Forage Nutrient Variability Associated with Hypomagnesemia and Hypocalcemia

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Abstract

Seasonal variation of crown mineral concentrations in cool-season forages and hypomagnesemia (grass tetany), as well as hypocalcemia (milk fever) risk, were assessed using the grass tetany index (GTI) and dietary cation-anion difference (DCAD). Thirty-three cultivars from orchardgrass, fes-tulolium, smooth brome grass, perennial ryegrass, meadow fescue, tall fescue, alfalfa, white clover and red clover were grown on Andisol in northern Japan. Plants were harvested thrice, and mineral contents were analyzed by energy reflectance X-ray spectrometry (XRF). Across harvests, highest K, Ca, and S contents were observed in alfalfa. The highest Mg content was recorded in white clover which was optimum for legumes and adequate for livestock. In contrast, Na and Cl contents were higher in perennial ryegrass and orchardgrass, respectively, irrespective of cultivars. Regardless of cultivars, K, Mg and Cl contents in forages across the harvests were adequate for grazing animals. Cultivars of Festulolium, perennial ryegrass, meadow fescue, alfalfa, white clover and red clover were grown on Andisol in northern Japan. Plants were harvested thrice, and mineral contents were analyzed by energy reflectance X-ray spectrometry (XRF). Across harvests, highest K, Ca, and S contents were observed in alfalfa. The highest Mg content was recorded in white clover which was optimum for legumes and adequate for livestock. In contrast, Na and Cl contents were higher in perennial ryegrass and orchardgrass, respectively, irrespective of cultivars. Regardless of cultivars, K, Mg and Cl contents in forages across the harvests were adequate for grazing animals. Cultivars of Festulolium, perennial ryegrass, meadow fescue, alfalfa, and clover species did not show any GT risks over season. On the other hand, all cultivars of orchardgrass, smooth brome grass, and tall fescue species showed GT risk [(K/(Ca + Mg) > 2.2] in the first harvest. Across harvest, the average value of DCAD in perennial ryegrasses was lowest among species. Our results suggested that perennial ryegrass and alfalfa species are the suitable cool-season forages for grazing animals in temperate regions of Japan.

Subject Areas

Agricultural Science

Keywords

Animal Disorders, Grass Tetany Index, Ryegrass, Alfalfa, Potassium,
1. Introduction

Forage quality is important to support adequate growth, health and nutrition of grazing animals. While forages acquire minerals from their surrounding environments to ascertain life cycle, an excess and a deficiency of minerals affect forage growth and quality [1]. The excessively high or low concentrations of minerals in forages might endanger and be able to adversely affect grazing animal development [2]. The forage nutrient quality induced major animal disorders have been reported including pasture bloat, nitrate poisoning, grass tetany, milk fever, laminitis, acute bovine pulmonary emphysema, or atypical interstitial pneumonia and agalactia [3]-[8].

Grass tetany (hypomagnesemia) and milk fever (hypocalcemia) are two major non-infectious fatal metabolic disorders in dairy cattle, and frequently occur when the animals are being fed with forages containing imbalanced ratios of essential mineral nutrients. Annual losses of animals due to GT in the United States [9], Ireland [10], New Zealand [11], Australia [12], and Japan [13] are well documented. In Japan, cattle especially cows are often affected by hypocalcaemia [14]. Sanchez [15] reported that at a critical level, about 5% - 7% of dairy cows are affected by hypocalcaemia and, at a subclinical level, an estimated 66% are affected by hypocalcaemia in the United States. It is estimated that 5% of dairy cows are affected by hypocalcaemia in Australia [16].

The risk of GT and milk fever can be assessed by calculating the GTI of forages and the DCAD’s. The calculation of GTI involves the mineral elements such as Mg, K, and Ca [17] [18]. Forages containing 0.2% Mg are considered adequate to meet the Mg requirement of dairy cattle. However, the excess absorption of Mg by herbivores is negatively affected by K, and Ca interferes with the K and Mg absorption as well i.e., GTI calculation was done as GTI = [K/(Ca + Mg)] [17]. The risk of GT incidence increases exponentially when the cattle graze forages with a GTI, above 2.2, as based on moles of charge. Unlike, several other equations used in the calculation of DCAD involving mineral elements Na, Mg, P, S, Cl, K, and Ca, respectively [19] [20] [21]. Ender et al. [19] reported their approach is widely compatible in dairy cattle nutrition because it is well correlated with urinary pH and is predictive of clinical milk fever. Based on the simplified strong ion model and the meta-analysis [22] [23], it is suggested that the formula used by Ender et al. [19] is the most effective one for predicting the risk of milk fever. Conversely, Charbonneau et al. [24] inferred that the formula introduced by Goff et al. [21] was most closely correlated with urinary pH and milk fever.

Mineral nutrient concentrations in forages vary with biotic- and abiotic factors, as well as their growing seasons. A wide range of analytical techniques is used in determining mineral nutrients of forage grasses. The most popular me-
Methods for measuring plant nutrients are: 1) spectrophotometry, flame photometry, atomic absorption spectrophotometry (AAS), and inductively coupled plasma mass spectrometry (ICP-MS) or inductively coupled plasma optical-emission spectroscopy (ICP-OES); and 2) energy dispersive X-ray microanalyzer (EDX), energy reflectance X-ray spectrometry (XRF), and near-infrared reflectance spectroscopy (NIRS). While all those methods are reliable, nutrient specific chemical digestion of samples with expensive analytical grade reagents are required for the methods of analysis belongs to group a. In contrast, in group b, the methods do not require any digestions. Therefore, methods in group b are less expensive, laborious and time consuming when compared to the methods in group a.

The XRF method is an elemental analysis technique that has been tested for precise elemental analysis in several fields including plant samples [25]-[30], medicine and pharmacy, biochemical research, quality control systems, oil and fuel industries [31], environmental pollution [32], forensic sciences [33], and food industries [34].

Our research hypothesis is that dried, and ground forage samples can be predictively analyzed and interpreted for the nutrient elements by the XRF method in a rapid and cost-effective manner. The objective of our study was to determine mineral nutrient concentrations of forage grasses and interpret the results to evaluate the incidence of GT and milk fever associated with nutrient imbalance disorder.

2. Materials and Methods

2.1. Experiment and Plant Material

Thirty-three cultivars from nine forages commonly grown in the northern Japan, including six grass species, namely orchardgrass (Dactylis glomerata L.), festulolium (Festulolium hercynicum (wein) Banfi, Galasso, Foggi, Kopecky & Ardenghi (Festuca rubra x Lolium pratense), smooth brome grass (Bromus inermis Leyss.), perennial ryegrass (Lolium perenne L.), meadow fescue (Festuca pratensis L.), tall fescue (Festuca arundinacea Schreb.); and three legumes, namely alfalfa (Medicago sativa L.), white clover (Trifolium repens L.), and red clover (Trifolium pratense L.) were used. The experiment was a randomized complete block design in split-split-plot arrangement, with nine forage species (whole plots), 33 cultivars (subplots), and three harvests (sub-subplots) with each treatment combination replicated four times.

