Title: Inclusion of Alfalfa Hay in Diets for Non-lactating Dairy Cows During the Prepartum Period

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Abstract: The objectives of this study were to determine dry matter intake, urine pH, Ca concentration in blood, Ca output in urine, and incidence of hypocalcemia from pregnant and non-lactating dairy cows consuming diets containing either grass hay (GH) or alfalfa hay (AH) with the inclusion of either calcium chloride (CL) or polyhalite mineral (PO) during the prepartum period. Eighty pregnant and non-lactating Holstein cows were fed 1 of the 4 experimental diets during the prepartum period. Diets included either GH or AH and CL or PO as the acidogenic product. All diets had a dietary cation-anion difference (DCAD) below -190 mEq/kg DM. Grass hay contained 7.5% CP, 74.9% NDF, 0.36% Ca, 0.09% Na, 1.88% K, 0.38% Cl, and 0.15% S. Alfalfa hay contained 19.6% CP, 45.6% NDF, 1.52% Ca, 0.16% Na, 2.5% K, 0.77% Cl, and 0.32% S. Cows consuming GH tended to consume more dry matter than cows consuming AH but dry matter intake did not differ between acidogenic products. Urine pH decreased below 6.5 for all diets, although cows consuming the GHPO had the greatest urine pH. The concentration of Ca in plasma decreased substantially around calving but neither the hay type nor the acidogenic product affected it. Urinary Ca output was lesser for cows consuming the GHPO diet. Cows consuming diets containing AH had a greater incidence of normocalcemia (37 and 40% for AHCL and AHPO, respectively) than cows consuming diets containing GH (20 and 25% for GHCL and GHPO, respectively). The conclusions of this study are that the inclusion of alfalfa hay in prepartum diets does not necessarily increase the incidence of hypocalcemia and that the cation-anion difference of the alfalfa hay is a determinant of whether alfalfa hay fits in a prepartum feeding program for pregnant and non-lactating dairy cows.

Introduction

Dairy cows transitioning from a pregnant and non-lactating stage to a lactating stage after calving have a great risk of suffering metabolic diseases. Periparturient paresis, also known as milk fever or clinical hypocalcemia, is a metabolic disease that occurs when the concentration of Ca in blood drops below 5.5 mg/dl (Goff, 2008) due to the utilization of Ca by the mammary gland to secrete colostrum. Because clinical hypocalcemia can impair muscle and nerve function, cows suffering periparturient paresis are recumbent unless this recumbency is reverted through an intravenous administration of Ca. Even though any cow with blood Ca concentrations below 5.5 mg/dL can be defined as clinically hypocalcemic, not every clinically hypocalcemic cow will show periparturient paresis (Hendriks et al., 2020). Subclinical hypocalcemia is another metabolic disease that cows may have during the transition period, and this occurs when the concentration of Ca in blood is between 5.5 and 8.0 mg/dL. Because no clinical signs exist, subclinical hypocalcemia can only be diagnosed after measuring the concentration of Ca in blood daily during the 4 days after calving (Neves et al, 2017; McArt and Neves, 2020). Hypocalcemia, in either its clinical or subclinical forms, can affect cow welfare and productivity.

Feeding prepartum diets with a negative dietary cation-anion difference (DCAD) to non-lactating pregnant cows is a frequent practice to reduce the risk of hypocalcemia in the postpartum (Ender et al. 1971; Goff et al. 2004). The DCAD is determined by the difference in chemical equivalents between the cations Na^+ and K^+ and the anions Cl^- and SO_4^{2-} (Ender et al., 1971). To obtain a negative DCAD, acidogenic ingredients containing Cl^- and SO_4^{2-} equivalents in greater proportions than Na^+ and K^+ equivalents are typically fed to non-lactating cows before calving (Lopera et al., 20018; Richardson et al., 2021), although the success of obtaining diets with a negative DCAD is highly-dependent on the concentrations of cations in the other ingredients included in the ration. In this regard, forages with great concentrations of K may demand greater inclusions of acidogenic ingredients to obtain a negative DCAD.

