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Chemical Composition of *Agropyron desertorum* as Related to Grass Tetany

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ABSTRACT

Grass tetany, a Mg deficiency of ruminants, accounts for significant economic losses to Western cattlemen during the spring grazing period on *Agropyron desertorum*. This nutritional deficiency may also occur when ruminants graze other temperate grasses, but the soil-plant-animal factors leading to the problem are not well understood.

The objective of this study was to provide definitive data relating seasonal changes in the chemical composition of forage to the occurrence of grass tetany on semiarid grasslands.

The field experiment was established on two calcareous soils where grass tetany had previously occurred. *Agropyron desertorum* forage was harvested from both sites at regular intervals during the spring tetany period in each of 5 years. The forage samples were analyzed for mineral elements, N, total water-soluble carbohydrates (TWSC), higher fatty acids (HFA), ash alkalinity, and aconitic acid.

High levels of N and HFA, which are known from other studies to reduce Mg availability to the animal, coincided with the occurrence of tetany. The low Mg concentrations measured in the forage probably provided only marginal levels of available Mg to the grazing animals. A rapid increase in the ratio of N/TWSC coincided with the onset of tetany, and may be the primary factor which indirectly decreases Mg availability and precipitates the occurrence of tetany. Although the relationship of the protein/energy imbalance to grass tetany has been suggested previously, as far as the authors are aware this is the first time that N/TWSC values obtained during the growing season have been documented in conjunction with the occurrence of grass tetany.

Additional index words: **Crested wheatgrass, Hypomagnesemia.**

HYPOMAGNESEMIA (grass tetany) is a Mg deficiency of ruminants which results in considerable animal death loss throughout the world's temperate regions. Occurring generally in the spring, the deficiency is associated with low concentrations of Mg in forage and coincides with accelerating growth rates in grass. Nitrogen and K fertilization of grass or grass-legume pastures increases the tetany potential (3, 4). Physiological and environmental stresses, such as lactation, old age, transportation, cold temperature, and insufficient dry matter intake, increase the animals' susceptibility to tetany (3, 4, 7, 9).

During the 1960's, grass tetany killed large numbers of animals that grazed annual winter pastures in California, cool-season grasses in the eastern United States, and the fall growth of winter cereals in the southern Great Plains (4). Most of these locations are relatively humid, and soils are coarse-textured, neutral to acid, and have low soil Mg.

Grass tetany also occurs on calcareous semiarid grass ranges of the western United States (6, 16). In these areas, tetany or wheatgrass poisoning, as it is known locally, occurs generally in late spring when cattle graze monocultured crested wheatgrass (*Agropyron desertorum* (Fisch.) Schult.) and occasionally when they graze native range. This paper reports the seasonal changes in chemical composition of the wheatgrass forage in relation to the occurrence of grass tetany.

Conclusions, based on this and other work (3, 4, 7, 9, 10) are made concerning the relative importance of various chemical factors of crested wheatgrass forage in the etiology of grass tetany. In addition, chemical composition of the forage is discussed in view of the nutritional requirements of lactating beef animals (11).

MATERIALS AND METHODS

The field study was located on the broad alluvial fans of the San Jacinto seedings in northeastern Nevada, about 80 km south of Twin Falls, Idaho. The native cold desert shrub-bunchgrass vegetation had been replaced in 1952 with crested wheatgrass which was continued as a monoculture crop through the study period. The spring grazing period begins about mid-April and continues into late May and early June, but the timing is highly variable depending upon forage availability. The average 280-mm precipitation at this 1,650-m elevation may occur as either snow or rain during winter and early spring. Rainfall generally ceases in mid-June and perennial grasses mature by early July. Regrowth occasionally occurs during fall months if soil moisture conditions are favorable.

Field Procedures

The two pasture sites selected for intensive study were located 5 km apart on calcareous soils: one a coarse, loamy, Durixerollic camborthid; and the other a loamy, skeletal Haploxerollic durorthid. Both have been described elsewhere (6).

Gravimetric soil-water content values (0 to 20 cm depth) were determined for both sites and then converted to mean water potential by referencing water retentivity versus water potential curves for the respective soil. Data for these curves were obtained by equilibrating previously saturated, undisturbed soil cores (5-cm outside diameter × 2.5-cm long) on the pressure plate membrane to −0.1, −0.3, −1, and −15 bars. Desorption values were determined from triplicate soil samples representing each 5-cm depth increment.