2.2. Plant Growth Management

Grasses were grown in Nation Livestock Breeding Centre, Shimokuriyagawa (39°41’42.7”N, 140°58’32.8”S; altitude 308 m), Shizukuishi, Morioka, Japan. The mean monthly air temperature, precipitation, and total sunshine were 17.3°C, 165 mm, and 145 hr, respectively (Japan Meteorological Agency). Forages grown on an Andisol in the northern Japan where soils are dominated by sandy loam in texture with pH (<5.5), soil organic carbon (60 g·kg⁻¹), bulk density (~0.54...
Mg·m$^{-3}$), P retention capacity (~94%), and with a melanic index of 1.61, respectively [35] [36]. Forages were grown by following the standard management practices, typically, a total of 150, 240, and 150 kg N, P$_2$O$_5$, and K$_2$O·ha$^{-1}$, respectively were applied in early spring and immediately after each harvest.

2.3. Plant Harvest and Analysis

Forages were harvested three times at 6 cm cutting height while maintaining a growing period of 60 days per cycle. Plant samples were dried at 80$^\circ$C for 24 hr in a force-air oven and ground through a 0.5 mm mesh with a cyclone mill. A uniform surface of pellet was made by taking 0.5 to 1.0 g of milled and sieved sample in a circular plastic ring (coherent disc of 2.5 cm) and pressing 15 tons of pressure using a hand jack-hydraulic from the Wright Tool Company, MI, USA [27] [28]. A concentration of nutrient elements viz., K, Ca, Mg, Na, phosphorus (P), sulfur (S), and chlorine (Cl) on both sides of pellet were measured with a live time of 100S using a XRF (XRF, JEOL Co., JSX-3220 Element Analyzer according to Hutton and Norrish [25] and Norrish and Hutton [26].

2.4. Parameter Calculation

The risk of hypomagnesemia (GT) and hypocalcemia (milk fever) were calculated by calculating the GT and DCAD of forages. The GTI was calculated as per [17]:

$$\text{GTI} = \frac{\text{K}}{(\text{Ca} + \text{Mg})}. \quad (1)$$

Three different approaches used to calculate DCAD were:

$$\text{DCDA1 (Ender et al., 1971)} = (\text{Na} + \text{K}) - (\text{Cl} + \text{S}) \quad (2)$$
$$\text{DCDA2 (NRC, 2001)} = (\text{Na} + \text{K} + 0.15\text{Ca} + 0.15\text{Mg}) - (\text{Cl} + 0.6\text{S} + 0.5\text{P}) \quad (3)$$
$$\text{DCDA3 (Goff et al., 2004)} = (\text{Na} + \text{K}) - (\text{Cl} + 0.6\text{S}) \quad (4)$$

where, GTI expressed in moles of charge basis (cmolc$^\circ$·kg$^{-1}$ of DM) and DCAD is expressed in millimoles of charge basis (cmolc$^\circ$·kg$^{-1}$ of DM).

2.5. Statistical Analysis

Mean separations between and among harvests within a species and cultivar were obtained with analysis of variance and F-protected Least Significant Difference (LSD) procedures [37] at P ≤ 0.05. The skewness and kurtosis of all data were also calculated. Skewness and kurtosis were computed to detect a degree of a dataset’s symmetry or lack of symmetry, and the degree of peakedness of a distribution and the evaluation was done according to Joanes and Gill [38] and Westfall [39], respectively. Pearson correlation coefficients were also calculated. All calculations were performed with pooled data by using PROC GLM [40] and Microsoft Excel 365.

3. Results and Discussion

3.1. Summary of Statistics

To the best of our knowledge, this is the first time we analyzed a wide range of
forages mineral nutrients using the XRF method. Therefore, it is important to detect a degree of a dataset's symmetry or lack of symmetry and the degree of peakedness of a distribution. The skewness and kurtosis were computed because of departing from an accepted standard of the analytical data (Table 1). Data set over harvests for Mg, Na, and P were highly skewed; K, Ca, GTI, and Ca/P were moderately skewed; and S, Cl, K/Mg, and DCAD were slightly skewed. In contrast, within the dataset the average of three harvests, all parameters were platykurtic, except for Mg and P, which were leptokurtic. The range of mineral concentrations across harvest was the widest for K (10.2 Yatsunami and 34.3 Toyomidorì g·kg⁻¹ of DM) and the narrowest for P (1.73 Felina and 3.58 Northwhite g·kg⁻¹ of DM). The range of DCAD across harvest was the widest for DCAD2 (87 Yatsunami and 609 Makiwaba cmolc·kg⁻¹ of DM) followed by DCAD3 (133 Yatsunami and 580 Makiwaba cmolc·kg⁻¹ of DM) and the narrowest for DCAD1 (82 Yatsunami and 520 Makiwaba cmolc·kg⁻¹ of DM), respectively.

Table 1. Summary statistics (n = 99; number of observations) of nutrients¹ and their interaction².

| Elements | Harvest | Skewness | Kurtosis | SD³ | Mean | Minimum | Maximum |
|----------|---------|----------|----------|-----|------|---------|---------|
| K        | 1       | -1.04    | 0.35     | 6.37| 21.69| 6.75    | 30.5    |
|          | 2       | -0.01    | -0.26    | 6.41| 26.57| 13.0    | 41.6    |
|          | 3       | -0.14    | -0.98    | 7.52| 20.55| 7.3     | 32.2    |
|          | Av      | -0.55    | -0.30    | 6.17| 22.94| 10.2    | 34.3    |
| Ca       | 1       | 1.03     | -0.20    | 6.43| 6.84 | 0.97    | 23.6    |
|          | 2       | 0.77     | -1.04    | 4.19| 7.38 | 2.8     | 16.56   |
|          | 3       | 0.87     | -0.89    | 5.49| 7.39 | 1.94    | 20.2    |
|          | Av      | 0.90     | -0.73    | 5.32| 7.20 | 2.5     | 20.1    |
| Mg       | 1       | 2.02     | 7.83     | 0.54| 1.90 | 1.02    | 4.1     |
|          | 2       | 2.13     | 7.74     | 0.98| 3.07 | 1.86    | 7.1     |
|          | 3       | 1.91     | 4.52     | 0.88| 2.73 | 1.36    | 5.6     |
|          | Av      | 3.30     | 14.74    | 0.65| 2.57 | 1.62    | 5.5     |
| Na       | 1       | 1.11     | -0.06    | 1.62| 1.75 | 0.19    | 5.33    |
|          | 2       | 1.44     | 1.10     | 1.59| 1.75 | 0.17    | 5.75    |
|          | 3       | 1.37     | 0.68     | 1.93| 1.90 | 0.07    | 9.60    |
|          | Av      | 1.20     | -0.05    | 1.61| 1.80 | 0.26    | 5.31    |
| P        | 1       | 1.93     | 7.66     | 0.41| 1.54 | 0.79    | 3.22    |
|          | 2       | 0.96     | 0.48     | 0.62| 2.86 | 1.97    | 4.51    |
|          | 3       | 0.53     | 0.94     | 0.46| 2.22 | 1.41    | 3.57    |
|          | Av      | 1.88     | 6.53     | 0.35| 2.21 | 1.73    | 3.58    |
| S        | 1       | 0.69     | 0.41     | 0.52| 1.28 | 0.41    | 2.64544 |