Alfalfa (Medicago sativa), in the form of fresh grass, hay or silage, is a forage commonly used in rations for dairy cattle. The relatively high DM yields and the high concentrations of CP and energy make alfalfa one of the best forages to feed dairy cattle. A drawback of alfalfa when included in prepartum rations, however, may be the high concentration of K (Joyce et al., 1997). The high concentration of K of alfalfa can challenge the possibility of obtaining a negative DCAD and, therefore, alfalfa may be less desirable to be included in diets for pregnant and non-lactating dairy cows in the prepartum period (Ferreira, 2017; Goof et al., 2007; Horst et al., 2008).

A negative DCAD can still be obtained when using ingredients with high concentrations of K as long as anion equivalents are provided. For example, even though polyhalite mineral contains ~12% K, Ferreira et al. (2019) and Richardson et al. (2021) fed polyhalite in rations (DCAD < -150 mEq/kg DM) for pregnant and non-lactating cows in the prepartum period and observed that urine pH was successfully decreased to values below 6.5. These observations imply that ingredients having relatively high concentrations of K may be incorporated in rations for pregnant and non-lactating cows in the prepartum period as long as a negative DCAD can be achieved.

In this study, we hypothesized that alfalfa hay can be included in prepartum diets for pregnant and non-lactating cows without increasing hypocalcemia as long as a negative DCAD can be obtained through the use of acidogenic products. Therefore, the objectives of this study were to determine the dry matter intake, urine pH, Ca concentration in blood, Ca output in urine, and incidence of hypocalcemia from pregnant and non-lactating dairy cows consuming diets containing either grass hay (GH) or alfalfa hay (AH) with the inclusion of either calcium chloride (CL) or polyhalite mineral (PO) during the prepartum period.

Materials and Methods

The study was conducted at Virginia Tech Dairy Complex in Blacksburg, VA, from February 2021 to March 2022. All procedures were approved by the Institutional Animal Care and Use Committee of Virginia Tech (Protocol No. 20-158). Eighty pregnant and non-lactating Holstein cows (34±7 days relative to expected calving date and 749±73 kg BW) approaching their second or greater calving (Table 1) were housed within 2 pens and trained to eat through an electronic gate feeding (American Calan Inc., Northwood, NH). Each pen contained 8 individual feeding tubs with their specific gate. Ten cohorts of 8 cows each were selected as cows approached their expected calving date (ECD). Cows were selected based on their proximity to calving and their parity (2nd calving or greater). When the first cow in a cohort reached 35 d before her expected calving date (d -35), the whole cohort was transferred from a pasture paddock to a pen within in a compost-bedded pack barn. Once in the pen, cows were fed once daily (8:00 am) a far-off diet for 14 d. Dry matter intake was not recorded during this 14-d training period to the Calan gates.

Table 1. Count of cows by parity and treatment.[†]

Parity	GHCL	GHPO	AHCL	AHPO
2	13	10	7	8
3	3	7	4	2
4	2	0	6	5
5	1	3	2	3
6	0	0	0	0
7	1	0	0	2
Average	2.8	2.8	3.2	3.6

[†] GHCL = grass hay and calcium chloride as acidogenic product, GHPO = grass hay and polyhalite as acidogenic product, AHCL = alfalfa hay and calcium chloride as acidogenic product, AHPO = alfalfa hay and polyhalite as acidogenic product.

When the first cow in a cohort reached 21 d before her expected calving date (d -21), cows were fed 1 of the 4 experimental diets (Table 2) according to a 2 × 2 factorial arrangement of treatments, in which hay type (AH vs. GH) and the acidogenic product (CL vs. PO) were the experimental factors. Grass hay (Table 3) was grown on-site and harvested in round-bales that were chopped using a hay chopper (Roto Grind 760, Burrows Enterprises, LLS; Greeley, CO). Large square-bales of AH (Table 3) were obtained from the Great Plains region and chopped using a vertical mixer (NDEco FS600; Sioux Falls, SD). Calcium chloride was the acidogenic product commonly included in prepartum diets (i.e., positive control) at the Virginia Tech Dairy Complex, and PO was an acidogenic product under evaluation for prepartum diets (Ferreira et al., 2019; Richardson et al., 2021).

