second animal showed labored breathing, heart rate of 190 and rectal temperature of 40.1°C. The animal was immediately treated with 200 mg thiamine via the juglar and 200 mg thiamine intramuscularly. After 30 minutes the animal was much improved. On the 6th day of infusion rectal temperature again reached 40.1°C, heart rate increased to 180 and respiratory distress was apparent. An intramuscular injection of 200 mg thiamine was made. Improvement was noted in temperature, heart rate, ECG and breathing after 90 minutes. No more thiamine was administered and on the 9th day of infusion, the same symptoms as before returned. Heart rate was over 180, breathing was difficult and rectal temperature was 41.2°C. Thiamine (200 mg) was administered intramuscularly. On the tenth day of infusion the animal was taken off test.

TRIAL V

Two growing fistulated wether lambs weighing 37.3 kg (lamb #1) and 42.3 kg (lamb #2) were adapted to an all concentrate diet in pens for a period of 3 weeks and then placed in the same metabolism crates used in trial IV.

Ambient temperature was again held at 22.5°C. After a 2-day adjustment period to the crates, both lambs were infused at the base energy level shown on table 3. Thiamine (thiamine hydrochloride) was added to the liquid ration at the rate of 100 mg per day. After two days for adjustment to the liquid ration a metabolism and nitrogen balance study was started. Both lambs were weighed at the start of the trial and at 7-day intervals thereafter. Lithium sulfate was added at each weighing to measure rumen liquid volume. Urine and fecal samples were taken daily. Blood samples were withdrawn periodically to measure hematocrit. Rumen fluid was sampled daily and aliquots frozen for VFA determination. After metabolism data had been gathered for 7 days the energy level of the ration was raised to the 125% base level (table 3). The energy level was raised again to 150% of base after 14 days of test with the thiamine level being raised to 150 mg per day. This level of energy was fed for 7 days and the energy was again raised to 175% of base. Within 24 hours after the increase to 175% of base it was apparent that rumen pH could not practically be held above 5.0. The energy level was reduced on the 25th day of infusion to the 150% level (table 3). On the 25th day of infusion, animal #2 died and was taken to Dykstra Veterinary Hospital for necropsy. On the 33rd day of infusion thiamine supplementation of the diet was ceased. The lamb died on the 41st day of infusion and was removed to Dykstra Veterinary Hospital for necropsy.

RESULTS AND DISCUSSION

TRIAL I

The milo and urea ration was designed to prepare the animal as much as possible for the high carbohydrate infusion diet. The amount fed during the nitrogen balance study furnished an average of 21.6 grams of nitrogen per day (table 4). Average nitrogen excretion in the combined feces and urine was approximately 21.8 grams of nitrogen per day, giving a net result of -0.17 gm per day.

During the infusion study, it was necessary to strain the liquid ration through four layers of cheesecloth in order to prevent clogging of the infusion tube. This procedure was maintained in all further infusion studies. Pipette washers were chosen as ration containers because their narrow base diameters allowed the stirring blades to almost cover the base and prevent settling out of ration components. No difficulty with settling was encountered in any of the trials. Six liters was chosen as ration volume because it seemed to be the least volume to adequately dilute the ration without unduly upsetting the normal urine volume of the animal. During infusion the urine volume increased from approximately 1500 ml per day on the conventional ration to about 4000 ml per day on the infusion diet. Feces remained solid throughout the trial.

On the 10th day of infusion, rumen pH was observed to drop to 4.7. It was decided to cut the amount of starch and sugar in the ration by one half to 115 gm sugar and 230 gm starch. At this level of energy rumen pH rose to 6.2 where it remained with little variability for the rest of the trial.

Nitrogen balance data (table 4) revealed a continuing negative nitrogen balance during the first three weeks of infusion. On the 21st day of infusion the amount of urea was increased from 4 to 5 gm per day and the amount of

TABLE 4

NITROGEN BALANCE STUDIES

Trial I

Week	Intake[2]	Feces[2]	Urine[2]	N-Balance[2]	Coeficient of Digestion (N)
		Trial I Conventional Ration[1]			
1	151.43	32.35	120.30	-1.23	78.10
		Trial I Liquid Ration[3]			
1	68.04	27.68	52.76	-12.40	59.36
2	52.92	16.32	52.69	-16.09	69.14
3	52.92	11.50	51.22	-9.80	78.21
4	55.76	6.66	50.04	-0.94	88.09

1/ 800 gm ground milo - 8 gm urea
2/ gm nitrogen per day
3/ 6 liters per day, composition as in table 1

casein from 40 to 50 gm per day, providing an increase of two gm of nitrogen per day. This increase resulted in an almost equilibrium state of nitrogen balance during the fourth week of infusion (-0.13 gm per day). Ruminal VFA analysis showed an increase in the acetate:propionate ratio during the infusion phase of the trial (table 5). While propionate and butyrate stayed within reasonable ranges, the acetate concentration was higher than could be expected for this type of high carbohydrate ration. Energy balance studies showed the infusion diet to have a digestibility of 93.0% for the last three weeks of infusion (table 6). The urea in the liquid ration could have contributed to the negative N-balance seen in this trial since it could have been absorbed from the rumen and excreted in the urine.

Microscopic examination of the rumen fluid samples revealed a decrease in protozoal numbers until protozoa disappeared during the second week of infusion. The ratio of cocci to rods increased with the continuation of infusion.

Distention of the rumen as seen by Parks (1970) was not observed during this trial, possibly because liquid volume was decreased from 12 to 6 liters per day.

The animal was taken off trial after 30 days of infusion due to an infection around the canula. This animal had been fistulated for several months and the muscles around the canula had stretched allowing rumen fluid to leak onto the surrounding tissues. On advice of the examining veterinarian, the animal was killed with an intravenous injection of pentabarbitol and a necropsy performed. All internal organs appeared normal. The rumen showed no perakeritosis and no liver abscesses were evident. Absence of digesta distal to the rumen indicated almost complete digestion and absorption in the rumen. The brain was not examined.