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|          | 2    | 0.93 | 2.64 | 0.76 | 2.42 | 0.95  | 4.74  | 3.25  | S444 |
|----------|------|------|------|------|------|-------|-------|-------|------|
|          | 3    | 0.21 | −0.78| 0.60 | 2.01 | 1.01  | 3.52  | S444  |      |
|          | Av   | −0.13| 0.003| 0.47 | 1.91 | 1.00  | 2.83  | S444  |      |
| Cl       | 1    | 0.37 | −1.08| 2.55 | 8.06 | 4.29  | 12.6  |      |      |
|          | 2    | 0.27 | −1.07| 3.10 | 8.96 | 3.87  | 15.5  |      |      |
|          | 3    | 0.16 | −1.37| 1.71 | 6.69 | 3.91  | 9.56  |      |      |
|          | Av   | 0.15 | −1.30| 2.29 | 7.90 | 4.02  | 12.2  |      |      |
| GTI      | 1    | 0.59 | −0.82| 1.12 | 1.70 | 0.31  | 4.06  |      |      |
|          | 2    | 0.74 | −0.20| 0.48 | 1.21 | 0.39  | 2.31  |      |      |
|          | 3    | 0.94 | 0.52 | 0.50 | 1.03 | 0.34  | 2.46  |      |      |
|          | Av   | 0.52 | −0.99| 0.64 | 1.31 | 0.35  | 2.61  |      |      |
| K/Mg     | 1    | 0.02 | −0.25| 4.51 | 12.07| 3.75  | 20.79 | S444  |      |
|          | 2    | 0.08 | −1.29| 3.99 | 9.61 | 3.04  | 16.43 | S444  |      |
|          | 3    | 0.36 | −0.53| 3.61 | 8.08 | 3.24  | 16.08 |      |      |
|          | Av   | −0.26| −1.11| 3.46 | 9.92 | 3.63  | 15.13 |      |      |
| Ca/P     | 1    | 0.81 | −1.00| 3.34 | 4.09 | 1.11  | 11.07 |      |      |
|          | 2    | 4.42 | 22.68| 0.89 | 0.91 | 5.46  | 0.24  |      |      |
|          | 3    | 0.91 | −0.70| 2.22 | 3.26 | 1.27  | 8.17  | S444  |      |
|          | Av   | 0.75 | −1.23| 1.94 | 2.75 | 1.03  | 6.34  | S444  |      |
| DCAD1    | 1    | −0.65| −0.17| 113  | 325  | 91    | 506   |      |      |
|          | 2    | −0.10| −1.39| 168  | 353  | 72    | 606   |      |      |
|          | 3    | 0.29 | −1.03| 138  | 295  | 79    | 529   |      |      |
|          | Av   | −0.26| −1.01| 127  | 324  | 82    | 520   |      |      |
| DCAD2    | 1    | −0.39| −0.73| 137  | 357  | 92    | 599   |      |      |
|          | 2    | −0.10| −1.40| 185  | 368  | 63    | 647   |      |      |
|          | 3    | 0.44 | −1.06| 165  | 327  | 89    | 630   |      |      |
|          | Av   | −0.03| −1.12| 153  | 351  | 87    | 609   |      |      |
| DCAD3    | 1    | −0.60| −0.24| 119  | 357  | 112   | 557   |      |      |
|          | 2    | 0.05 | −1.40| 161  | 414  | 144   | 668   |      |      |
|          | 3    | 0.40 | −1.04| 145  | 345  | 128   | 602   |      |      |
|          | Av   | −0.10| −1.04| 128  | 372  | 133   | 580   |      |      |

|          |      |      |      |      |      |      |      |      |      |
|          |      |      |      |      |      |      |      |      |      |

1Mineral content in g∙kg⁻¹ DM; 2DCAD in mmolc∙kg⁻¹ DM; 3SD (standard deviation).

### 3.2. Crown Mineral Nutrient Concentration

The concentration of both essential and beneficial mineral nutrients (K, Ca, Mg, Na, P, S, and Cl) varied consistently among forage species and cultivars across
harvests (Table 1 & Table 2). In general, legumes are higher in K than grasses, which could be a disadvantage depending on the concentration of other nutrients. Across the harvests, mean K content of all forages was 22.6 g∙kg⁻¹ and ranged from a high of 28.2 to a low of 13.6 g∙kg⁻¹ in alfalfa and perennial ryegrass, respectively (Table 2). Banowetz et al. [41] observed that the perennial ryegrass accumulated a significantly lower concentration of K in a high rainfall environment. However, K concentration in forages grown in temperate Andisol was reportedly adequate for beef cattle, sheep, and red deer [42] [43] [44] [45] [46]. McDowell and Valle [47] reported that K deficiencies very uncommon in cool-season forages. According to NRC [48], the K concentration in forages required to meet nutritional requirements for beef cattle was 5.76 g∙kg⁻¹. On average, the K concentration of our tested grasses and legumes is 2 - 5 folds higher than the requirement of beef cattle. The higher levels ensured the suitability of these forages for lactating cattle, as they excrete large amounts of K via milk [49].

Table 2. Mineral nutrient across three harvests (g·ka⁻¹) in forages grown in temperate Andisol of Japan.

| Species*   | Cultivar   | K   | Ca   | Mg   | Na   | P   | S   | Cl  |
|------------|------------|-----|------|------|------|-----|-----|-----|
| Orchardgrass | Akimidori  | 18.7| 3.03 | 2.21 | 4.62 | 2.09| 1.95| 11.35|
|            | Akimidori II | 22.2| 3.39 | 2.40 | 1.95 | 2.20| 1.88| 10.25|
|            | Kitamidori | 17.0| 3.31 | 2.57 | 4.02 | 2.17| 1.76| 9.69 |
|            | Wasemidori | 17.1| 3.16 | 2.23 | 4.94 | 2.21| 1.73| 10.59|
|            | Natsumidori | 20.8| 3.25 | 2.59 | 3.64 | 2.15| 1.56| 11.00|
|            | Potomac     | 25.8| 3.30 | 2.52 | 0.77 | 2.09| 1.51| 10.89|
|            | Frontier    | 24.9| 2.81 | 1.68 | 1.20 | 1.86| 1.36| 9.26 |
|            | Makibamidori | 26.0| 3.48 | 1.75 | 0.81 | 2.03| 1.32| 10.52|
|            | Okamidori   | 28.4| 3.33 | 1.90 | 1.10 | 2.18| 1.48| 10.47|
|            | Toyomidori  | 34.3| 3.40 | 2.21 | 1.20 | 2.25| 1.87| 12.15|
| Festulolium | Felina      | 23.3| 2.57 | 2.46 | 0.99 | 1.73| 1.53| 9.52 |
|            | Evergreen   | 20.7| 4.52 | 2.45 | 0.26 | 2.28| 1.85| 8.22 |
| Smooth bromegrass | Aicap | 24.7| 3.63 | 1.66 | 0.33 | 1.85| 1.24| 7.35 |
| Perennial ryegrass | Yatsuboku | 10.8| 3.69 | 1.91 | 5.31 | 2.04| 1.92| 9.39 |
|            | Yatsukaze  | 10.6| 4.67 | 2.45 | 5.22 | 2.63| 2.67| 7.79 |
|            | Yatsunami  | 10.2| 5.54 | 2.35 | 2.94 | 2.44| 2.06| 6.39 |
|            | Yatsuuyutaka | 13.1| 5.14 | 2.39 | 4.54 | 2.23| 1.90| 7.08 |
|            | Friend      | 16.4| 4.58 | 2.33 | 2.34 | 2.56| 2.42| 8.52 |
|            | Kusaboshi   | 20.4| 3.72 | 2.11 | 0.70 | 2.65| 2.17| 9.91 |
| Meadow fescue | Riguro   | 25.1| 4.10 | 2.37 | 0.98 | 1.89| 1.40| 7.66 |
| Tall fescue | Hokuryo    | 26.9| 2.51 | 2.37 | 0.43 | 1.82| 1.61| 6.63 |
In general, plants must have assimilated to a certain concentration of nutrients to achieve their establishment and optimized growth. It is reported that forage grass and legume yields often suffered if they contained less than 20 g K∙kg\(^{-1}\) DM [50]. The maximum dry-matter yield in cool-season forages was observed at 28 g K∙kg\(^{-1}\) in dry matter. However, a higher K uptake antagonistically reduces the uptake of Ca and Mg by plants. The high K concentration in forages suppresses Mg and Ca absorption by ruminants [51].