The experimental diets were formulated using CPM Dairy (version 3.0.8.1; CAHP Software Information; Philadelphia, PA). Cow input for ration formulation included a BW equal to 720 kg, 37 months of age, 260 days pregnant, and a body condition score of 3.5. Formulation constraints included DMI (≥100% requirement), metabolizable energy (≥100% requirement), metabolizable protein (≥100% requirement), dietary forage (≥60% DM), hay inclusion (≥20% forage), dietary NDF (35%<NDF<40%), dietary NFC (≤40%), and dietary cation-anion difference (DCAD; approximately -160 mEq/kg DM). Rations were formulated after obtaining the Na, K, Cl, and S concentrations of the corn silage and the AH and GH. Mineral concentrations for concentrates were obtained from the feed library of CPM Dairy. The acidogenic products were then included into pelleted concentrate mixtures prepared by a commercial feed mill (Big Spring Mill, Inc; Elliston, VA).

The experimental diets were mixed and delivered daily (~8:00 am) using a mobile mixer (Data Ranger American Calan) equipped with a scale. Feed refusals (~5%) were collected daily with the same equipment, and DMI was estimated daily as the difference between feed delivered and feed refused. A preliminary mixing test was performed to ensure adequate mixing with small amounts of TMR (i.e., amount enough for feeding 2 cows). For this, 1 L of a 50 mM solution of LaCl3 was sprayed onto 15 kg of pelleted concentrate and mixed. Then, a small batch (~115 lb) of representative TMR was prepared and mixed for 3 minutes, dispensed on a tarp, and sampled (n = 10) for analysis of La concentration (Richardson et al., 2021). With a coefficient of variation equal to 8.2%, we considered this mixing process appropriate for mixing small batches.

Cows were weighed weekly, and the weights of 3 to 4 weeks were averaged to obtain the covariate BW. Urine and blood samples were collected before feeding on d -21, -14, -7, and 0 relative to ECD. To collect samples within 12 h of calving, cows were monitored every night through 3 cameras (Ring Spotlight Cam Plus; Santa Monica, CA). Within 12 h of calving, clinical signs of hypocalcemia (i.e., cold ears, wobbly gait or recumbency) were also monitored and recorded for all cows (Goff and Koszewski, 2018). In addition, blood samples were collected on d 1, 2, and 3 after calving (Neves et al., 2017; McArt and Neves, 2020). Urine samples were collected after vulva stimulation, and urine pH was determined immediately after collection using a portable pH meter (Hanna Checker, Hanna Instruments, Woonsocket, RI, USA). After collection, urine samples were filtered through grade 1 qualitative filter paper (Whatman, GE Healthcare Bio-sciences, Pittsburgh, PA, USA) and stored at -20 ◦C until Ca analysis. Blood samples were collected by venipuncture of the coccygeal vessel into 10-mL vacutainer tubes containing heparin as anticoagulant. Blood samples were centrifuged at 3000 × g for 10 min at 20 ◦C, and plasma was collected and stored at -20 ◦C until Ca analysis. Plasma samples were diluted (1:50) in a 7 mM solution of LaCl3 as described previously (Richardson et al., 2021). Urine samples were diluted (1:25) in a solution of 2% nitric acid (v/v). Diluted samples were analyzed for total Ca using inductively coupled plasma optical emission spectrometry (Spectro Arcos II ICP-AES, SPECTRO Analytical Instruments GmbH, Germany) using 318 µm of wavelength. The concentration of Ca in urine was normalized using urinary creatinine concentration, which was determined using a colorimetric assay (Creatinine kit 500701, Cayman Chemical, Ann Arbor, MI, USA) after a 1:20 dilution of urine. Calcium output was estimated as described in Eq. [1],

$$\text{Ca output} = 0.029 \times \text{BW} \times \text{Ca urine} \quad [1]$$

where Ca output is the daily output of Ca in g/d, 0.029 is the constant daily clearance of creatinine in g/kg BW (Valadares et al., 1999), BW is the body weight of the cow in kg, and Ca urine is the concentration of Ca in urine normalized by the concentration of creatinine in urine as g Ca/g creatinine.

Samples of feed ingredients were collected weekly and composited by cohort of cows (10 cohorts in total). All composited samples were dried to constant weight at 55 ◦C in a forced-air oven and ground to pass through a 1-mm screen of a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA). Ash concentration was determined after burning samples in a furnace (Thermolyne 30400, Barnstead International, Dubuque, IA, USA) for 3 h at 600 ◦C (Method 942.05, AOAC, 2019). Crude protein concentration was calculated as percent N × 6.25 after combustion analysis (Method 990.03, AOAC, 2019) using a Vario El Cube CN analyzer (Elementar Americas, Inc., Mount Laurel, NJ, USA). Ash-free NDF was determined using the Ankom200 Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Sodium sulfite and α-amylase (Ankom Technology) were included for NDF analysis (Ferreira and Mertens, 2007). Starch concentration was determined using the acetate buffer method of Hall (2009) with α-amylase from Bacillus licheniformis (FAA, Ankom Technology) and amyloglucosidase from Aspegillus niger (E-AMGDF, Megazyme International, Wicklow, Ireland). Concentrations of Na, K, Cl, and S were determined in a commercial laboratory (Cumberland Valley Analytical Services, Waynesboro, PA, USA).