TABLE 5

RUMINAL VFA CONCENTRATIONS

Trial I

Day	Ration	Acetic (uM/ml)	(M%)[3]	Propionic (uM/ml)	(M%)	Butyric (uM/ml)	(M%)	A:P[4]
1	Conv.[1]	39.35	68.85	5.82	10.18	11.98	20.96	6.76
2		35.81	52.48	21.98	32.21	10.44	15.30	1.63
3		39.59	50.36	34.50	43.80	4.51	5.73	1.14
4		42.84	49.93	39.43	45.95	3.57	4.16	1.09
5		44.27	42.69	54.73	52.77	4.70	4.53	0.81
	means	40.37	52.86	31.29	36.98	7.04	10.13	
1	Liquid[2]	37.98	49.97	33.89	44.59	4.13	5.43	1.12
2		39.68	54.22	28.60	39.08	4.90	6.69	1.38
3		38.56	48.28	30.60	38.31	10.71	13.40	1.26
4		38.56	45.29	39.43	46.31	7.14	8.39	0.97
5		44.27	42.12	52.97	50.88	7.85	7.46	0.83
6		53.55	45.08	52.38	44.10	12.85	10.82	1.02
7		57.12	43.72	53.55	40.99	19.98	15.29	1.06
8		68.78	44.51	57.67	37.32	28.06	18.16	1.19
9		74.45	45.11	54.02	32.73	36.55	22.12	1.37
10		34.27	49.17	32.04	45.97	3.38	4.85	1.06
11		34.26	37.51	25.30	41.24	1.78	2.90	1.35
12		27.13	54.18	19.42	38.78	3.51	7.01	1.39
13		25.70	53.30	18.24	37.83	4.28	8.87	1.40
14		22.13	63.30	10.00	28.60	2.83	8.09	2.21

TABLE 5 (continued)

Day	Ration	Acetic (uM/ml)	Acetic (M%)[3]	Propionic (uM/ml)	Propionic (M%)	Butyric (uM/ml)	Butyric (M%)	A:P[4]
15	Liquid[5]	27.13	54.18	19.42	38.78	3.51	7.01	1.39
16		26.44	60.09	13.68	31.55	3.08	8.78	2.17
17		36.91	56.73	20.30	31.20	7.85	12.06	1.81
18		34.78	46.87	24.16	32.56	15.25	20.56	1.43
19		38.19	66.91	18.88	33.08	18.91	33.13	2.02
20		44.78	64.92	12.22	17.72	11.99	17.37	3.67
21		58.65	70.59	12.22	14.70	12.28	14.76	4.79
22		43.98	62.48	16.11	22.89	10.29	14.62	2.72
23		42.00	64.90	13.03	22.95	8.18	12.95	3.12
24		34.10	62.32	13.60	24.85	7.02	12.83	2.50
25		27.96	49.89	14.44	25.77	13.64	24.33	1.93
26		36.83	60.92	9.99	16.52	13.64	22.56	3.68
27	Liquid[6]	38.20	67.83	5.44	9.66	12.88	22.51	7.02
28		38.91	72.47	3.88	7.22	10.90	20.30	10.02
	Means	40.19	52.87	25.20	33.15	10.62	13.97	

1/ 800 gm per day milo + urea ration
2/ 8 liters per day, composition shown in table 1
3/ Molar percent
4/ Acetate:propionate ratio
5/ 110% of base energy
6/ 135% of base energy

TABLE 6

ENERGY BALANCE STUDIES

Trial I

Week	Intake[2]	Feces[2]	Absorbed[2]	Coeficients of Digestion
		Trial I Conventional Ration[1]		
1	29.997	3.398	26.599	88.67
		Trial I Liquid Ration[3]		
1	26.063	3.624	22.439	86.09
2	10.887	0.638	10.249	94.14
3	10.887	0.904	9.983	91.70
4	11.207	0.860	10.333	92.20

1/ 800 gm ground milo - 8 gm urea
2/ Megacalories per week
3/ 6 liters per day, composition as in table 1

TABLE 7

SERUM MINERAL CONCENTRATIONS

Trial I

Day	Ration	Na	K	Mg ug/ml	Ca	P	Cu	Zn
1	Conventional[1]	3196.0	17.0	27.6	98.9	64.1	1.2	0.96
3		3222.0	18.0	28.6	104.6	58.7	0.9	0.74
5		3114.0	16.3	29.2	130.0	58.0	3.0	0.71
7		3250.0	15.3	24.3	98.5	55.2	2.2	0.69
3	Liquid[2]	2980.0	16.0	27.0	99.0	59.4	5.8	0.52
8		2680.0	15.5	25.0	99.0	58.0	1.3	0.47
18		2575.0	15.8	26.8	101.0	65.0	1.5	0.52
25		3150.0	17.1	37.8	100.0	60.0	2.0	0.72
27		3000.0	16.8	39.0	110.0	58.3	1.8	0.80
28		3200.0	17.2	40.5	103.0	57.9	1.3	0.77

1/ 800 gm ground milo - 8 gm urea
2/ 6 liters per day, composition as in table 1

TABLE 8

HEMOTOLOGY

Blood HCt, Hb, Serum protein and Serum urea

Trial I

Day	Ration	HCt (%)	Hb (mg/100ml)	Serum protein (mg/100ml)	Serum urea (mg/100ml)
1	Conv.[1]	33	10.7		
3		33	11.9		
5		33	12.5		
8		32	12.2		21.6
3	Liquid[2]	32	11.4	7.17	
8		32	12.3		26.0
10		29	13.7		
18		33	12.4		
25		29	13.6		24.4
28		29	13.6	7.34	

1/ 800 gm milo - 8 gm urea
2/ 6 liters per day, composition shown in table 1

Blood samples were taken during both phases of the trial and revealed no changes in serum mineral concentrations (table 7), serum protein or serum urea concentrations (table 8). Hematocrit and hemoglobin values suggest a slight hemodilution during the liquid phase of the trial although these changes were very small.

TRIAL II

The conventional diet used in this trial was the same as in trial I. Nitrogen balance data shown in table 9 indicates that this diet resulted in a positive nitrogen balance of 3.51 gm per day.

When liquid infusion was begun, it was found that the Silastic tubing would permit reduction of the liquid volume from 6 liters to 4 liters per day. Since the normal water intake and urine volume for this animal were very small, this reduction was made to minimize disruption of body functions. The animal was weighed just prior to the start of infusion and once every seven days afterward. Linear regression analysis of lithium sulfate measurements showed little change in rumen liquid volume during the liquid phase of the infusion study. Average rumen volume for the trial was 3.929 L. The animal lost an average of 1.39 kg per week while on the infusion ration suggesting that the ration was low in energy.

Nitrogen balance data for the infusion phase of the trial (table 9) shows that the animal was in a slightly negative balance for the second and third weeks of infusion and in a very negative balance (-7.21 gm per day) during the fourth week. Energy balance data (table 10) indicates that the digestibility coeficient for this animal was much lower on the liquid ration than the animal in trial I.

