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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, festulolium, smooth bromegrass, 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, and clover species did not show any GT risks over season. On the other hand, all cultivars of orchardgrass, smooth bromegrass, 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, Temperate Andisol

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 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 interpretated 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 prantese*), smooth bromegrass (*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 $g \cdot kg^{-1}$), bulk density (~0.54

$Mg \cdot m^{-3}$), 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_2O_5, and $K_2O \cdot ha^{-1}$, 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˚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]:

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

Three different approaches used to calculate DCAD were:

$$\text{DCDA1 (Ender \textit{et al.}, 1971)} = (\text{Na} + \text{K}) - (\text{Cl} + \text{S}) \quad (2)$$

$$\text{DCDA2 (NRC, 2001)} = (\text{Na} + \text{K} + 0.15\text{Ca} + 0.15\text{Mg}) - (\text{Cl} + 0.6\text{S} + 0.5\text{P}) \quad (3)$$

$$\text{DCDA3 (Goff \textit{et al.}, 2004)} = (\text{Na} + \text{K}) - (\text{Cl} + 0.6\text{S}) \quad (4)$$

where, GTI expressed in moles of charge basis ($cmolc_+ \cdot kg^{-1}$ of DM) and DCAD is expressed in millimoles of charge basis ($cmolc_+ \cdot kg^{-1}$ 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 $P \leq 0.05$. 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 ($10.2_{\text{Yatsunami}}$ and $34.3_{\text{Toyomidori}}$ g·kg^{-1} of DM) and the narrowest for P (1.73_{Felina} and $3.58_{\text{Northwhite}}$ g·kg^{-1} of DM). The range of DCAD across harvest was the widest for DCAD2 ($87_{\text{Yatsunami}}$ and 609_{Makiwaba} cmolc·kg^{-1} of DM) followed by DCAD3 ($133_{\text{Yatsunami}}$ and 580_{Makiwaba} cmolc·kg^{-1} of DM) and the narrowest for DCAD1 ($82_{\text{Yatsunami}}$ and 520_{Makiwaba} cmolc·kg^{-1} of DM), respectively.

Table 1. Summary statistics (n = 99; number of observations) of nutrients[1] and their interaction[2].

Elements	Harvest	Skewness	Kurtosis	SD[3]	Mean	Minimum	Maximun
K	1	−1.04	0.35	6.37	21.69	$6.75_{\text{Yatsunami}}$	30.5_{Aicap}
	2	−0.01	−0.26	6.41	26.57	$13.0_{\text{Yatsuboku}}$	$41.6_{\text{Toyomidori}}$
	3	−0.14	−0.98	7.52	20.55	$7.3_{\text{Yatsukaze}}$	$32.2_{\text{Toyomidori}}$
	Av	−0.55	−0.30	6.17	22.94	$10.2_{\text{Yatsunami}}$	$34.3_{\text{Toyomidori}}$
Ca	1	1.03	−0.20	6.43	6.84	0.97_{Frontier}	$23.6_{\text{Northwhite}}$
	2	0.77	−1.04	4.19	7.38	2.8_{Hokuryo}	$16.56_{\text{Northwhite}}$
	3	0.87	−0.89	5.49	7.39	1.94_{Felina}	$20.2_{\text{Northwhite}}$
	Av	0.90	−0.73	5.32	7.20	2.5_{Hokuryo}	$20.1_{\text{Northwhite}}$
Mg	1	2.02	7.83	0.54	1.90	1.02_{Frontier}	$4.1_{\text{Northwhite}}$
	2	2.13	7.74	0.98	3.07	$1.86_{\text{Tsuyuwakaba}}$	$7.1_{\text{Northwhite}}$
	3	1.91	4.52	0.88	2.73	1.36_{Aicap}	$5.6_{\text{Northwhite}}$
	Av	3.30	14.74	0.65	2.57	1.62_{Aicap}	$5.5_{\text{Northwhite}}$
Na	1	1.11	−0.06	1.62	1.75	$0.19_{\text{Kusaboshi}}$	$5.33_{\text{Yatsukaze}}$
	2	1.44	1.10	1.59	1.75	$0.17_{\text{Evergreen}}$	$5.75_{\text{Wasemidori}}$
	3	1.37	0.68	1.93	1.90	$0.07_{\text{Evergreen}}$	$9.60_{\text{Yatsuyutaka}}$
	Av	1.20	−0.05	1.61	1.80	$0.26_{\text{Evergreen}}$	$5.31_{\text{Yatsuboku}}$
P	1	1.93	7.66	0.41	1.54	0.79_{Frontier}	$3.22_{\text{Northwhite}}$
	2	0.96	0.48	0.62	2.86	$1.97_{\text{Makimidori}}$	$4.51_{\text{Yatsukaze}}$
	3	0.53	0.94	0.46	2.22	1.41_{Aicap}	$3.57_{\text{Northwhite}}$
	Av	1.88	6.53	0.35	2.21	1.73_{Felina}	$3.58_{\text{Northwhite}}$
S	1	0.69	0.41	0.52	1.28	0.41_{Frontier}	2.645444