Prior to designing the experiment, a statistical power analysis was performed using the POWER procedure of SAS (SAS version 9.4, SAS Institute Inc., Cary, NC, USA). Considering a statistical power equal to 0.80 and a probability of committing Type I error (α) equal to 0.10, 38 cows per treatment within a main effect were deemed sufficient to detect a 2.5-kg/d difference in DMI between two treatments. One cow consuming the AHCL died of pulmonary emphysema while in the experiment, leaving 79 experimental units for the final statistical analysis.

Variables were analyzed using the MIXED procedure of SAS (SAS version 9.4) using repeated measures (cow was the subject and day was the repeated measure). The statistical model for evaluating DMI in the pre-partum period included BW as a covariate [fixed; degrees of freedom (df) = 1], the effect of hay (fixed; df = 1), the effect of acidogenic product (fixed; df = 1), the interaction of hay × acidogenic product (fixed; df = 1), the effect of day (fixed; df = 21), the day × hay type interaction (fixed; df = 21), the day × acidogenic product interaction (fixed; df = 21), the day × hay type × acidogenic product interaction (fixed; df = 21), and the random residual error. The statistical models for evaluating urine pH, Ca concentration in plasma and urinary Ca output in the pre-partum period included the effect of hay type (fixed; df = 1), the effect of acidogenic product (fixed; df = 1), the interaction of hay type × acidogenic product (fixed; df = 1), the effect of day (fixed; df = 3, df = 7, and df = 4 for urine pH, Ca concentration in plasma, and urinary Ca output, respectively), the day × hay type interaction (fixed; df = 3, df = 7, and df = 4), the day × acidogenic product interaction (df = 3, df = 7, and df = 4), the day × hay type × acidogenic product interaction (fixed; df = 3, df = 7, and df = 4), and the random residual error.

Project Objectives and Corresponding Results

Objective	Corresponding Result
Dry matter intake	Cows consuming GH tended to consume more dry matter than cows consuming AH (11.6 vs 10.8 kg/d). Dry matter intake did not differ between acidogenic products (11.2 kg/d), and no interaction existed between hay type and acidogenic products.
Urine pH	Feeding the diets with a negative DCAD decreased urine pH, and this occurred for all four diets. Urine pH decreased to a lesser extent for the cows consuming the GHPO diet than for the rest of the cows.
Ca concentration in blood	The concentration of Ca in plasma decreased substantially around calving but neither the hay type nor the acidogenic product affected it. No interaction existed between these factors or between time and any of these factors.
Ca output in urine	An interaction existed between hay type and acidogenic product, and the urinary Ca output was greatest for cows consuming the GHCL diet.
Incidence of hypocalcemia	Only one cow had acute signs of clinical hypocalcemia (i.e., peripartum paresis). However, 13 out of the 79 cows had calcium concentrations in plasma below 5.5 mg/dL at least once between 0 and 3 d after calving. In addition, 44 out of 79 cows had calcium concentrations in plasma between 5.5 and 8.0 mg/dL at least once between 0 and 3 d after calving

Results and Discussion

On a dry matter basis, GH contained 7.5% CP and 74.9% NDF, whereas the AH contained 19.6% CP and 45.6% NDF (Table 3). Regarding minerals, GH contained 0.36% Ca, 0.09% Na, 1.88% K, 0.38% Cl, and 0.15% S, whereas AH contained 1.52% Ca, 0.16% Na, 2.5% K, 0.77% Cl, and 0.32% S (Table 3). The GH had a cation-anion difference equal to 289 mEq/kg DM, whereas the AH had a cation-anion difference equal to 292 mEq/kg DM (Table 3).