The 15-cm soil and 30-cm air temperatures were measured at both sites and these data were supplemented with 1.5-m air temperatures (dotted lines, Fig. 1) from the climatological station at Contact, Nevada, 20 km southwest of the study sites. Moving 5-day means were computed for all max-min air and soil temperatures, and plotted for the fifth day.

Plant sampling frequency varied from year to year, but bracketed the potential tetany period. Grass spears were manually plucked or clipped 2 cm above the stem base of clones selected at random, placed into polyethylene bags, immediately frozen with dry ice (solid CO_2), and stored at −20 C until freeze-dried. Water content of the freeze-dried material was generally less than 1% as determined by weight loss over P_2O_5 dessicant. Fresh samples were weighed before and after freeze-drying to determine dry matter content. Dried samples were ground to pass a 40-mesh sieve and stored in glass bottles at ambient temperatures.

Laboratory Procedures

Plant samples were dry-ashed overnight in pyrex beakers at 500 C, and subsequently treated to assure the loss of carbonates and nitrates and to dehydrate the silica which was removed by filtration through Whatman No. 42 (T. Greweling, 1966. The

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Chemical Analysis of Plant Tissue, Cornell University Agron. Mimeo. 6622). The filtrate was analyzed for Na, K, Mg, and Ca by atomic absorption. Aliquots reserved for Mg and Ca analysis contained 1,500 ppm Sr to minimize Al and P interference with Ca. Total P was determined by the colorimetric molybdenum-blue method (1), modified by substituting 1-amino-2-napthol-4-sulfonic acid for $SnCl_2$ as reducing agent. All 1971 samples and selected samples from previous years were wet-ashed with nitric:perchloric (1:1, v:v) and the above procedures again used, except that the vanomolybdate method (1) was modified to use a wavelength of 420 nm rather than 470 nm. Aluminum in a select few of the wet-ashed samples was determined by atomic absorption.

Chloride was determined by potentiometric titration with $AgNO_3$ (1). Nitrate-N was determined by the phenoldisulfonic acid method in 1967 and 1968 (1) and later by the nitrate-electrode method (8), except that extraction time was increased from 5 to 25 minutes. Sulfate-S was extracted from 0.5-g plant samples by shaking for 25 minutes with 50 ml 0.35 *N* acetic acid plus 0.5 g carbon black (Elf 1, channel black), and filtered. SO_4-S was then determined by the $BaSO_4$ turbidimetric technique (15).

Ash and ash alkalinity (18) values were obtained after ignition at 550 C for 2 hours after reaching temperature. Higher fatty acids (HFA) were determined by titration following saponification and separation into a petroleum ether phase (5). Cations minus anions (C — A) were calculated on a millequivalent basis as ($Na^+ + K^+ + Mg^{++} + Ca^{++}$) minus ($Cl^- +$ total P as $H_2PO_4^- + NO_3^- + SO_4^=$). The ratio K/(Ca + Mg) was calculated on a millequivalent/kg basis.

Total aconitic acid was determined by polarography (12). Total water-soluble carbohydrates (TWSC) were determined as reducing sugars (7). Total N (including NO_3) was determined by the Kjeldahl method.

Physical and chemical data are reported as means for the two study sites unless indicated otherwise. Forage dry matter concentration was calculated as dry weight divided by fresh weight × 100. Chemical data are given on a dry matter basis.

The paired T test (14) was used to test various treatments for significant differences. Simple correlation-regression analyses were performed on some parameter pairs (14).

RESULTS AND DISCUSSION

Incidence of Grass Tetany

Tetany was observed during the third week in May 1967, simultaneously with an observed acceleration in forage growth. Nineteen cows died on the San Jacinto seedings, and a high incidence of tetany was reported throughout Utah, Nevada, and Idaho at that time. In 1968, forage growth was initially retarded but increased significantly as soil moisture improved. The general incidence of tetany was less in 1968 than in 1967, but five cases of tetany resulting in death were observed in the study area. Very few cases of tetany were reported during 1969 in Utah, Nevada, and Idaho because of droughty conditions. A few cases were reported during the second week in May 1969; however, most of these occurred 400 km south of the study area where soil moisture conditions were more desirable. The temporary improvement in soil moisture status shown for April 22, 1969 (Fig. 1), resulted from artificially sprinkling the research plots with 4 cm of water during the previous 4-day period.