Blood analysis showed that serum mineral concentrations (table 11) did not change between the two phases of feeding. Hematocrit and hemoglobin

TABLE 9

NITROGEN BALANCE STUDIES

Trial II

Week	Intake[2]	Feces[2]	Urine[2]	N-Balance[2]	Coeficient of Digestion
		Trial II Conventional Ration[1]			
1	95.64	15.79	55.30	24.55	83.49
		Trial II Liquid Ration[3]			
1	69.72	13.84	91.96	-36.08	80.14
2	66.34	14.40	58.51	- 6.57	78.29
3	69.72	21.05	52.31	- 3.64	69.80
4	69.72	28.08	92.12	-50.48	59.87

1/ 800 gm milo - 8 gm urea
2/ gm Nitrogen per week
3/ 4 liters per day

TABLE 10

ENERGY BALANCE STUDIES

Trial II

Week	Intake[2]	Feces[2]	Absorbed[2]	Coeficient of Digestion
		Trial II Conventional Ration[1]		
1	29.997	5.427	24.570	81.91
		Trial II Liquid Ration[3]		
1	10.887	3.892	6.995	64.25
2	10.887	2.887	8.000	73.48
3	12.096	4.427	7.669	63.40
4	12.096	5.396	6.700	44.61

1/ 800 gm milo - 8 gm urea
2/ Megacalories per week
3/ 4 liters per day, composition as shown in table 1

TABLE 11

SERUM MINERAL CONCENTRATIONS

Trial II

Day	Ration	Na	K	Mg ug/ml	Ca	P	Cu	Zn
3	Conv.[1]	3185.0	185.0	33.0	172.5	61.5	1.90	0.65
1	Liquid[2]	3300.0	179.6	31.4	127.4	55.0	1.80	0.92
7		3589.0	189.8	42.3	119.5	50.7	2.16	0.74
14		3200.0	162.0	31.3	150.7	50.2	2.32	0.70
21		3260.0	160.0	31.0	148.6	53.9	1.92	0.80
28		3320.0	172.4	32.5	149.5	58.1	2.00	0.75

1/ 800 gm milo - 8 gm urea
2/ 4 liters per day, composition as in table 1

values (table 12) seemed to indicate a slight hemodilution although values remained within the "normal" range (Merck Veterinary Manual, 1957). Average rumen pH throughout the liquid phase of the trial was approximately 6.4± 0.2 after the first week of infusion during which rumen pH was around 5.8.

On the 15th day of infusion, the total energy level of the ration was increased to 110% by increasing the amounts of starch and sugar in the ration. Two days after this increase the animal developed labored breathing and a rectal temperature of 40.9°C. It was immediately taken to Dykstra Veterinary Hospital for examination. No apparent cause for the respiratory distress and increase in temperature could be found. An antibiotic was administered intramusculary and a blood sample drawn for examination. The animal improved after about four hours at the clinic and was returned to the infusion ration at the 110% level. The blood sample revealed nothing to explain the condition. On the 27th day of infusion the energy level of the ration was increased to 135% of the base level. On the 28th day the animal appeared very dull, showed difficulty in breathing and had a rectal temperature of 41.1°C. Approximately 12 hours later, on the 29th day of infusion, the animal died and was taken to Dykstra Veterminary Hospital for necropsy. The necropsy revealed fluid in lungs, trachea, pericardial sac and thorax. All other organs appeared normal. The skull was not opened.

Ruminal VFA analysis showed the acetate:propionate ratio to be higher on the liquid ration than on the conventional diet. Total VFA concentration increased with each increase in energy level of the liquid ration (table 13).

TRIAL III

The animals used in this trial were young and growing, and not as mature as those used in trials I and II. About 30 days after the fistulation

TABLE 12

HEMOTOLOGY

Blood HCt, Hb, Serum protein and Serum urea

Trial II

Day	Ration	HCt (%)	Hb (mg/100ml)	Serum protein (mg/100ml)	Serum urea (mg/100ml)
4	Conv.[1]	34	11.4	6.53	25.0
2	Liquid[2]	34	11.8		
8		28	13.7		20.7
15		32	13.6		11.5
22		24	10.0	6.92	19.9
28		24	9.8		18.4

1/ 800 gm milo - 8 gm urea
2/ 4 liters per day, composition shown in table 1

TABLE 13

RUMINAL VFA CONCENTRATIONS

Trial II

Day	Ration	Acetic (uM/ml)	Acetic (M%)[3]	Propionic (uM/ml)	Propionic (M%)	Butyric (uM/ml)	Butyric (M%)	A:P[4]
1	Conv.[1]	55.00	52.24	42.12	40.01	8.16	7.72	1.29
2		61.88	54.77	41.58	36.80	9.52	8.43	1.49
3		63.12	52.72	39.42	33.56	14.96	12.72	1.60
4		53.90	54.12	36.53	36.68	9.20	9.24	1.47
5		50.14	50.62	39.12	39.49	9.80	9.89	1.28
	means	56.81	53.15	39.75	37.19	10.33	9.66	
1	Liquid[2]	34.71	57.45	19.21	31.79	6.50	10.76	1.80
3		42.98	62.40	15.90	23.08	10.00	14.52	2.70
4		60.79	65.58	19.91	21.48	12.00	12.94	3.06
5		96.89	63.95	33.18	21.90	21.43	14.14	2.92
6		79.26	58.31	42.03	30.96	14.57	10.73	1.88
7		66.84	63.42	21.00	17.00	35.67	20.78	3.18
8		37.89	57.50	14.50	22.01	13.50	20.49	2.61
9		36.84	65.97	4.00	7.16	15.00	26.86	9.21
10		36.84	50.43	7.00	9.57	29.25	40.03	5.26
11		18.95	61.22	4.00	12.92	8.00	25.85	4.73
12		23.16	83.73	3.00	10.85	1.50	5.42	7.72
13		47.37	74.75	10.00	15.78	6.00	9.47	4.73
14		27.37	78.47	3.00	8.60	4.50	12.90	9.12
	means	46.91	61.93	15.13	19.98	13.69	18.08	

TABLE 13 (continued)

Day	Ration	Acetic (uM/ml)	(M%)[3]	Propionic (uM/ml)	(M%)	Butyric (uM/ml)	(M%)	A:P[4]
15[3]		54.13	60.53	19.78	22.12	15.52	17.35	2.73
16		40.53	69.54	11.00	18.87	6.75	11.58	3.68
17		45.79	64.46	14.50	20.41	9.75	13.72	3.15
18		45.79	66.56	14.00	20.35	9.00	13.08	3.27
19		34.74	61.23	7.00	12.34	15.00	26.44	4.96
20		48.19	59.13	16.65	20.43	16.66	21.44	2.89
21		56.68	62.26	20.19	22.18	14.16	15.56	2.80
22		61.00	60.75	27.75	27.63	11.66	11.44	2.19
23		36.80	58.53	11.82	18.80	14.25	22.66	3.11
24		42.50	68.44	12.28	17.05	17.25	23.95	3.09
25		43.40	68.60	14.01	22.09	6.00	9.46	3.46
	means	46.32	62.56	15.36	20.74	12.36	16.69	
27[6]		66.60	52.28	28.04	22.01	32.75	25.71	2.38
28		87.75	67.34	21.80	16.73	20.75	15.92	4.00
29		84.50	67.45	25.02	19.97	15.75	12.57	3.38
	means	79.62	62.37	24.95	19.54	23.08	18.08	

1/ 800 gm milo - 8 gm urea
2/ 4 liters per day - 230 gm starch + 115 gm sugar
3/ Molar percent
4/ Acetate:propionate ratio
5/ 4 liters per day - 278 gm starch + 132 gm sugar
6/ 4 liters per day - 310 gm starch + 155 gm sugar

procedure, both lambs were placed in metabolism crates and adapted to a milo and urea diet shown on table 3 for a period of one week. A nitrogen and energy balance trial was then conducted on this conventional ration. The animals showed positive N balances of 2.52 and 0.16 gm per day respectively for animals 1 and 2. Energy balance data is shown on table 15.