Cows consuming GH tended to consume more dry matter than cows consuming AH (11.6 vs 10.8 kg/d; $P = 0.07$; Figure 1). Dry matter intake did not differ between acidogenic products (11.2 kg/d; $P = 0.21$), and no interaction existed between hay type and acidogenic products ($P = 0.41$; Figure 1). Dry matter intake decreased towards calving ($P < 0.01$), and no interactions existed between day and hay or acidogenic product ($P > 0.51$).

Feeding the diets with a negative DCAD decreased urine pH ($P < 0.01$), and this occurred for all four diets (Figure 2). As reflected by the interaction between hay type and acidogenic product ($P = 0.08$), urine pH decreased to a lesser extent for the cows consuming the GHPO diet than for the rest of the cows, which lead to significant main effects for hay type ($P < 0.01$) and anionic product ($P < 0.01$).

Table 2. Ingredient inclusion and nutritional composition of prepartum diets containing either grass hay (GH) or alfalfa hay (AH) and calcium chloride (CL) or polyhalite (PO).

	GHCL	GHPO	AHCL	AHPO
Ingredient Inclusion, % DM				
Corn silage	38.1	38.0	40.5	39.8
Grass hay	22.7	22.6	-	-
Alfalfa hay	-	-	20.7	20.4
Corn grain	10.9	10.8	7.6	7.5
Soybean meal (48% CP)	10.9	10.4	4.2	4.0
Dry distillers' grains w/solubles	7.3	6.8	7.2	7.1
Soybean hulls	7.3	7.2	17.1	16.8
Calcium chloride (dihydrate)	2.5	-	2.5	-
Polyhalite	-	3.8	-	4.0
Mycotoxin binder[†]	0.182	0.181	0.180	0.177
Selenium product[‡]	0.027	0.027	0.027	0.027
Mineral and vitamin mix[¶]	0.045	0.045	0.045	0.044
Vitamin ADE mix[††]	0.100	0.099	0.099	0.097
Vitamin E (IU/g)	0.036	0.036	0.036	0.035
Ionophore[‡‡]	0.014	0.014	0.013	0.013
Nutritional Composition,[¶¶] % DM				
Organic matter	92.1 ± 0.9	92.3 ± 0.3	92.6 ± 0.8	92.4 ± 0.5
Crude protein	13.1 ± 0.7	12.9 ± 2.4	14.2 ± 1.2	14.4 ± 1.4
Neutral detergent fiber	41.2 ± 3.3	41.9 ± 2.4	39.2 ± 1.7	37.5 ± 2.2
Starch	22.0 ± 7.2	21.6 ± 6.7	20.4 ± 2.1	21.7 ± 6.8
Calcium	1.10 ± 0.12	0.85 ± 0.05	1.38 ± 0.02	1.20 ± 0.14
Sodium	0.07 ± 0.01	0.09 ± 0.01	0.09 ± 0.05	0.11 ± 0.02
Potassium	1.29 ± 0.17	1.63 ± 0.10	1.33 ± 0.18	1.54 ± 0.22
Chloride	1.31 ± 0.53	0.26 ± 0.03	1.56 ± 0.04	0.91 ± 0.44
Sulfur	0.32 ± 0.26	0.95 ± 0.06	0.21 ± 0.02	0.61 ± 0.22
DCAD,[†††] mEq/kg DM	-209 ± 30	-207 ± 45	-190 ± 34	-194 ± 57

[†] Mycosorb (Alltech, Inc; Nicholasville, KY).

[‡] Sel-Plex 600 (Alltech, Inc).

[¶] Contained 22.3% calcium; 7.5% magnesium; 2.8% potassium; 3.9% sulfur; 1.5% manganese; 1.5% zinc; 9,500 ppm iron; 2,500 ppm copper; 200 ppm iodine; 200 ppm cobalt; 66 ppm selenium; 227,273 IU/kg Vitamin A; 136,364 IU/kg Vitamin D3; 636 IU/kg Vitamin E.

[††] Contained 3500 IU/kg Vitamin A; 950 IU/kg Vitamin D3; 2000 IU/g Vitamin E.

[‡‡] Rumensin 90 (Elanco Animal Health; Greenfield, IN).

[¶¶] Mean ± SD (n = 8).

[†††] DCAD: dietary cation-anion difference.