Grass tetany did occur throughout the Intermountain and Great Basin States during the spring periods of 1970 and 1971, but the severity was not as great as in 1967. Grass tetany did not occur, however, on the San Jacinto seedings during the 1970-71 period. In 1970, grazing was terminated early because of the availability of higher elevation range. The excellent forage growth in 1971 was not spring grazed but, instead, was grazed as dry standing forage in the fall. There was insufficient forage to warrant grazing the San Jacinto seedings in 1972 because of droughty conditions.

Forage growth rates were not measured directly but can be inferred from the dry matter content (Fig. 1) which closely reflected soil water availability. For example, percentage dry matter decreased following the improvement in soil water status on May 9, 1968. This decrease in dry matter concentration accompanied an increase in plant tillering and a gradual increase in leaf length. Grass tetany generally occurs when mean daily air tempertures are between 5 and 15 C and grass is growing rapidly (4). Adequate soil moisture, as well as optimum temperature, must be present for rapid forage growth. Either of these factors may frequently be limiting forage growth in a given year under semiarid conditions. Thus, the intensity of grass tetany varies from year to year. Just by evaluating temperature and soil moisture conditions, tetany might have been anticipated in May 1967, April 1 or mid-May 1968, about May 1, 1970, and late April to late May 1971.

Forage Chemical Composition

Plant material grown during the five seasons on the camborthid site had significantly (P = 0.05) greater Ca (0.06%) and significantly less Mg (0.025%), NO_3-N (20 ppm) and Cl (0.10%) than did plant material from the durorthid site. Forage from the camborthid site generally contained more P (300 ppm), greater ash alkalinity (30 to 40 meq/kg) and greater water-soluble carbohydrates (1.5%), but it had less ash (1.6%) and less K (0.1%) than forage from the durorthid site. These data generally supported the field observations that the camborthid site provided better growing conditions because of more favorable microenvironment and soils than the other site. Other parameters were not consistently different between the two sites. Results discussed in the remaining portion of this paper deal with mean values for the two sites.

The mean forage calcium concentration varied between 0.4 and 0.5% (Fig. 1) during the early part of each season. This value generally declined to about 0.3% as plants matured, but even this level is considered adequate for lactating beef cows. Concentration of Mg generally decreased during the season and averaged only half of the 0.2% level above which tetany seldom occurs (3, 4). Potassium, while not high compared with that of many forages (4), varied between 1.5 and 2.5% and generally decreased with time but was adequate for ruminant needs.

The frequency of grass tetany increases rapidly as forage K/(Ca + Mg) values exceed 2.2 (4, 7, 9). In the present study the ratio was greater than 2.2 during the early part of 1967, which was the worst season for grass tetany. The ratio was also greater than 2.2 during the late part of the 1968 and 1971 grazing season. The ratio was less than 2.0 during several occurrences of tetany, so a high K/(Ca + Mg) ratio was not essential for the observance of grass tetany. Correlation analysis indicated that variations in the K/(Ca + Mg) values were significantly related to variation in Ca values but not to variation in Mg values. The 2.2 threshold, therefore, may not