The maintenance level of energy used in both the conventional and liquid phases of this trial was calculated according to metabolic body size. The fact that lamb #1 gained 0.45 kg and lamb #2 lost 0.9 kg during the first week of infusion indicates that the energy level of the ration was very close to maintenance.

Nitrogen balance and energy balance data shown in tables 14 and 15 suggests that neither lamb achieved a positive N balance on the infusion ration although #1 was very close during days 8 and 9, and #2 was much improved during the second week of infusion.

At the end of the first week of infusion, both lambs appeared healthy. Rectal temperatures were normal. The feces of both lambs were loose but not to the point of diarrhea. On the 8th day of infusion the energy level of the ration was raised to 125% of maintenance. By the end of the ninth day, both lambs had rectal temperatures of 40.6^{o}C and appeared very dull. Both died in an eight hour period on the tenth day.

Necropsy showed slight accumulations of froth in the tracheas of both lambs. No other abnormalities could be found. Again the skulls were not opened. Throughout the trial no significant changes could be found in serum mineral (table 16), serum protein or serum urea. A very slight hemodilution was again noted in HCt data (table 17).

TABLE 14

NITROGEN BALANCE STUDIES

Trial III

Week	Intake[2]	Feces[2]	Urine[2]	N-Balance[2]	Coeficient of Digestion
		Trial III Conventional Ration[1]			
		Lamb #1			
1	56.00	3.97	34.35	+17.66	92.88
		Trial III Liquid Ration[2]			
1	55.76	2.83	68.81	-15.88	94.94
2	19.92	1.87	12.86	+ 5.19	90.61
		Trial III Conventional Ration[1]			
		Lamb #2			
1	56.00	5.59	49.30	+ 1.10	90.01
		Trial III Liquid Ration[3]			
1	55.76	8.31	67.60	-20.15	85.09
2	19.92	3.65	16.06	+ 0.21	81.69

1/ 560 gm milo - 5 gm urea
2/ gm N per week
3/ maintenance level for week 1 (table 3)
125% maintenance for week 2

TABLE 15

ENERGY BALANCE STUDIES

Trial III

Week	Intake[2]	Feces[2]	Absorbed[2]	Coeficients of Digestion
		Trial III Conventional Ration[1]		
		Lamb #1		
1	16.870	1.574	15.296	90.66
		Trial III Liquid Ration[3]		
1	17.108	874	16.234	94.89
2	6.517	581	5.986	91.85
		Trial III Conventional Ration[1]		
		Lamb #2		
1	16.870	1.931	14.939	88.55
		Trial III Liquid Ration[3]		
1	17.108	2.308	14.800	86.51
2	6.517	1.629	4.888	75.00

1/ 560 gm milo - 5 gm urea
2/ megacalories per week
3/ maintenance level for week 1
125% maintenance for week 2

TABLE 16

SERUM MINERAL CONCENTRATIONS

Trial III

Day	Ration	Na	K	Mg ug/ml	Ca	P	Cu	Zn
				Lamb #1				
5	Conv.[1]	2920.0	160.0	24.6	119.0	55.0	1.81	0.85
7	Liquid[2]	3100.0	182.4	31.3	123.9	55.0	1.76	0.86
				Lamb #2				
5	Conv.[1]	3182.0	173.0	24.6	119.5	57.5	2.25	0.80
7	Liquid[2]	3120.0	165.0	31.0	120.6	54.4	2.18	0.90

1/ 560 gm milo - 5 gm urea
2/ 4 liters per day, composition as in table 3

TABLE 17

HEMOTOLOGY

Blood HCt, Hb, Serum protein and Serum urea

Trial III

Day	Ration	HCt (%)	Hb (mg/100ml)	Serum protein (mg/100ml)	Serum urea (mg/100ml)
			Lamb #1		
3	Conv.[1]	33	12.5	6.04	25.92
7	Liquid[2]	31	11.8		24.40
			Lamb #2		
3	Conv.[1]	34	13.8	5.43	26.16
7	Liquid[2]	32	12.4	8.24	22.08

1/ 560 gm milo - 5 gm urea
2/ 4 liters per day, composition as in table 3

TRIAL IV

This trial was designed to investigate the possible involvement of a thiamine deficiency in the unexplained deaths of animals in trials II and III. Since previous trials showed the liquid ration to maintain serum mineral, serum protein and serum urea concentrations within normal limits, no serum analyses were conducted during this trial. Trials I, II and III indicated that the condition was related to energy level of the ration, therefore, the energy level of the ration was increased by 25% on the second day of infusion in order to reproduce the syndrome as quickly as possible.

The first animal showed symptoms on the 4th day of infusion. The lamb was comatose when thiamine was injected. Within two hours after administration of thiamine, rectal temperature had dropped to 40.0°C, heart rate was 165, and the animal appeared to be more alert. The animal was able to stand three hours after injection of thiamine. Electrocardiograms were examined by a cardiologist[1] from the College of Veterinary Medicine showed a definite cardiac response to administration of thiamine. With the onset of symptoms an increase in the length of the QRS complex could be seen in the ECG charts indicating some interruption in conductivity of the myocardial cells. A decrease in heart rate was also noted with the administration of thiamine. The animal relapsed about four hours after medication and died approximately 10 hours later. Histological studies revealed that death was due to polioencephalomalacia. Brain slides showed neuronal necrosis in the cerebral cortex along with capillary activation. Apparently the brain was too badly damaged at the time of thiamine injection to permit recovery.

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[1] Thanks are extended to Dr. Stanley Harris, Department of Surgery and Medicine, for aid in interpreting Electro Cardiographic data.

Approximately 12 hours after the death of the first lamb the second lamb showed similar symptoms with temperature 40.2°C and heart rate of 190. The animal was not yet comatose. Thiamine (200 mg IV and 200 mg IM as before) was injected and an improvement seen in 30 minutes. Heart rate slowed to 120 and temperature dropped to 39.5°C (0.4°C above normal for this animal) within 3½ hours after medication. Again a lengthening of QRS interval with the onset of symptoms and a shortening of the QRS soon after injection of thiamine was seen.

Twenty eight hours after the first injection of thiamine, rectal temperature again rose to 40.0°C, although heart rate rose only to 120. Since the condition of the animal was not as severe as before, no thiamine was injected IV. Heart rate decreased to 90 by 1½ hours after medication and rectal temperature dropped to 39.4°C within 10 hours after injection. The animal remained alert with heart rate and rectal temperature within normal limits until the 9th day of infusion when heart rate increased to 180 and rectal temperature rose to 40.2°C. Thiamine (200 mg) was injected IM. After two hours rectal temperature was 39.6°C, heart rate was 90 and breathing was improved. On the tenth day of infusion heart rate was 90 and rectal temperature 39.4°C. The lamb was taken off test and returned to pen feeding. The development of PEM in the first lamb and the response of the second lamb to thiamine injections seemed to show the involvement of thiamine deficiency in the deaths of lambs in trials II and III. Further the fact that these lambs were ruminants and should have been synthesizing thiamine, together with the sudden onset of symptoms indicates that an antimetabolite rather than a frank thiamine deficiency is present.