### 3.3. Calcium Concentration

Among forages, mean Ca concentration was 7.6 g∙kg\(^{-1}\) (Table 2). Legumes registered 3 - 7 folds higher Ca concentration than grasses. Excess Ca in forage can...
lead to “big head disease” in creating Ca deficiency as oxalates bind with excess Ca to form insoluble Ca-oxalate and reduce its digestibility. Regardless of cultivars, Ca concentration was highest in clover and lowest in tall fescue. With the exception of the orchardgrass Frontier, Festulolium Felina, and tall fescue Hokuryo, most of the grasses contained Ca content just above the concentration that was critical to meet the requirements for beef cattle and sheep [45] [48] [52]. Calcium concentration in forage grass species was below the concentration to meet requirements for beef cattle; however, Ca content typically peaked well above recommended levels in forage legumes [43] [44]. Calcium deficiencies are rare among grazing cattle with even they are heavily lactating of foraging on rapid-growing herbage which grown on sandy, acidic or organic soils [53]. Our findings partially supported this claim.

3.4. Magnesium Concentration

Mg concentration in forages was varied among species and cultivars (Table 1 & Table 2). Pooled across harvests and cultivars, mean Mg concentration was 2.2 g·kg⁻¹ in forage grass and 3.32 g·kg⁻¹ in forage legume, which met the requirement for livestock [20] [48]. Magnesium concentration was lowest in first harvest followed by third harvest, and the highest occurred in the second harvest regardless of forage species and cultivars. Across the harvests, the lowest Mg concentration was in Aicapat 1.66 g·kg⁻¹, which was adequate for sheep but inadequate for beef cattle and red deer. Unlike, the highest Mg concentration was in North white at 5.08 g·kg⁻¹, the concentration necessary to meet requirements for beef cattle, sheep, and red deer [43] [44] [45] [52]. The dietary Mg concentration should be 3.5 - 4 g·kg⁻¹, which allows the cow to take advantage of passive absorption of Mg across the rumen wall, and the parathyroid hormone (PTH) interacts effectively with its receptors on bone and kidney cells [50]. Smith et al. [54] delineated that Mg concentration was 1.9 g·kg⁻¹ when herbage K was ≥25 g·kg⁻¹; whereas Ca concentration continues to decrease to 6 g·kg⁻¹ while forage K content increases to 65 g·kg⁻¹.

3.5. Sodium Concentration

Pooled across harvests, Na concentration varied considerably among the grasses and cultivars (Table 1 & Table 2). Evergreen cultivar of Festulolium showed the lowest and Yatsuboku perennial ryegrass showed the highest Na concentration. Among the orchardgrass species, the highest and lowest Na concentrations were recorded in Wasemidori (4.94 g·kg⁻¹) and Potomac (0.77 g·kg⁻¹), respectively, while the mean Na concentration among the forages was 1.3 g·kg⁻¹. The recommended concentration [20] [43] [44] [52] [48] of Na in forage for sheep, beef cattle, and red deer is 1.0, 1 - 2, and 0.7 g·kg⁻¹, respectively. In orchardgrass species, all cultivars were sufficient Na concentration for sheep, cattle, and red deer. In alfalfa species, only Natsuwakaba cultivar showed adequate Na for sheep and beef cattle. However, Evergreen, Aicap, Makimidori, and Hokuryo had Na con-
centration inadequate for livestock. The United States National Research Council [20] recommended that the Na concentration of 1.2 g·kg\(^{-1}\) is needed in a diet for a late gestation cow. Sodium is primarily a constituent of extracellular fluids in animals, and assists in maintaining osmotic pressure, acid-base equilibrium, nutrient passage into cells, and water metabolism, in conjunction with K and Cl [49]. Generally, animals have the potentiality to preserve Na; however, lactating individuals suffer from a lack of Na in their diet, as large amounts of Na passed away via milk extraction. Therefore, lactating animals require a spontaneous supply of Na through diet. Persistent inadequacies of Na in diets may cause loss of appetite, decreased growth or weight loss, unthrifty appearance, and reduced milking in ruminants [47].

3.6. Phosphorus Concentration

The maximum and minimum values for P were 0.79 g·kg\(^{-1}\) in Frontier and 4.51 g·kg\(^{-1}\) in Yatsukaze during first and second harvests, respectively (Table 1). Mean P concentration among forage grasses and legumes across harvests were 2.02 and 2.53 g·kg\(^{-1}\), respectively, and the concentrations were higher than required need for sheep and cattle [48] [52], which was 1.94 g·kg\(^{-1}\) (Table 2). However, the P levels were constantly lower than the requirements for red deer [43] [44]. Phosphorus is considered as one of the deficient nutrients for livestock [55] and P deficiencies are more pronounced in tropical grasses than temperate forages [56]. Phosphorus deficiency results in a decrease in reproduction in cattle [47]. With a serious P deficiency, lactating cows may not enter oestrus until they cease milking or are supplemented with P [57]. In our experiment, while conducted on Andisol, a high P retention soil, we did not find any P deficiencies in forages for beef cattle and sheep. Instead, among the forages, the general trend was that P levels equaled or exceeded cattle requirements with the exception of the Frontier cultivar of orchardgrass species, and all cultivars of smooth bromegrass, red clover, meadow fescue, and tall fescue species.