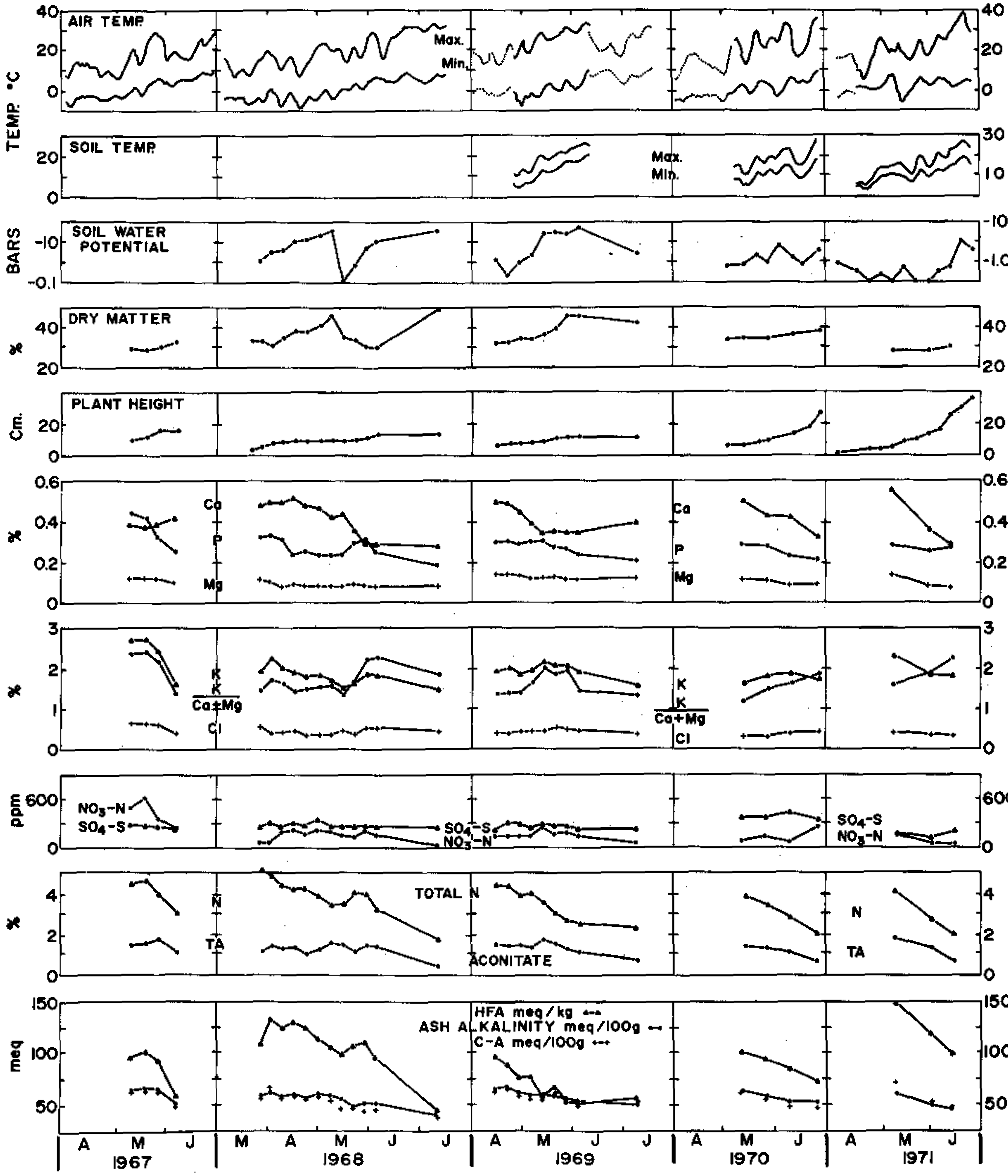

Fig. 1. Environmental parameters, growth characteristics, and mean chemical composition of *Agropyron desertorum* forage during five successive seasons.

be very helpful in describing a potential Mg tetany forage under conditions of this study.

Analysis of wet-ashed samples indicated that Na concentrations declined as the season progressed (data not shown). For example, the 1971 crested wheatgrass forage contained 69, 52, and 48 ppm Na for the three successive sampling dates. Seasonal averages were 34 ppm Na in 1970 and 56 ppm Na in 1971. Dry-ashed samples had widely varying Na values (200 to 2,000 ppm), suggesting contamination from the ashing glassware. The Na concentrations found in wet-ashed samples were admittedly deficient for animal requirements and thus might be related to the occurrence of tetany (4) since 0.2 to 0.3% dietary Na is required in the transport of Mg through the intestinal membrane (9). However, cattle in this area had free access to NaCl blocks or licks, although this did not insure adequate Na intake.

Crested wheatgrass forage, harvested during the serious outbreak of tetany in 1967, contained 10 to 15 ppm Al. In the southern Great Plains, however, the 500 to 1,000 ppm Al found in a few forage samples of winter-grazed cereals and grass was suggested as a causative agent in the occurrence of grass tetany (2). In New Zealand (13) and in the senior author's laboratory, values in excess of 200 ppm Al or Fe in the forage have been attributed to soil contamination. The authors are unaware of any well-documented evidence implicating dietary Al in the etiology of grass tetany. However, in studies referred to by Underwood (17), animals supplemented with large quantities of several different Al sources developed a gastrointestinal irritation and eventually rickets due to Al interference with P absorption.