Continued

	2	0.93	2.64	0.76	2.42	$0.95_{\text{Makimidori}}$	$4.74_{\text{Yatsukaze}}$
	3	0.21	−0.78	0.60	2.01	1.01_{Aicap}	3.25_{5444}
	Av	−0.13	0.003	0.47	1.91	$1.00_{\text{Makimidori}}$	2.83_{5444}
Cl	1	0.37	−1.08	2.55	8.06	$4.29_{\text{Makimidori}}$	$12.6_{\text{Natsumidori}}$
	2	0.27	−1.07	3.10	8.96	$3.87_{\text{Makimidori}}$	$15.5_{\text{Toyomidori}}$
	3	0.16	−1.37	1.71	6.69	$3.91_{\text{Makimidori}}$	$9.56_{\text{Yastuboku}}$
	Av	0.15	−1.30	2.29	7.90	$4.02_{\text{Makimidori}}$	$12.2_{\text{Toyomidori}}$
GTI	1	0.59	−0.82	1.12	1.70	$0.31_{\text{Northwhite}}$	4.06_{Frontier}
	2	0.74	−0.20	0.48	1.21	$0.39_{\text{Northwhite}}$	2.31_{Hokuryo}
	3	0.94	0.52	0.50	1.03	$0.34_{\text{Northwhite}}$	$2.46_{\text{Toyomidori}}$
	Av	0.52	−0.99	0.64	1.31	$0.35_{\text{Northwhite}}$	$2.61_{\text{Toyomidori}}$
K/Mg	1	0.02	−0.25	4.51	12.07	$3.75_{\text{Yatsunami}}$	20.79_{Aicap}
	2	0.08	−1.29	3.99	9.61	$3.04_{\text{Northwhite}}$	16.43_{Euver}
	3	0.36	−0.53	3.61	8.08	$3.24_{\text{Makimidori}}$	$16.08_{\text{Toyomodori}}$
	Av	−0.26	−1.11	3.46	9.92	$3.63_{\text{Northwhite}}$	$15.13_{\text{Toyomidori}}$
Ca/P	1	0.81	−1.00	3.34	4.09	$1.11_{\text{Akimidoti II}}$	$11.07_{\text{Tsuyuwakaba}}$
	2	4.42	22.68	0.89	0.91	$5.46_{\text{Northwhite}}$	0.24_{Hokurio}
	3	0.91	−0.70	2.22	3.26	1.27_{Potomac}	8.17_{5444}
	Av	0.75	−1.23	1.94	2.75	1.03_{Frontier}	6.34_{5444}
DCAD1	1	−0.65	−0.17	113	325	91_{Friend}	506_{Makiwaba}
	2	−0.10	−1.39	168	353	$72_{\text{Yatsunami}}$	606_{Euver}
	3	0.29	−1.03	138	295	$79_{\text{Yatsukaze}}$	$529_{\text{Hisawakaba}}$
	Av	−0.26	−1.01	127	324	$82_{\text{Yatsunami}}$	520_{Makiwaba}
DCAD2	1	−0.39	−0.73	137	357	92_{Friend}	599_{Makiwaba}
	2	−0.10	−1.40	185	368	$63_{\text{Yatsunami}}$	647_{Euver}
	3	0.44	−1.06	165	327	$89_{\text{Yatsunami}}$	630_{Makiwaba}
	Av	−0.03	−1.12	153	351	$87_{\text{Yatsunami}}$	609_{Makiwaba}
DCAD3	1	−0.60	−0.24	119	357	$112_{\text{Yatsunami}}$	557_{Makiwaba}
	2	0.05	−1.40	161	414	$144_{\text{Yatsuboku}}$	668_{Euver}
	3	0.40	−1.04	145	345	$128_{\text{Yatsunami}}$	$602_{\text{Hisawakaba}}$
	Av	−0.10	−1.04	128	372	$133_{\text{Yatsunami}}$	580_{Makiwaba}

[1]Mineral content in $g \cdot kg^{-1}$ DM; [2]DCAD in $mmolc \cdot kg^{-1}$ DM; [3]SD (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
	Yatsuyutaka	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