3.7. Sulphur Concentration

Highest and lowest S concentrations were recorded in Yatsukaze during the second harvest (4.74 g·kg\(^{-1}\) DM) and Frontier during the first harvest (0.41 g·kg\(^{-1}\) DM). As for the mean across harvests, S concentration of all forages was 1.72 g·kg\(^{-1}\), and the values ranged from a low of 1.63 in grasses to a high of 1.80 g·kg\(^{-1}\) in legumes (Table 1 & Table 2). Among forages, alfalfa and perennial ryegrass had S concentration above 2 g·kg\(^{-1}\). On the contrary, orchardgrass, Festulolium, smooth bromegrass, white clover, red clover, meadow fescue, and tall fescue were consistently low in S. To ensure adequate S nutrition for rumen, microbial amino acids, B-vitamins, proteins, and sulfhydryl bonds for some enzymes synthesis, dietary S must be kept above 2.2 g·kg\(^{-1}\) DM. To avoid possible neurological problems associated with S toxicity, the diet S should be kept below 4 g·kg\(^{-1}\) DM [58] [59]. Conversely, the growth of cattle restricts the long-term consum-
tion of high S diet, as increased dietary S affect Ca, K, Mg, Cu, Mn, Se, and Zn concentrations, thus limiting their absorption, retention, availability, and use by the animals [60] [61] [62]. Gooneratne et al. [63] reported that the consumption of a S and Mo-enriched diet for two months caused both the biliary and urinary Cu excretion to significantly increase in Angus and Simmental heifers. Mayland [58] indicated that in Canada and the western United States the excess S, whether consumed from diet or from drinking water, increased the risk of blind staggerers or clinical Polioencephalomalacia (PEM) in livestock. To avoid the deleterious effects of high-S diets on cattle health, recently the NRC [45] recommended 1.5 g S∙kg⁻¹ DM in cattle diets with maximum tolerable limits ranging from 3 to 5 g S∙kg⁻¹ DM for diets containing less than 15% forage or at least 40% forage, respectively. It is reported that the S concentration of 10.1 and 6.2 g∙kg⁻¹, respectively in diets is expected to cause either fatal death of cattle, comatose, or blind and head-pressing symptoms, respectively [64]. In our experiment, mean values across the harvests, none of the cultivars showed more than 3 g S∙kg⁻¹.

3.8. Chlorine Concentration

The required Cl concentration in diets for sheep was 1.0 g∙kg⁻¹ and for beef cattle, it was 2 g∙kg⁻¹. On the other hand, the growth of cool-season grass is optimized when the biomass Cl concentration is between 1 to 5 g∙kg⁻¹ DM [48]. In our study, the mean Cl concentration among grasses across three harvests was 8.2 g∙kg⁻¹. Among the forage grasses, the highest Cl concentration was in orchardgrass species (10.6 g∙kg⁻¹), whereas the lowest Cl content was in tall fescue (6.6 kg∙kg⁻¹). The Cl concentration in grasses ranked as: orchardgrass >Festuclium> perennial ryegrass > meadow fescue > smooth bromegrass > tall fescue.

Among the forage legumes, the highest Cl was in white clover (6.7 g∙kg⁻¹) followed by alfalfa 95.4 g∙kg⁻¹), and the lowest was in red clover 94.0 g∙kg⁻¹). Higher Cl concentration was observed in grasses when compared to legumes. Banowetz et al. [41] observed that orchardgrass had the least concentration of Cl compared to perennial ryegrass and tall fescue, respectively grown on silt loam soil in the high rainfall western Oregon. However, in our experiment, we measured higher Cl concentration in orchardgrass when compared to perennial ryegrass and tall fescue grown in temperate Andisol of Japan.

3.9. Grass Tetany Index

The GTI (Hypomagnesemia) is a non-infectious metabolic disorder in ruminants grazing on forages with nutrient imbalances (K, Ca, and Mg). When the forage K/(Ca+Mg) increases to above 2.2, the risk of GT in ruminants increases exponentially [17]. Results showed the values of GTI were harvest and species dependent (Table 1 and Table 3) and highest values were measured in first harvest followed by second harvest, and the lowest were in third harvest irrespective of forage species/cultivars. In orchardgrass species, except Kitamidori and Natsumidori, all other cultivars showed GTI values greater than 2.2 in the
Table 3. Grass tetany index (molc-kg⁻¹ DM) and mineral ratio of forages grown in temperate Andisol of Japan.

| Species               | Cultivar  | GTI¹   | K/Mg Harvest | Ca/P Harvest |
|-----------------------|-----------|--------|--------------|--------------|
|                       |           | 1   | 2  | 3  | 1   | 2  | 3  | 1   | 2  | 3  |
| Orchardgrass          | Akimidori | 2.31a² | 1.22b | 0.79c | 12.21a | 6.66b | 4.12b | 1.44ab | 1.25b | 1.75a |
|                       | Akimidori II | 2.83a | 1.15b | 0.96b | 14.78a | 6.21b | 5.31b | 1.11c | 1.58a | 1.77a |
|                       | Kitamidori | 2.15a | 0.95b | 0.61c | 12.09a | 5.05b | 3.44b | 1.42b | 1.39b | 1.85a |
|                       | Wasemidori | 2.52a | 0.94b | 0.74b | 12.71a | 5.06b | 3.95b | 1.22b | 1.58a | 1.38ab |
|                       | Natsumidori | 2.14a | 1.20b | 1.01b | 11.38a | 6.13b | 5.88b | 1.47a | 1.59a | 1.47a |
|                       | Potomac   | 2.34a | 1.37b | 1.66b | 12.92a | 7.97b | 8.91b | 1.91a | 1.70ab | 1.26 |
|                       | Frontier  | 4.06a | 1.77b | 1.37b | 20.54a | 10.32b | 7.25c | 1.21b | 1.63a | 1.49ab |
|                       | Makibamidori | 3.93a | 1.43c | 1.79b | 21.17a | 9.59b | 11.86b | 1.16b | 1.96a | 1.69a |
|                       | Okamidori | 3.70a | 1.76b | 1.66b | 19.69a | 10.93b | 9.89b | 1.23b | 1.71a | 1.45ab |
|                       | Toyomidori | 3.04a | 2.17b | 2.46b | 16.13a | 13.43a | 16.44a | 1.54ab | 1.63a | 1.37b |
| Festulolium            | Felina    | 2.11a | 1.59b | 1.77b | 12.28a | 8.09b | 8.82b | 1.56a | 1.54a | 1.34a |
|                       | Evergreen | 1.48a | 1.02a | 1.30a | 11.03a | 6.29c | 9.21b | 2.31a | 1.69b | 2.15a |
| Smooth bromegrass      | Aicap     | 2.99a | 2.08b | 1.16c | 21.02a | 14.93b | 10.41c | 1.59b | 1.78b | 2.73a |
| Perennial ryegrass     | Yatsuboku | 1.35a | 0.63b | 0.59b | 8.58a  | 4.01b  | 3.93b | 1.86a | 1.68a | 1.99a |
|                       | Yatsukaze | 0.67a | 0.63a | 0.53b | 5.37a  | 3.98b  | 3.65b | 2.43a | 1.44c | 2.08b |
|                       | Yatsunami | 0.46b | 0.68a | 0.46b | 3.92a  | 4.76a  | 3.58a | 3.83a | 1.71c | 2.38b |
|                       | Yatsuyutaka | 0.51b | 1.04a | 0.57b | 4.42ab | 6.89a  | 3.95b | 4.39a | 1.57c | 2.31b |
|                       | Friend    | 0.81b | 0.99ab| 1.04a | 5.81a  | 6.63a  | 6.69a | 2.48a | 1.48b | 1.88b |
|                       | Kusaboshi | 1.63a | 1.29a | 1.29a | 11.14a | 8.31b  | 7.64b | 1.91a | 1.19b | 1.38b |
| Meadow fescue          | Riguro    | 1.82a | 1.93a | 1.09b | 11.94a | 10.9a  | 7.77b | 1.95b | 1.79b | 2.85a |
| Tall fescue            | Hokuryo   | 2.55a | 2.31a | 1.59b | 13.65a | 11.73ab| 8.74b | 1.57a | 1.22a | 1.45a |
|                       | Southerncross | 2.95a | 1.89b | 1.86b | 17.67a | 10.92b | 9.78b | 1.54a | 1.56a | 1.35a |
| Alfalfa                | Natsuwakaba | 0.74b | 0.92a | 0.93a | 13.40a | 13.61a | 16.15a | 9.09a | 5.09b | 5.29b |
|                       | Kitawakaba | 0.92a | 0.84a | 0.86a | 16.43a | 12.98b | 12.49b | 7.09a | 5.96b | 6.11ab |
|                       | Tsuyuwakaba | 0.56c | 0.91a | 0.78ab| 10.59a | 13.32a | 11.58a | 11.1a | 4.94b | 5.52b |
|                       | 5444      | 0.54b | 0.87a | 0.52b | 10.32b | 14.79a | 6.92c | 9.87a | 4.94b | 8.13a |
|                       | Tachiwakaba | 0.74b | 1.01a | 0.77b | 14.98a | 16.43a | 10.99b | 8.81a | 4.74c | 6.14b |
|                       | Makiwakaba | 0.87ab| 0.97a | 0.77b | 13.58a | 13.59a | 12.17a | 7.59a | 4.71b | 7.76a |
|                       | Hisawakaba | 0.79b | 1.00a | 0.81b | 13.05a | 13.87a | 9.29b  | 7.55a | 4.48b | 4.82b |
|                       | Vertus    | 0.86b | 1.07a | 0.82b | 12.39a | 14.85a | 9.41b  | 6.67a | 4.01b | 5.27ab |
|                       | Euver     | 0.63c | 1.23a | 0.84b | 9.27c  | 16.69a | 12.59b | 10.0a | 4.13c | 6.19b |
| White clover           | Northwhite | 0.31a | 0.39a | 0.34a | 4.43a  | 3.04a  | 3.45a | 7.34a | 4.19b | 5.66b |
| Red clover             | Makimidori | 0.66ab| 0.71a | 0.40b | 7.69a  | 8.33a  | 3.25b | 8.46a | 6.00c | 7.19b |