Phosphorus concentrations varied from 0.2 to 0.4% and seemed to parallel K concentrations. The P levels appeared adequate for beef cows because Ca/P values ranged between 0.9 and 2.2 (Fig. 1). Chloride concentrations were relatively stable, being similar to those values of 0.5% reported in northwestern Nevada (16). Nitrate-N levels were as high as 600 ppm in 1967, but were never greater than 300 ppm at any other time during the study. The concentration of SO_4-S ranged from 200 to 400 ppm. In our opinion, concentrations of Cl, P, NO_3-N, and SO_4-S did not appear abnormal for dietary requirements of lactating cows. It is not expected that these inorganic anions were involved in causing grass tetany (4, 9).

Ash alkalinity values were as high as 70 meq/100 g but decreased to approximately 40 meq/100 g by the end of the growing season. Values for cations minus anions (C — A) were parallel to those for ash alkalinity but were generally a little lower. For the same range of values of ash alkalinity and C — A, Van Tuil, Lampe, and Dijkshoorn (18) made a similar observation for crops grown in the Netherlands. The discrepancy between the two methods is not known, since theoretically they measure the same property. Either measurement should give a value equivalent to the organic acid content of the plant (18). The organic acid anions are produced by the plants in order to maintain electrical neutrality and often occur in concentrations proportional to plant growth rate (18).

Aconitic acid was determined because of previous implications in the occurrence of tetany (4). Crested wheatgrass forage contained about 1.5% aconitic acid and this value occasionally increased to 2%, but concentration changes were not associated with the occurrence or absence of tetany. Aconitic acid accounted for more than 50% of the total organic acid concentration as inferred from ash alkalinity and C — A values. In agreement with earlier work (4) the *trans* isomer was present in much larger quantities than was the *cis* form. The organic acids in forage, e.g., malic, citric, and *trans*-aconitic acid, may have strong affinities for both Ca and Mg (10). However, their survival in the rumen and subsequent passage into the small intestine where Mg is absorbed is very doubtful (10). It is expected, however, that organic acids may be important in the grass tetany syndrome under conditions of marginal Mg supply.

Total N in plant material ranged from 2 to 5%, decreasing as the season progressed. There was about 4% N in forage in 1967, 1968, and 1969 when tetany was reported. This N level could have directly or indirectly reduced the level of available Mg to the animal (4, 7, 9). There are several ways in which increased N in the forage could be decreasing the Mg available to ruminants (4). Rapid rumen ammonification of high N forage in the absence of adequate energy material may increase the ingesta pH, thus decreasing Mg solubility. The forage N levels often paralleled concentrations of HFA and organic acids and these could complex Mg making it less available to the animal, especially at higher pH's.

High values of total water-soluble carbohydrate were encountered in the first samples harvested each season, but they decreased with time, physiological age of forage, or both. It has been suggested that high crude protein/energy ratios predispose animals to grass tetany (9). Very high forage N levels have been implicated in the etiology of tetany (9), but protein/energy data have not been reported. The occurrence of grass tetany in our study coincided closely with the periods when the ratio of total N/TWSC exceeded 0.3 (Fig. 2). The threshold value of 0.3 may not be as important as the steepness of the rising leg of the N/TWSC, indicative of a rapid departure in the values for these two dietary components. This dietary imbalance between N and readily available energy may result in the rapid build-up of NH_4-N in the rumen. The subsequent increase in rumen fluid pH with the possible formation of magnesium ammonium phosphate, tricarboxylic acid salts of Mg, and Mg soaps would thus reduce the availability of dietary Mg to the ruminant.

Diets having adequate levels of readily fermentable carbohydrates allow for rapid depletion of rumen NH_3 resulting from high protein feeds. Adequate carbohydrate also enhances the formation of volatile fatty acids. The net effect is thought to reduce rumen fluid pH and thus increase the absorption and effective availability of dietary Mg and Ca (7). This thesis was checked by Wilson et al. (19) with lactating cows grazing fresh grass. They found that the plasma Mg levels of cows receiving a starch supplement were not depressed as much as Mg levels of cows not receiving the supplemental carbohydrate. Thus, reduced levels of readily available energy, especially in the presence of high N levels, would reduce Mg availability.