The concentration of Ca in plasma decreased substantially around calving ($P < 0.01$; Figure 3) but neither the hay type ($P = 0.86$) nor the acidogenic product ($P = 0.81$) affected it. No interaction existed between these factors ($P = 0.37$) or between time and any of these factors ($P > 0.65$). For urinary Ca output (Figure 4), an interaction existed between hay type and acidogenic product ($P = 0.01$), and the urinary Ca output was the greatest for cows consuming the GHCL diet.

Only one cow had acute signs of clinical hypocalcemia (i.e., peripartum paresis). However, 13 out of the 79 cows had calcium concentrations in plasma below 5.5 mg/dL at least once between 0 and 3 d after calving (Figure 5). In addition, 44 out of 79 cows had calcium concentrations in plasma between 5.5 and 8.0 mg/dL at least once between 0 and 3 d after calving (Figure 5).

Table 3. Nutritional composition† of forages.

	Corn silage	Grass hay	Alfalfa hay
Dry matter, % as-fed	36.3 ± 3.0	86.0 ± 9.8	86.8 ± 7.4
Organic matter, % DM	97.1 ± 0.4	93.9 ± 0.7	90.2 ± 1.1
Crude protein, % DM	7.8 ± 0.7	7.5 ± 1.3	19.6 ± 2.0
Neutral detergent fiber, % DM	38.6 ± 3.7	74.9 ± 2.6	45.6 ± 4.4
Starch, % DM	33.9 ± 3.4	1.9 ± 0.9	2.4 ± 0.9
Calcium, % DM	0.20 ± 0.04	0.36 ± 0.04	1.52 ± 0.13
Sodium, % DM	0.01 ± 0.01	0.02 ± 0.02	0.16 ± 0.10
Potassium, % DM	0.75 ± 0.14	1.88 ± 0.21	2.50 ± 0.45
Chloride, % DM	0.08 ± 0.04	0.38 ± 0.09	0.77 ± 0.16
Sulfur, % DM	0.10 ± 0.04	0.15 ± 0.02	0.32 ± 0.02
Cation-Anion Difference, mEq/kg DM	109 ± 32	290 ± 46	292 ± 58

† Mean ± SD (n = 8).

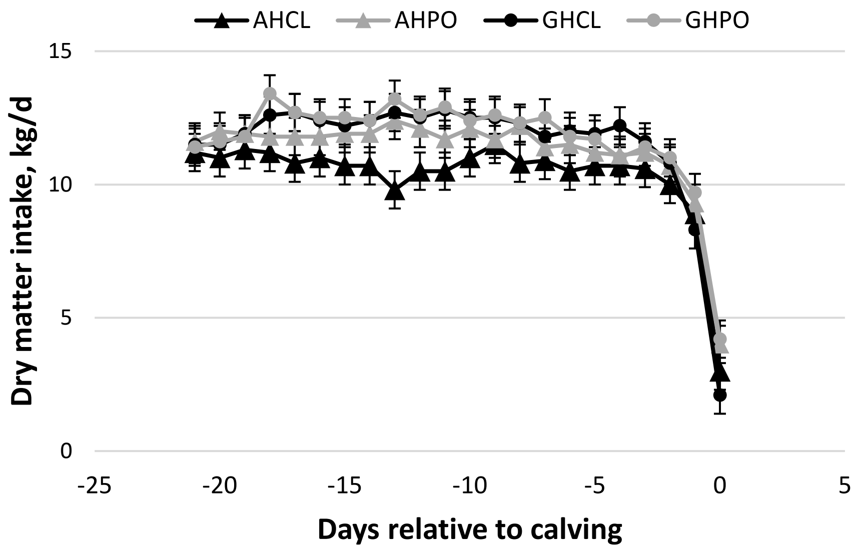

Figure 1. Dry matter intake of pregnant and non-lactating dairy cows during the prepartum period. Cows consumed diets containing grass hay and calcium chloride as an acidogenic product (GHCL; DCAD = -209 mEq/kg DM), grass hay and polyhalite as an acidogenic product (GHPO; DCAD = -207 mEq/kg DM), alfalfa hay and calcium chloride as an acidogenic product (AHCL; DCAD = -190 mEq/kg DM), or alfalfa hay and polyhalite as an acidogenic product (AHPO; DCAD = -194 mEq/kg DM). Error bars represent standard errors of the means (SEM).