Continued

	Southerncross	25.8	2.69	2.03	1.02	1.80	1.60	6.59
Alfalfa	Natsuwakaba	29.0	13.92	2.03	1.33	2.29	2.19	6.01
	Kitawakaba	27.6	12.99	1.82	0.46	2.05	2.00	5.10
	Tsuyuwakaba	25.7	14.72	2.10	0.72	2.19	2.24	5.16
	5444	24.5	16.20	2.20	0.97	2.22	2.83	5.69
	Tachiwakaba	28.2	14.07	1.80	0.93	2.27	2.27	5.53
	Makiwakaba	30.5	14.27	2.23	0.70	2.21	2.42	4.98
	Hisawakaba	29.3	13.25	2.15	0.85	2.46	2.34	5.07
	Vertus	31.0	13.19	2.62	0.43	2.58	2.68	5.91
	Euver	27.9	12.75	2.27	0.92	2.07	2.35	5.49
White clover	Northwhite	19.7	20.14	5.08	2.03	3.58	2.04	6.67
Red clover	Makimidori	20.2	12.32	2.73	0.66	1.74	1.00	4.02
Average								
Orchardgrass		23.5	3.2	2.2	2.4	2.1	1.6	10.6
Festulolium		22.0	3.5	2.5	0.6	2.0	1.7	8.9
Smooth bromegrass		24.7	3.6	1.7	0.3	1.9	1.2	7.4
Perennial ryegrass		13.6	4.6	2.3	3.5	2.4	2.2	8.2
Meadow fescue		25.1	4.1	2.4	1.0	1.9	1.4	7.7
Tall fescue		26.4	2.6	2.2	0.7	1.8	1.6	6.6
Alfalfa		28.2	13.9	2.1	0.8	2.3	2.4	5.4
White clover		19.7	20.1	5.1	2.0	3.6	2.0	6.7
Red clover		20.2	12.3	2.7	0.7	1.7	0.7	4.0
Grasses		22.6	3.6	2.2	1.4	2.0	1.6	8.2
Legumes		22.7	15.5	3.3	1.2	2.5	1.7	5.4
Cool season forages		22.6	9.5	2.8	1.3	2.3	1.7	6.8

*Data averege of four replications.

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 coos-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 $g \cdot kg^{-1}$ in forage grass and 3.32 $g \cdot kg^{-1}$ 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 $g \cdot kg^{-1}$, 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 $g \cdot kg^{-1}$, 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 $g \cdot kg^{-1}$, 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 $g \cdot kg^{-1}$ when herbage K was ≥25 $g \cdot kg^{-1}$; whereas Ca concentration continues to decrease to 6 $g \cdot kg^{-1}$ while forage K content increases to 65 $g \cdot kg^{-1}$.

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 $g \cdot kg^{-1}$) and Potomac (0.77 $g \cdot kg^{-1}$), respectively, while the mean Na concentration among the forages was 1.3 $g \cdot kg^{-1}$. 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 $g \cdot kg^{-1}$, 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 concentration 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 consumption 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 staggers or clinical Polioencephalomalacia (PEM) in livestock. To avoid the deleterious effects of high-S diets on cattle health, recently the NRC [45] recommended1.5 g $S\cdot kg^{-1}$ DM in cattle diets with maximum tolerable limits ranging from 3 to 5 g $S\cdot kg^{-1}$ 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 $g\cdot kg^{-1}$, 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 $S\cdot kg^{-1}$.

3.8. Chlorine Concentration

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

Among the forage legumes, the highest Cl was in white clover (6.7 $g\cdot kg^{-1}$) followed by alfalfa 95.4 $g\cdot kg^{-1}$), and the lowest was in red clover 94.0 $g\cdot kg^{-1}$). 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	GTI[1]			K/Mg			Ca/P		
		Harvest			Harvest			Harvest		
		1	2	3	1	2	3	1	2	3
Orchardgrass	Akimidori	2.31a[2]	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

Continued

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

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

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 practices 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 (H_2O) 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

[78]. 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

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

Species	Cultivar	DCAD1[1]			DCAD2[2]			DCAD3[3]		
		Harvest			Harvest			Harvest		
		1	2	3	1	2	3	1	2	3
Orchardgrass	Akimidori	288a[4]	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

Festulolium	Felina	341a	286b	207c	333a	298b	216c	369a	334a	244b
	Evergreen	267a	126c	190b	261a	126c	199b	290a	193b	238a
Smooth bromegrass	Aicap	475a	451a	165b	458a	431a	167b	505a	488a	190b
Perennial ryegrass	Yatsuboku	203a	75b	89b	199a	73b	107b	222a	144b	145b
	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
	Kusaboshi	130a	153a	126a	125a	109a	124a	161a	224a	187b
Meadow fescue	Riguro	416a	490a	242	405b	506a	252c	444b	542a	267c
Tall fescue	Hokuryo	474b	554a	233c	480b	551a	231c	507b	606a	268c
	Southerncross	446b	560a	258c	447b	559a	256c	480b	612a	290c
Alfalfa	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	429c	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										
Orchardgrass		318	309	293	315	305	285	343	362	338
Festulolium		304	206	198	297	212	208	330	263	241
Smooth bromegrass		475	451	165	458	431	167	505	488	190
Perennial ryegrass		129	129	142	136	118	151	153	218	192
Meadow fescue		416	490	242	405	506	252	444	542	267
Tall fescue		460	557	246	464	555	243	494	609	280
Alfalfa		405	512	453	506	564	547	454	572	523
White clover		313	263	253	419	351	352	347	331	304
Red clover		395	452	313	460	495	417	406	468	342
Forages		350	357	214	346	354	218	378	414	251
Legumes		371	409	340	462	470	439	402	457	389
Cool season forages		361	383	277	404	412	328	390	435	320

[1]Ender *et al.* (1971); [2]NRC (2001); [3]Goff *et al.* (2004). [4]Values in columns across parameters within harvest for each cultivar with the same etters are not significantly different at $P \leq 0.05$.

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

Table 5. Pearson correlation coefficients among the parameters of forages (pooled data of three harvests)*.

	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

*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 compered 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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