TRIAL V

This trial was conducted with three objectives in mind: to test the adequacy of the infusion ration over an extended period of time at different energy levels; to test the effect of adding thiamine to the ration; and finally to produce polioencephalomalacia by removing thiamine supplementation from the ration of one animal.

Nitrogen balance data (tables 18 and 19) shows that both animals were in a positive N-balance after the first week of infusion. Coefficients of digestion for crude fiber and ether extract were not computed on the liquid rations since no crude fiber or ether extract was contained in the ration. However endogenous crude fat in concentrations of around 3% did appear in proximate analysis of feces from the infusion ration as did crude fiber at approximately the 2% level. Each animal lost 3.18 kg during the first week. The weight loss may have been due partly to the animals adjusting to the infusion ration and partly to the fact that the 100% base level (Table 3) was below maintenance for these animals. However, this level of energy was used during the first week of infusion because a higher level would probably have caused acidosis. Lithium sulfate data showed little change in rumen liquid volume for both lambs throughout the liquid phase of the trial. Average rumen liquid volumes were 4.020 L and 2.408 L for lambs 1 and 2 respectively. During the second week of infusion with energy at the 125% base level lamb #1 lost 0.45 kg while lamb #2 gained 0.45 kg. This would indicate that the ration was just meeting maintenance requirements. Highest gains were made during the third week of infusion when gains were 4.3 kg and 4.1 kg respectively for the week. Energy level for the third week was 150% of base (Table 3).

Twenty four hours after the increase to 175% base level of energy rumen pH fell to 4.6 in lamb #1 and 4.8 in lamb #2. Apparently the rumen could not

TABLE 18

NITROGEN BALANCE STUDIES

Trial V

Lamb #1

Day	Ration	Intake[1]	Feces[1]	Urine[1]	N-Balance[1]
4	Liquid[2]	9.96	2.70	7.75	-0.49
5		9.96	2.43	7.88	+0.35
6		9.96	2.40	7.00	+0.56
7		9.96	4.00	8.84	-2.88
8	Liquid[3]	9.96	3.10	9.10	-2.44
9		9.96	2.35	7.08	+0.56
10		9.96	3.68	9.96	-3.38
11		9.96	2.70	6.98	+0.28
12		9.96	3.63	5.17	+0.26
13		9.96	4.99	5.29	-0.32
14		9.96	3.10	7.68	-0.82
15		9.96	1.78	7.32	+0.86
16	Liquid[4]	9.96	1.99	7.23	+0.04
17		9.96	1.99	5.78	+2.19
18		9.96	0.74	7.04	+2.08
19		9.96	2.46	3.30	+4.20
20		9.96	2.60	11.02	-3.66
21		9.96	2.19	8.09	-0.32
22		9.96	----[6]	8.34	+1.62
23		9.96	----[6]	8.87	+1.09

TABLE 18 (continued)

Day	Ration	Intake[1]	Feces[1]	Urine[1]	N-Balance[1]
24	Liquid[5]	9.96	3.01	4.70	+2.66
25	Liquid[4]	9.96	2.97	5.51	+1.87
26		9.96	2.48	6.84	+0.51
27		9.96	2.60	7.38	-0.02
28	Liquid[4]	9.96	3.40	6.20	+0.36
29		9.96	2.91	6.28	+0.75
30		9.96	2.33	9.32	-1.69
31		9.96	1.76	5.91	+2.29
32		9.96	2.80	7.45	-0.29
33		9.96	2.25	6.04	+1.67
34		9.96	----[6]	7.89	+2.07
35		9.96	----[6]	6.42	+3.54
36		9.96	----[6]	7.56	+2.40
37		9.96	----[6]	12.10	-2.44
38		9.96	----[6]	21.07	-11.11
39		9.96	----[6]	18.87	-8.91

1/ Expressed as gm per day
2/ 100% base level (table 3)
3/ 125% base level (table 3)
4/ 150% base level (table 3
5/ 175% base level (table 3)
6/ On these days the lamb was in a state of diarrhea and separation of urine and feces was impossible. Analysis was made on a sample of urine and feces combined.

TABLE 19

NITROGEN BALANCE STUDIES

Trial V

Lamb #2

Day	Ration	Intake[1]	Feces[1]	Urine[1]	N-Balance[1]
4	Liquid[2]	9.96	3.38	11.25	-4.68
5		9.96	3.46	9.25	-2.75
6		9.96	3.20	10.07	-3.31
7		9.96	6.65	15.63	-12.32
8	Liquid[3]	9.96	2.55	6.75	-0.66
9		9.96	3.81	11.72	-5.51
10		9.96	1.57	3.91	+4.88
11		9.96	2.12	7.50	+0.36
12		9.96	1.87	7.92	+0.15
13		9.96	2.08	11.41	-3.53
14		9.96	1.20	7.86	+1.20
15		9.96	3.01	5.05	+1.90
16	Liquid[4]	9.96	3.98	5.63	+0.35
17		9.96	----[6]	7.52	+2.44
18		9.96	----[6]	6.67	+3.29
20		9.96	----[6]	7.28	+2.68
21		9.96	3.09	10.01	-3.13
22		9.96	3.51	6.66	-0.21

TABLE 19 (continued)

Day	Ration	Intake[1]	Feces[1]	Urine[1]	N-Balance[1]
24	Liquid[5]	9.96	2.33	8.85	-1.11
25	Liquid[4]	9.96	3.86	16.98	-9.96

1/ Expressed as gm per day
2/ 100% base level (table 3)
3/ 125% base level (table 3)
4/ 150% base level (table 3)
5/ 175% base level (table 3)
6/ On these days the lamb was in a state of diarrhea and separation on urine and feces was impossible. Analysis was made on a sample of urine and feces combined.

metabolize the amount of starch in the 175% energy level ration. Necropsy on lamb #2 revealed that death was due to a rupture in the rumen. Approximately 300 gm of undigested starch was found caked in the rumen. The fact that rumen liquid volume in lamb #2 was smaller than in lamb #1 could have contributed to the inability to digest the starch. Histology revealed no evidence of polioencephalomalacia or any other organ damage.