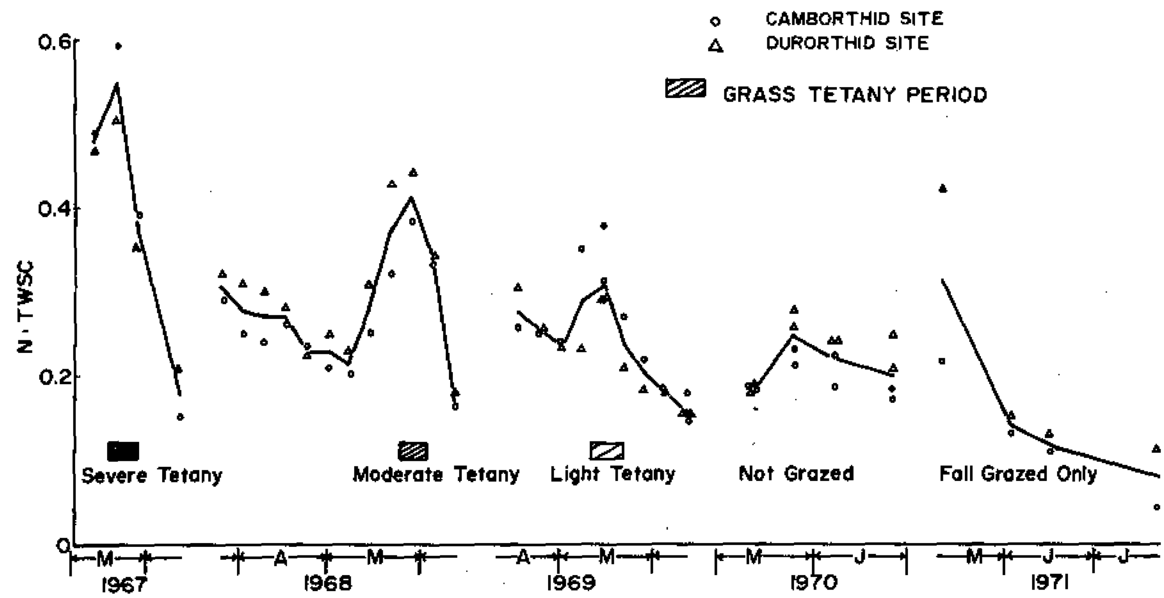

Fig. 2. Seasonal changes in the ratio total N/total water-soluble carbohydrates over five seasons and the relationship to the occurrence of grass tetany.

Quite a number of dietary and animal physiological factors have been considered in the etiology of grass tetany (3, 4, 7). While some of these may be important under conditions of this study, it would appear that the rapid changes in the N/TWSC may have been the triggering factor inducing the tetany.

Higher fatty acids (e.g., palmitic and linolenic) generally decreased during the season and, like K and total N, responded to the replenished soil water supply received during May 1968 (Fig. 1). Higher fatty acids can reduce Mg availability by forming insoluble Mg soaps (4, 9). Maximum concentrations of HFA did not necessarily coincide with the occurrence of tetany (Fig. 1); however, the effect of this factor, as well as others, in the etiology of grass tetany may be gradual. The review articles by Grunes, Stout, and Brownell (4) and Molloy (9) indicate that concentrations of both N and HFA were correlated. This was due at least in part to the fact that concentrations of both N and HFA decreased during the season. That the HFA values in this study were significantly correlated with N corroborates previous findings (5), but this may not be a direct relationship. We are now aware that auto-oxidation of HFA occurs during ambient storage of freeze-dried material (unpublished information by the authors; A. J. Metson, personal communication). Thus the 1968 and 1971 samples which were analyzed very shortly after completion of spring harvesting showed generally higher HFA values (Fig. 1) than did the 1967 samples analyzed in 1968 and the 1969 and 1970 samples analyzed in 1971. Consequently, some indeterminate analytical error is acknowledged; however, these data are valuable in a qualitative sense since they show the expected seasonal trends.

CONCLUSIONS

Crested wheatgrass forage is low in Mg throughout the season. From our experience on these and similar pastures, we know that reduced dry matter intake by the animal often limits Mg intake. Moreover, the apparent availability of Mg may be reduced by the accumulative effects of high levels of N, HFA, and N/TWSC and low percentage dry matter. Accelerating growth rates of forage, because of adequate temperatures and soil water, result in high levels of both forage N and HFA and low levels of TWSC. The N/TWSC values greater than 0.3 coincided with the occurrence of grass tetany. However, the sudden change in N/TWSC values rather than the absolute level may be the important criterion in precipitating grass tetany. If this hypothesis can be verified, then supplementing animals with Mg and a readily available energy source would reduce the incidence of tetany.

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