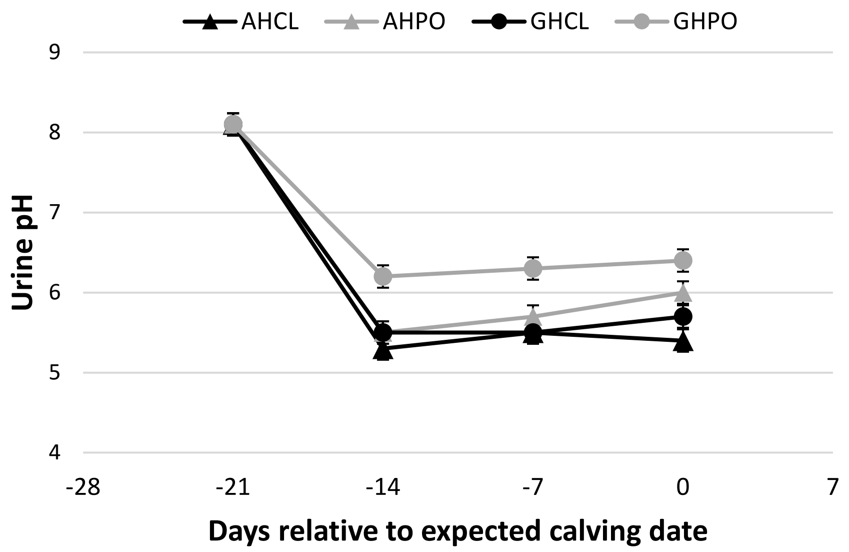

Figure 2. Urinary pH of pregnant and non-lactating dairy cows during the prepartum period. Cows consumed diets containing grass hay and calcium chloride as an acidogenic product (GHCL; DCAD = -209 mEq/kg DM), grass hay and polyhalite as an acidogenic product (GHPO; DCAD = -207 mEq/kg DM), alfalfa hay and calcium chloride as an acidogenic product (AHCL; DCAD = -190 mEq/kg DM), or alfalfa hay and polyhalite as an acidogenic product (AHPO; DCAD = -194 mEq/kg DM). Error bars represent standard errors of the means (SEM).

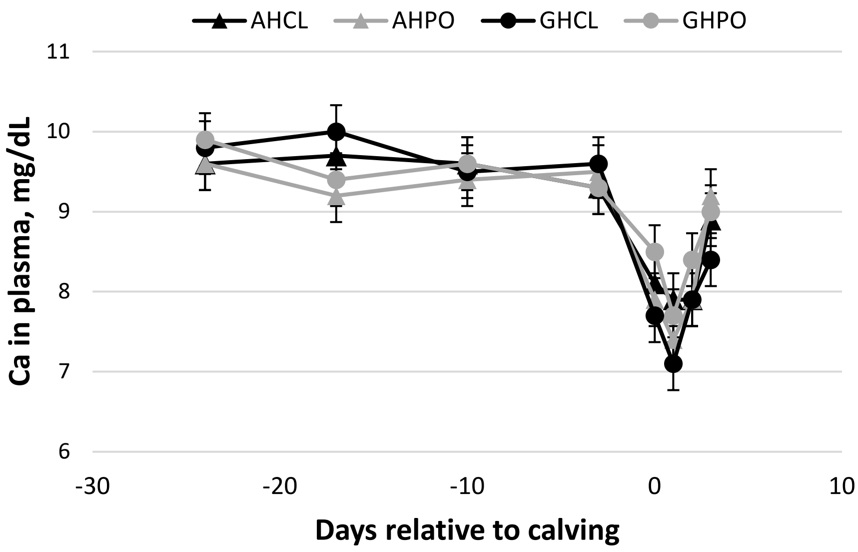

Figure 3. Concentration of Ca in plasma from pregnant and non-lactating dairy cows during the prepartum period. Cows consumed diets containing grass hay and calcium chloride as an acidogenic product (GHCL; DCAD = -209 mEq/kg DM), grass hay and polyhalite as an acidogenic product (GHPO; DCAD = -207 mEq/kg DM), alfalfa hay and calcium chloride as an acidogenic product (AHCL; DCAD = -190 mEq/kg DM), or alfalfa hay and polyhalite as an acidogenic product (AHPO; DCAD = -194 mEq/kg DM). Error bars represent standard errors of the means (SEM).