Lamb #1 remained in good condition and gained 0.23 kg per day during the fourth week of infusion. Rectal temperature increased approximately 2 degrees C while the 150% energy level was being infused. This was apparently due to increased metabolism. About 48 hours after thiamine was removed from the ration, the heart rate of lamb #1 increased from 100 to 180 where it remained for three days. Five days after the removal of thiamine from the ration heart rate increased to 220. The lamb was unable to stand, appeared very dull and showed difficulty in breathing. Occasional muscle spasms were observed. This condition continued until the lamb died nine days after the removal of thiamine from the ration. Examination of ECG charts revealed no change in length of QRS interval as had been noted in trial IV. Gross examination of the brain showed swelling of the cerebrum. Histological studies showed severe polioencephalomalacia. The fact that lamb #2 received exactly the same ration as lamb #1 for almost the same length of time with the only difference in treatment being the removal of thiamine from the ration of #2, shows quite clearly the ability of the infusion procedure to induce polioencephalomalacia in experimental animals. This should provide an execellent model for the study of PEM since specific antimetabolites were previously required to experimentally produce the syndrome. This data also seems to point to rumen bacteria as the source of thiaminase in diet-initiated PEM. Since the

animals received only the semi-purified liquid diet, no extraneous plant or animal source of thiaminase or thiamine antimetabolite was possible. Further the short time required to induce PEM with the liquid ration indicates that an antimetabolite (or antimetabolites) may be formed and is also of bacterial origin, possibly the result of a thiaminase altering the thiamine molecule.

Severe diarrhea was observed from 48 hours after the removal of thiamine from the ration until death occurred. This may have been the result of central nervous damage due to thiamine deficiency. Lactic acid analysis on urine-fecal mixtures for the last week on infusion showed that lactate excretion increased from 15.6 ug/ml on the 38th day of infusion to 134.0 ug/ml 12 hours before death. Volatile fatty acid analysis showed (table 21 and 22) a marked increase in the amount and molar per cent of butyrate in the rumen as energy was increased to the 150% base level. The data from this trial indicates that sheep can be satisfactorily maintained on this infusion ration if thiamine is added to the ration. The positive N-balance, weight gains and good condition of both lambs at death seem to show the ration to be nutritionally adequate. The ration could be improved, however, both from a standpoint of nutrition and also for ease of infusion. More protein could be added to the diet to make the N-balance more positive. Substituting a water soluble carbohydrate source such as corn sugar for insoluble corn starch would help eliminate plugging of infusion tubes, and would permit the infusion of much higher energy levels with much less water than would be possible with corn starch.

TABLE 20

RUMINAL LACTIC ACID CONCENTRATIONS

Trial	Lamb #	Day	Ration	Lactic Acid (ug/ml rumen fluid)
I		1	Liquid[1]	1.61
I		12	Liquid[1]	1.48
I		19	Liquid[2]	5.88
I		24	Liquid[2]	1.99
I		29	Liquid[2]	16.93
II		1	Liquid[2]	2.19
II		7	Liquid[2]	269.30
II		14	Liquid[2]	11.47
II		21	Liquid[3]	331.43
II		29	Liquid[4]	17.54
III	1	5	Conv.	6.26
III	1	7	Liquid[5]	241.00
III	1	9	Liquid[6]	355.95
III	2	5	Conv.	21.72
III	2	7	Liquid[5]	349.50
III	2	9	Liquid[6]	2.11
V	1	2	Liquid[5]	1.37
V	1	9	Liquid[6]	7.57
V	1	16	Liquid[7]	11.07
V	1	23	Liquid[7]	587.95
V	1	30	Liquid[7]	131.35

TABLE 20 (continued)

Trial	Lamb #	Day	Ration	Lactic Acid (ug/ml rumen fluid)
V	1	40	Liquid[7]	379.00
V	2	2	Liquid[5]	40.10
V	2	9	Liquid[6]	36.96
V	2	16	Liquid[7]	131.01
V	2	23	Liquid[7]	83.33

1/ Composition shown in table 1
2/ Composition shown in table 1
3/ 110% of level shown in table 1
4/ 125% of level shown in table 1
5/ 100% base level (table 3)
6/ 125% base level (table 3)
7/ 150% base level (table 3)

TABLE 21

RUMINAL VFA CONCENTRATIONS

Trial V

Lamb #1

Day	Ration	Acetic (uM/ml)	(M%)[3]	Propionic (uM/ml)	(M%)	Butyric (uM/ml)	(M%)	A:P[2]
2	Conv.	47.16	59.16	18.59	23.58	13.16	16.68	2.54
4	Liquid[1]	69.56	81.16	11.44	13.35	4.71	5.50	6.08
7		59.66	53.32	37.18	33.23	15.05	13.45	1.60
8	Liquid[4]	25.12	60.87	11.44	27.72	4.71	11.41	2.19
14		83.21	62.25	45.76	34.32	4.70	3.52	1.82
15		86.35	59.10	55.06	37.68	4.71	3.22	1.57
18	Liquid[5]	99.69	67.03	44.33	29.81	4.71	3.71	2.25
19		53.38	48.06	25.74	23.17	31.96	28.77	2.07
20		78.50	58.99	25.74	19.84	29.14	21.89	3.04
21		90.28	64.36	20.02	14.28	30.08	21.42	4.51
22		23.55	73.58	8.58	15.17	24.44	43.20	1.55
23		48.67	49.23	10.72	10.84	39.48	39.93	4.49
24	Liquid[6]	94.20	65.93	18.59	13.01	30.08	21.05	5.07
25	Liquid[5]	50.24	53.65	11.44	12.21	31.96	34.13	4.39
27		63.58	51.28	30.03	24.22	30.08	24.26	2.12
29		46.75	54.94	13.30	15.63	25.05	29.44	3.52
30		29.92	45.71	11.32	17.30	24.21	36.99	2.64
31		63.41	60.71	9.31	8.91	31.73	30.38	7.12
34		28.05	44.94	9.31	14.92	25.05	40.14	3.01

TABLE 21 (continued)

Day	Ration	Acetic (uM/ml)	(M%)[3]	Propionic (uM/ml)	(M%)	Butyric (uM/ml)	(M%)	A:P[2]
36	Liquid[5]	26.18	38.38	8.64	12.66	33.40	48.96	2.07
37		24.31	44.47	8.64	15.81	21.71	39.72	2.81
38		16.83	41.89	6.65	16.55	16.70	41.56	2.53

1/ 6 liters per day, composition shown in table 3 (100% base level)
2/ Molar percent
3/ Acetate:propionate ratio
4/ 125% base level (table 3)
5/ 150% base level (table 3)
6/ 175% base level (table 3)

TABLE 23

ENERGY BALANCE STUDIES

Trial V

Week	Intake[1]	Feces[1]	Absorbed[1]	Coeficient of Digestion
		Lamb #1		
1[2]	17.108	3.370	13.738	80.30
2[3]	21.380	1.564	19.826	92.70
3[4]	25.668	1.454	21.214	94.34
4[5]	26.273	1.944	24.429	92.98
		Lamb #2		
1[2]	17.108	5.051	12.059	70.48
2[3]	21.380	1.668	19.712	92.19
3[4]	25.668	2.077	25.591	91.90

1/ megacalories per week
2/ 100% base (table 3)
3/ 125% base (table 3)
4/ 150% base (table 3)
5/ 175% base for one day, 150% base 6 days