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|               | Average |          |          |          |          |          |          |          |
|---------------|---------|----------|----------|----------|----------|----------|----------|----------|
| **Orchardgrass** | 2.90    | 1.40     | 1.31     | 15.36    | 8.14     | 7.71     | 1.37     | 1.60     | 1.55     |
| **Festulolium** | 1.79    | 1.30     | 1.54     | 11.66    | 7.20     | 9.02     | 1.94     | 1.62     | 1.74     |
| **Smooth bromegrass** | 2.99    | 2.11     | 1.16     | 21.02    | 14.93    | 10.41    | 1.60     | 1.78     | 2.73     |
| **Perennial ryegrass** | 0.91    | 0.88     | 0.75     | 6.54     | 5.76     | 4.91     | 2.82     | 1.51     | 2.00     |
| **Meadow fescue** | 1.82    | 1.93     | 1.09     | 11.94    | 10.90    | 7.77     | 1.95     | 1.79     | 2.85     |
| **Tall fescue** | 2.75    | 2.10     | 1.72     | 15.66    | 11.33    | 9.26     | 1.56     | 1.39     | 1.40     |
| **Alfalfa** | 0.74    | 0.98     | 0.79     | 12.67    | 14.46    | 11.29    | 8.64     | 4.78     | 6.14     |
| **White clover** | 0.31    | 0.39     | 0.34     | 4.43     | 3.04     | 3.45     | 7.34     | 4.19     | 5.66     |
| **Red clover** | 0.66    | 0.71     | 0.40     | 7.69     | 8.33     | 3.25     | 8.46     | 6.00     | 7.19     |
| **Grasses** | 2.19    | 1.62     | 1.26     | 13.70    | 9.71     | 8.18     | 1.87     | 1.62     | 2.04     |
| **Legumes** | 0.57    | 0.69     | 0.51     | 8.26     | 8.61     | 6.00     | 8.15     | 4.99     | 6.33     |
| **Cool season forages** | 1.38    | 1.15     | 0.88     | 10.98    | 9.16     | 7.09     | 5.01     | 3.30     | 4.19     |

1GTI (Kemp and T Hart, 1957). 2Values in columns across parameters within harvest for each cultivar with the same letters are not significantly different at P ≤ 0.05.

first harvest. Aicap smooth bromegrass and Southern cross tall fescue also showed GTI values greater than 2.2 in first harvest and Hokuryo tall fescue showed a GTI value greater than 2.2 in both first and second harvests. In contrast, legumes (alfalfa and clover) and perennial ryegrass showed GTI values less than 2.2, irrespective of harvests.

Rahman et al. [65] reported that the GTI was extremely lower (0.42 to 0.52) in different grain legume forages grown in Japanese Andisol. The values of GTI were in order of tall fescue > smooth bromegrass > orchardgrass > meadow fescue > festulolium > perennial ryegrass > alfalfa > red clover > white clover. This result collaborates the importance of breeding high Mg content cultivars. An advancement has been made with breeding tall fescue [66] and orchardgrass [67] to overcome the GT risk in ruminants. The consistent results were observed by hydroponic experiment [68] as well as field experiment [69] in reduced GTI of high magnesium containing cultivars grown in temperate Andisol of Japan. It is worthwhile to mention that the new cultivars bred for high Mg content have resulted in reduced values of K/(Mg + Ca) in grasses used as a source of forage [28][70]. The GTI values of all grasses and legumes were lower than the GT risk, which ranked as: grasses>legumes>combined grass and legume. Research in the temperate region of Japan delineated that GTI depends on pasture management, as organic management showed higher GTI values than chemical-based pasture management especially for forage mixture, which showed lower GTI values over the harvests [27]. We concluded that 1) dual grazing pasture is better when compared to single grazing pasture to control GT and 2) the management prac-
tices can minimize the GT risk in ruminants. While in most situations, 2 g Mg·kg⁻¹ DM is considered adequate to meet Mg requirements for ruminants [51]. However, this recommended Mg level may not always be the case to reduce the GT risk especially in temperate Andisols. Andisols are enriched with 2:1 and 2:1:1 clays and fine-grained quartz, and their exchange sites are dominated by allophane, imogolite, and ferrihydrite enriched with Al- and (Fe)-humus complexes, having a pH (H₂O) range of 4.3 to 6.7 [71]. Aluminum in acid soil reduced Ca uptake by cool-season grasses, elevating the GTI [72].