TABLE 22

RUMINAL VFA CONCENTRATIONS

Trial V

Lamb #2

Day	Ration	Acetic (uM/ml)	Acetic (M%)[3]	Propionic (uM/ml)	Propionic (M%)	Butyric (uM/ml)	Butyric (M%)	A:P[2]
2	Conv.	71.06	53.99	50.54	38.40	10.02	7.61	1.41
7	Liquid[1]	36.46	49.01	21.28	28.60	16.70	22.45	1.71
8	Liquid[4]	38.34	47.36	29.26	36.14	13.36	16.50	1.31
14		91.63	52.48	44.56	25.52	38.41	22.00	2.06
15		78.54	58.37	19.28	14.33	36.74	27.30	4.07
18	Liquid[5]	46.75	46.52	5.32	5.29	48.43	48.19	8.79
19		35.53	44.19	10.64	13.23	34.24	42.58	3.34
20		59.84	59.52	10.64	10.58	30.06	29.90	5.62
21		18.70	35.24	9.31	17.54	25.05	47.21	2.01
22		71.99	68.01	7.98	7.54	25.88	24.45	9.02
23		44.88	54.45	6.65	8.06	30.90	37.49	6.75
24	Liquid[6]	56.10	57.75	9.31	9.58	31.73	32.66	6.02

1/ 6 liters per day, composition shown in table 3 (100% base level)
2/ Molar percent
3/ Acetate:propionate ratio
4/ 125% base level (table 3)
5/ 150% base level (table 3)
6/ 175% base level (table 3)

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ELECTROCARDIOGRAMS
TRIAL IV
Lamb #1
Day 6 of Infusion

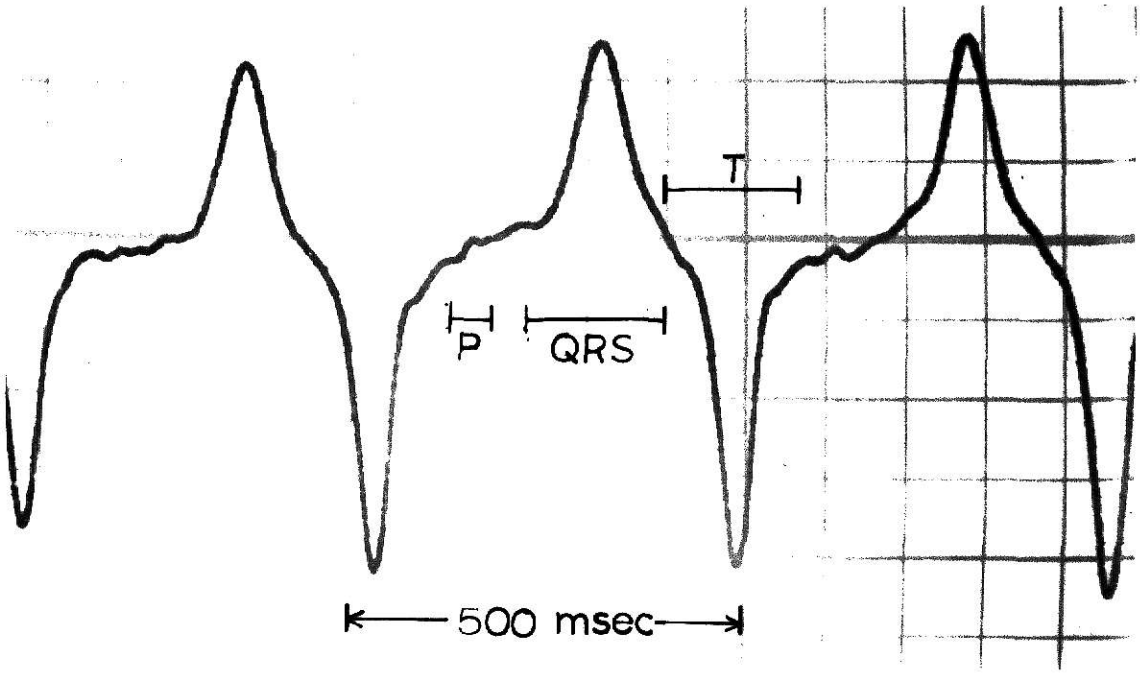

Figure 1. Fifteen minutes before injection of thiamine
P interval 53 msec.
QRS interval 180 msec.
T interval 174 msec.
Heart rate - 120

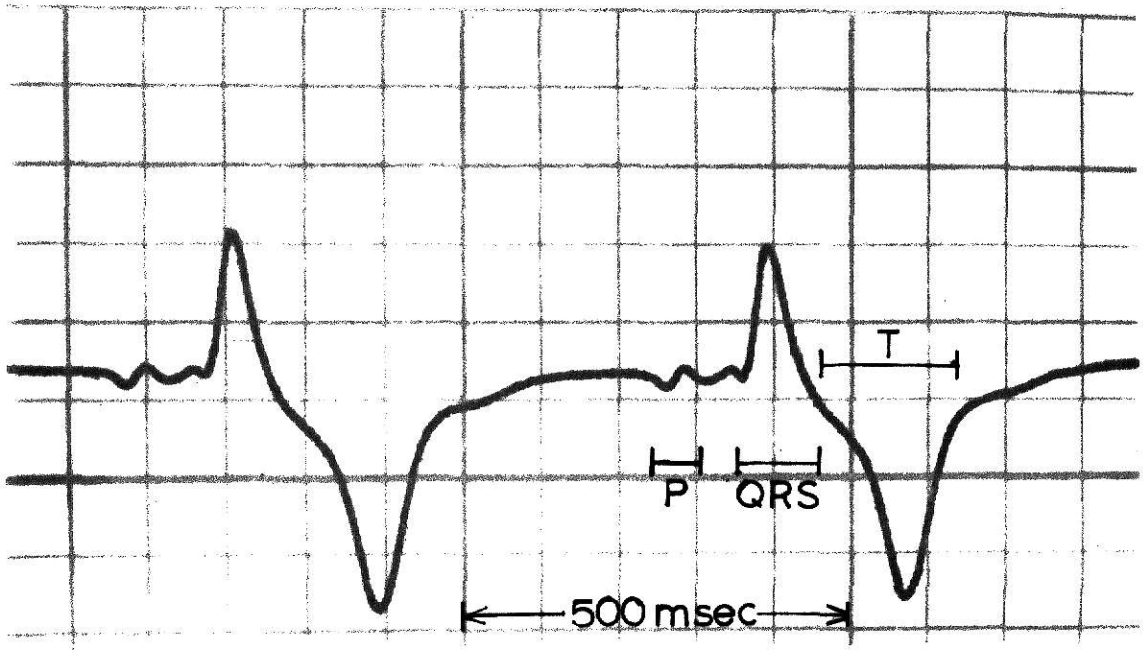

Figure 2. Ninety minutes after injection of thiamine (200 mg IM).
P interval 61 msec.
QRS interval 109 msec.
T interval 176 msec.
Heart rate - 90

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GROSS AND MICROSCOPIC LESIONS OF
POLIOENCEPHALOMALACIA

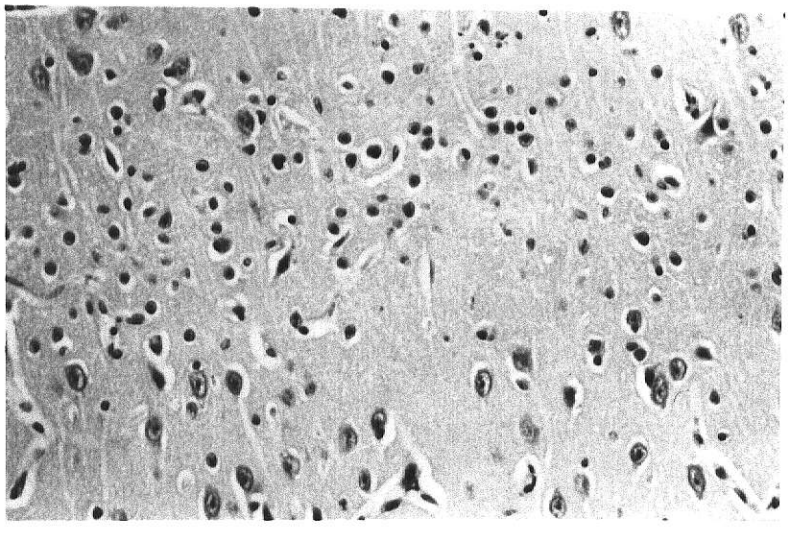

Figure 3. Photomicrograph of cerebral cortex (TRIAL V, Lamb #1). Note - cerebral edema and dead or dying Neurons (100X, H and E stain).

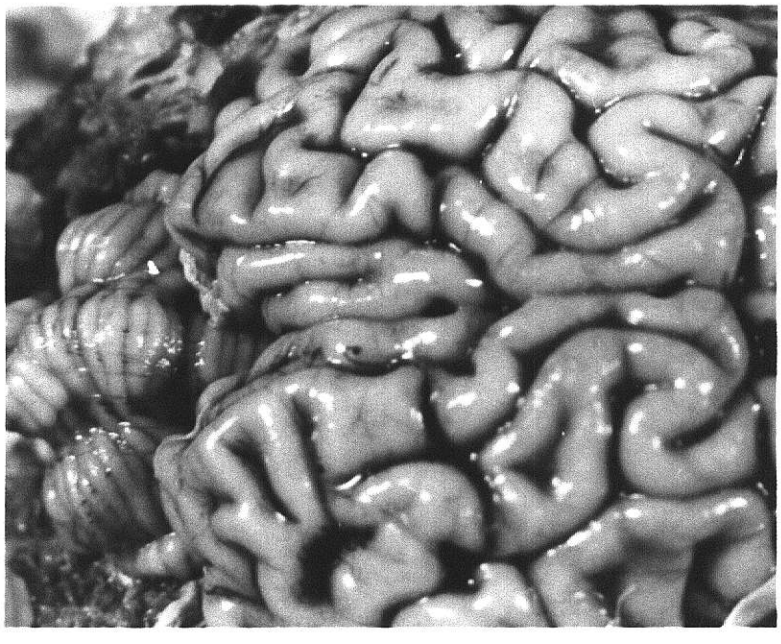

Figure 4. Cerebral hemisphere from which photomicrograph in figure 1 was taken, revealing swollen and flattened gyri.

SUMMARY

Five trials, four of which involved metabolism and nitrogen balance studies, were conducted in an attempt to 1) develop a complete liquid ration suitable for intraruminal infusion with the ultimate objective of determining the metabolic capabilities of animals for increased feed intake and 2) evaluate the effectiveness of the infusion ration in inducing polioencephalomalacia in experimental animals. The animals (fistulated wethers) were subjected to preliminary periods of from 1 to 3 weeks to adjust to the metabolism crates and concentrate rations. Measurements of rumen and blood components were used to evaluate the adequacy of the liquid infusion diet.

The mechanical problems encountered in the infusion technique have been solved. Serum mineral data shows no electrolyte imbalance during the liquid phase of feeding. Metabolism and nitrogen balance data indicate that the liquid ration is nutritionally adequate if thiamine is added to the ration. A negative nitrogen balance was seen when thiamine was not included in the ration. Volatile fatty acid (VFA) analysis shows the acetate:propionate ratio to be higher on the infusion ration than on conventional feeding. Total VFA concentrations (micromoles per ml) increased after each increase of energy in the infusion ration with an accompanying increase in the concentration and molar per cent of butyric acid.

The ration was proven to be effective in producing polioencephalomalacia in experimental animals. Polioencephalomalacia was shown by histological examination of brain tissue to have been produced in two lambs and implicated in the deaths of three other lambs from which the brains were not examined. Thiamine was shown to be effective in preventing polioencephalomalacia if added to the liquid ration and also to be effective in treating

animals with symptoms of polioencephalomalacia. A relationship between dietary level of energy and time required to produce polioencephalomalacia was also revealed. A thiaminase produced by rumen bacteria was suggested as the causitive agent in the syndrome.

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HYPERALIMENTATION IN LAMBS: A MODEL FOR THE STUDY OF POLIOENCEPHALOMALACIA

by

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Five trials, four of which involved metabolism and nitrogen balance studies, were conducted in an attempt to 1) develop a complete liquid ration suitable for intraruminal infusion with the ultimate objective of determining the metabolic capabilities of animals for increased feed intake and 2) evaluate the effectiveness of the infusion ration in inducing polioencephalomalacia in experimental animals. The animals (fistulated wethers) were subjected to preliminary adjustment periods of 1 to 3 weeks to metabolism crates and concentrate rations. Measurements of rumen and blood components were used to evaluate the adequacy of the liquid infusion ration.

Mechanical problems encountered in the infusion technique have been solved. Serum mineral data show no electrolyte imbalance during the liquid phase of feeding. Metabolism and nitrogen balance data indicate that the liquid ration is nutritionally adequate if thiamine is added to the ration. A negative nitrogen balance was seen when thiamine was not included in the diet. Volatile fatty acid (VFA) analysis showed the acetate:propionate ratio to be higher on the infusion ration than on conventional feeding. Total VFA concentrations (micromoles per ml) increased after each increase of energy in the infusion diet with an accompanying increase in the concentration and molar percentage of butyric acid.

The ration was proven to be effective in producing polioencephalomalacia in experimental animals. Polioencephalomalacia was shown by histological examination of brain tissue to have been produced in two lambs and implicated in the deaths of three other lambs from which the brains were not examined. Thiamine was shown to be effective in preventing polioencephalomalacia if added to the liquid ration and also to be effective in treating animals with clinical signs of polioencephalomalacia. A relationship between dietary level

of energy and time required to produce polioencephalomalacia was also revealed. A thiaminase produced by rumen bacteria was suggested as the causitive agent in the syndrome.