3.10. Potassium/Magnesium

The K/Mg ratio is the indication of uptake pattern between K and Mg in plants. The lowest K/Mg ratio was recorded in North white of white clover species in the second harvest, and highest was in Aicap of smooth bromegrass species across all the harvests (Tables 1-3). While the K concentration decreased with soil depth [73], deep-rooted plants are expected to extract more Mg than K from soil solution and this trend allows the K/Mg ratio to be lower, as more Mg will be available in the deeper root zone. Hackett [74] stated that a greater root distribution especially depth, branching, and numbers of secondary roots was observed related to soil P levels. Moreover, the uptake of water and nutrients is influenced by rooting depth, morphology, and architecture. Higher amounts of root mass in deeper soils, or a greater rooting depth, could improve the access to subsoil water [75], which could increase the Mg uptake by forages. Shewmaker et al. [76] observed that increased Mg concentration in growth media increases Mg but limits K uptake by boosting the Mg translocation mechanism in tall fescue, which affects K/Mg ratio in forages. Therefore, root characteristics, as well as nutrient concentrations at soil depths, are responsible for higher Mg acquisition and translocation. However, studies of cool-season root systems are scanty, and should be performed in detail.

3.11. Calcium/Phosphorus

Generally, Ca and P concentrations are strongly linked to support metabolic functions in animals. In the first harvest, the lowest and highest levels of Ca/P were in Akimidori II (1.11 g·kg⁻¹) and Tsuyuwakaba (11.07 g·kg⁻¹), respectively (Table 1). In the present experiment, we observed that having alfalfa in the diet may increase the Ca intake, as it contains higher Ca; however, its imbalances the Ca/P ratio (Table 2). The Ca/P ratio was low in forage grasses (1.84, across three harvests) and higher in forage legumes (6.49, across three harvests) than 2:1 (Table 3). Rahman et al. [65] observed a consistently lower Ca/P ratio in grain forage legumes grown in Japanese Andisol. Therefore, a diet of both forage grasses and legumes, with the addition of grain forage legumes, could be advantageous in balancing the Ca/P ratio. While uptake of water and nutrients is influenced by root length, depth, and root diameter, shallow rooting is advantageous for P assimilation [77] as most soil P is concentrated at the surface soil.
The ideal ratio of Ca/P is 2:1, however, 8:1 can be tolerated. Miller [79] suggested that the Ca/P ratio is not crucial unless the ratio is >7:1 or <1:1. We detected Ca/P lower than the optimum in forage grasses and higher in forage legumes. The Ca/P ratio was twofold higher than optimum, but lower than the tolerable limit in forage grass-legume mixtures. A high grain or grain byproduct-enriched diet may adversely affect the Ca/P ratio as grain or grain byproducts are normally very high in P. Due to P deficiency, cattle and sheep may be observed chewing on bones. Stone formation in the kidney of male sheep or cattle is common when the dietary Ca/P ratio is less than 2:1 [80].

3.12. Dietary Cation-Anion Difference

The DCAD varied significantly among species, cultivars, and harvests (Table 1 and Table 4). The trend of DCADs, calculated with different equations, is pretty much same. The DCAD3 showed the highest values in all cultivars (except Toyomidiri cultivar) of orchardgrass, Festulolium, perennial ryegrass, smooth bromegrass, and tall fescue species across the harvests. In contrast, the DCAD2 showed the highest values in alfalfa, clover, and meadow fescue across the harvests. The maximum acceptable value of DCAD ranged between 250 and 290 cmolc·kg⁻¹ DM for forage [81] [82]. The recommended values of DCAD1, DCAD2, and DCAD3 for diets are −50, 150, and −42 cmolc·kg⁻¹ DM, respectively [21] [81] [82]. In the second harvest, the values of cation-anion differences were wider and the highest and lowest values of DCAD1, DCAD2, and DCAD3 were recorded in Yatsunami and Euver, respectively. The values ranged from 72 to 606, 63 to 647, and 112 to 668 cmolc·kg⁻¹ DM for DCAD1, DCAD2, and DCAD3, respectively. Across the harvests, the values of DCAD1, DCAD2, and DCAD3 showed the highest values in alfalfa, clover, and meadow fescue across the harvests.

Table 4. Dietary cation-anion differences (DCAD, mmolc·kg⁻¹ DM) of forages grown in temperate Andisol of Japan.

| Species      | Cultivar | DCAD1¹ | DCAD2² | DCAD3³ |
|--------------|----------|--------|--------|--------|
|              |          | Harvest | Harvest | Harvest |
|              |          | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Orchardgrass | Akimidori | 288a¹ | 236a | 192b | 296a | 221b | 212b | 323a | 296a | 243b |
|              | Akimidori II | 301a | 228b | 214b | 294a | 236b | 221b | 335a | 289b | 260b |
|              | Kitamidori | 287a | 242a | 150b | 283a | 238b | 157c | 319a | 305a | 188b |
|              | Wasemidori | 268a | 261a | 214a | 264a | 259a | 211b | 288b | 311a | 274b |
|              | Natsumidori | 320a | 229b | 305a | 319a | 238b | 283b | 347a | 279b | 346a |
|              | Potomac | 321a | 252b | 305a | 338a | 246b | 279b | 357a | 295b | 339a |
|              | Frontier | 334a | 369a | 328a | 326b | 369a | 323b | 344b | 418a | 372b |
|              | Makibamidori | 340a | 271b | 354a | 328a | 260b | 338a | 357b | 313c | 394a |
|              | Okamidori | 345a | 453b | 371a | 336b | 440a | 349b | 364c | 501a | 415b |
|              | Toyomidori | 371b | 544a | 501a | 369c | 543a | 479b | 392c | 610a | 554b |
Continued

| Forage Type       | Cultivar       | 341b | 286b | 207c | 333a | 298b | 216c | 369a | 334a | 244b |
|-------------------|----------------|------|------|------|------|------|------|------|------|------|
| Festulolium       | Felina         | 341a | 286b | 207c | 333a | 298b | 216c | 369a | 334a | 244b |
| Smooth bromegrass | Aicap          | 475a | 451a | 165b | 458a | 431a | 167b | 505a | 488a | 190b |
| Perennial ryegrass| Yatsuboku      | 203a | 75b  | 89b  | 199a | 73b  | 107b | 222a | 144b | 214b |
|                   | Yatsukaze      | 120a | 139a | 79b  | 124a | 138a | 97b  | 152b | 257a | 130b |
|                   | Yatsunami      | 93a  | 72a  | 81a  | 110a | 63b  | 89ab | 112a | 159a | 128a |
|                   | Yatsuyutaka    | 136b | 216a | 291a | 164c | 208b | 297a | 159b | 299a | 327a |
|                   | Friend         | 91c  | 119b | 185a | 92c  | 115b | 195a | 114b | 223a | 238a |
| Meadow fescue     | Kusaboshi      | 130a | 153a | 126a | 125a | 109a | 124a | 161a | 224a | 187b |
| Tall fescue       | Riguro         | 416a | 490a | 242  | 405b | 506a | 252c | 444b | 542a | 267c |
| Alfalfa           | Hokuryo        | 474b | 554a | 233c | 480b | 551a | 231c | 507b | 606a | 268c |
|                   | Southerncross  | 446b | 560a | 258c | 447b | 559a | 256c | 480b | 612a | 290c |
|                   | Natsuwakaba    | 422b | 539a | 527a | 519b | 604a | 585a | 465b | 604a | 584a |
|                   | Kitawakaba     | 471a | 425a | 478a | 533a | 493b | 568a | 504a | 475b | 545a |
|                   | Tsuyuwakaba    | 353b | 386b | 476a | 487b | 429b | 560a | 403b | 432b | 548a |
|                   | 5444           | 326b | 447a | 229c | 468b | 505a | 374c | 392b | 512a | 310c |
|                   | Tachiwakaba    | 387c | 566a | 445b | 478c | 618a | 535b | 428b | 631a | 509a |
|                   | Makiwakaba     | 506a | 538a | 516a | 599b | 598b | 630a | 557b | 605a | 579b |
|                   | Hisawakaba     | 431b | 541a | 529a | 523b | 581a | 607a | 482b | 593a | 602a |
|                   | Vertus         | 410b | 563a | 466b | 501c | 601a | 556b | 469c | 632a | 539b |
|                   | Euver          | 341c | 606a | 414b | 448c | 647a | 509b | 382c | 668a | 487b |
| White clover      | Northwhite     | 313a | 263b | 253b | 419a | 351b | 352b | 347a | 331ab| 304b |
| Red clover        | Makimidori     | 365b | 433a | 313c | 440a | 485a | 417b | 386b | 456a | 342c |
| Average           |                | 318  | 309  | 293  | 315  | 305  | 285  | 343  | 362  | 338  |

1Ender et al. (1971); 2NRC (2001); 3Goff et al. (2004). 4Values in columns across parameters within harvest for each cultivar with the same letters are not significantly different at P ≤ 0.05.
DCAD3 in perennial ryegrass were lower than the maximum values for all forages. The average values of DCAD1, DCAD2, and DCAD3 were lower in forage grasses than in forage legumes. Pelletier et al. [82] also observed that the average value of DCAD3 for legumes was more than two fold higher than for grasses.

3.13. Role of Prime Nutrients on Grass Tetany and Dietary Cation-Anion Differences

In our studies, seven essential and beneficial elements (K, Ca, Mg, Na, P, S, and Cl) were used to calculate and evaluate GTI and DCAD’s. Notwithstanding the seven dietary ions caused animal health hazards, of which ion has the predominant impact in assembling the DCAD’s or GTI for ruminants. Pearson correlation coefficients were computed to evaluate the influence of prime nutrient elements on GTI or DCADs. Several significant correlations were identified among parameters (Table 5).

While Na concentration moderately and positively correlated with Cl (r = 0.46) and negatively with K (r = −0.77), Mg concentration positively and significantly correlated with P (r = 0.61). Likewise, Ca significantly and positively correlated with P (r = 0.46) and S (r = 0.49) but negatively with Cl (r = −0.78). DCAD1, DCAD2, and DCAD3 significantly and negatively correlated with Na (r = −0.64, −0.61, and −0.63, respectively) and Cl (r = −0.54, −0.66, and −0.56, respectively) but positively with K (r = 0.91, 0.85 and 0.91, respectively) and moderately with Ca (r = 0.48, 0.66 and 0.52, respectively). Among Mg, P, and S, the correlations were mostly negative and linearly non-significant (Table 3), which indicates that the influence of Mg, P, and S is minimal in calculating DCAD1, DCAD2, and DCAD3. It is not clear as to why there were no significant correlations between Mg, P, and S concentration with DCADs. In the calculating cation-anion

|     | Na  | Mg  | P   | S   | Cl  | K   | Ca  | DCAD1 | DCAD2 | DCAD3 | GT  |
|-----|-----|-----|-----|-----|-----|-----|-----|-------|-------|-------|-----|
| Na  | 1   | 0.218 | 0.205 | 0.138 | 0.464 | −0.772 | −0.318 | −0.637 | −0.614 | −0.625 | −0.432 |
| Mg  | 1   | 0.607 | −0.087 | 0.032 | −0.287 | 0.276 | −0.219 | −0.134 | −0.226 | −0.539 |
| P   | 1   | 0.534 | −0.001 | −0.246 | 0.416 | −0.291 | −0.176 | −0.246 | −0.499 |
| S   | 1   | −0.269 | −0.024 | 0.494 | −0.035 | 0.104 | 0.049 | −0.549 |
| Cl  | 1   | −0.297 | −0.780 | −0.536 | −0.660 | −0.558 | 0.492 |
| K   | 1   | 0.318 | 0.913 | 0.854 | 0.911 | 0.431 |
| Ca  | 1   | 0.482 | 0.658 | 0.523 | −0.669 |
| DCAD1 | 1 | 0.975 | 0.997 | 0.209 |
| DCAD2 | 1 | 0.983 | 0.005 |
| DCAD3 | 1 | 0.163 |
| GT  | 1   | 0.752 | 

*Shaded values are statistically significant (P < 0.05).
difference equation S either has been ignored [83] or dismissed [84] or included [85] [86]. The GTI significantly and negatively correlated with Mg (r = −0.54) and Ca (r = −0.67), as well as with Na (r = −0.43), P (r = −0.50) and S (r = −0.55), but positively with K (r = 0.43) and also with Cl (r = 0.49). Our results closely collaborated with the results of previous studies that the GT has been associated with the imbalances among K, Ca, and Mg concentrations in forages [87] [88]. Additionally, there is a potential contribution of Na, P, and S in GT risk that should be investigated. The GTI values were not correlated significantly either with DCAD1, or with DCAD2 and DCAD3. Research revealed that grasses bred for high Mg commitment had significantly higher shoot Mg concentrations compared to commercial cultivars. Conversely, high Mg-containing grass cultivars showed higher in Ca and lower in K concentration, which resulted a lower K/(Ca + Mg) ratio than the commercial cultivars [69]. Grass leaf contained 0.2% DM Mg provides sufficient Mg to protect against grass tetany incidence of ruminant [89]. On the other hand, cool season grasses grown in 5.0 mM of K level showed the highest differences in shoot Mg and K [90]. The study conferred that the tetany index in grass species is very much age/harvest specific [69]. The GT value depends on soil fertility [91] and nevertheless of grass species, increasing soil P level reduced the grass tetany risk and the value of GT became lowest at 5mM P level [68]. The milk production depends on dry mater intake (DMI) and body condition score (BCS) at calving. On the other hand, DMI depends on the digestibility components and nutritional factors. Increased the proportion of forage legumes compared to grasses in the diet of dairy cows boosted DMI thus increased milk yields [92].

4. Conclusion

The nutrient appraisal of cool-season forages of 33 cultivars across nine species over three harvests associated with animal health disorders, Cl was the nutrient that showed adequate concentration for forages. In contrast, K, Mg, and S concentrations were adequate for forage grasses. The highest values of GTI, as well as DCAD, were found either in the first or second harvest, and the lowest values were found in the third harvest irrespective of forage species and cultivars. The values of GTI in alfalfa and perennial ryegrass species were <2.2 across the harvests. The DCAD values in alfalfa and perennial ryegrass species were close to the recommended values for livestock diets. The current study confirms that grass/legume mixtures reduced the GTI and DCAD in forage growing in Andisols. While plant breeders developed grass cultivars to eliminate animal health disorders that are triggered by mineral imbalances, nevertheless, we recommend that 1) grass-legume balanced association, 2) rational pasture management, 3) species-cultivar specific forages, and 4) harvest-dependent grazing could be considered for economically viable and healthy livestock production.

Conflicts of Interest

The authors declare no conflicts of interest.
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