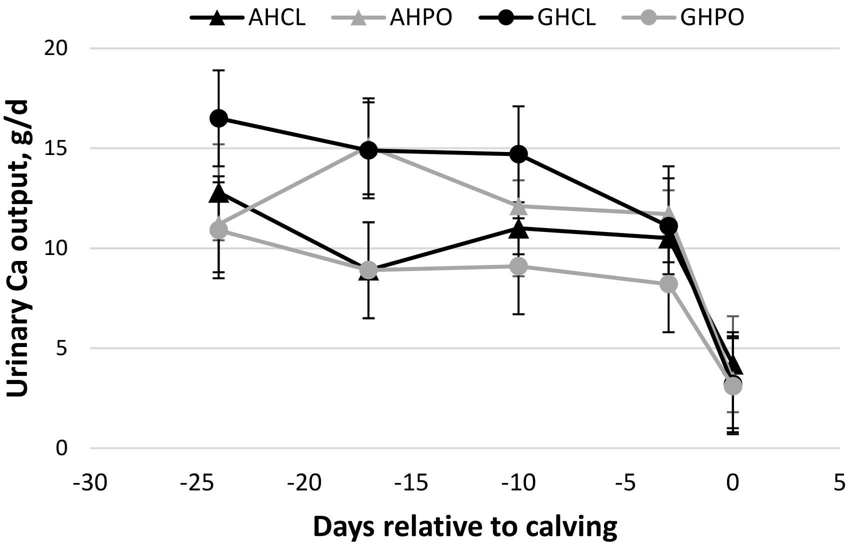

Figure 4. Urinary Ca output from pregnant and non-lactating dairy cows during the prepartum period. Cows consumed diets containing grass hay and calcium chloride as an acidogenic product (GHCL; DCAD = -209 mEq/kg DM), grass hay and polyhalite as an acidogenic product (GHPO; DCAD = -207 mEq/kg DM), alfalfa hay and calcium chloride as an acidogenic product (AHCL; DCAD = -190 mEq/kg DM), or alfalfa hay and polyhalite as an acidogenic product (AHPO; DCAD = -194 mEq/kg DM). Error bars represent standard errors of the means (SEM).

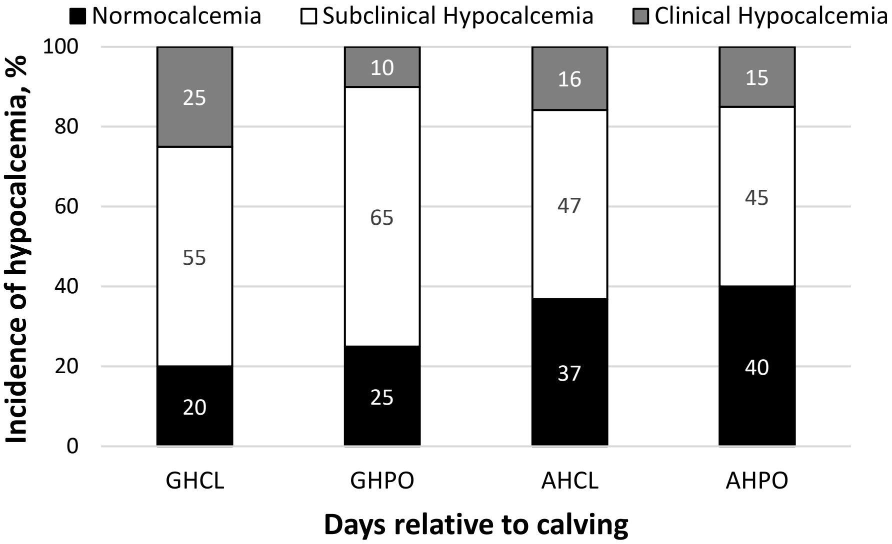

Figure 5. Incidence of normocalcemia ([Ca] > 8.0 mg/dL during the first 4 days after calving), subclinical hypocalcemia (5.5 < [Ca] < 8.0 mg/dL at least once in the first 4 days after calving), and clinical hypocalcemia ([Ca] < 5.5 mg/dL at least once in the first 4 days after calving) in pregnant and non-lactating dairy cows during the prepartum period. Cows consumed diets containing grass hay and calcium chloride as an acidogenic product (GHCL; DCAD = -209 mEq/kg DM), grass hay and polyhalite as an acidogenic product (GHPO; DCAD = -207 mEq/kg DM), alfalfa hay and calcium chloride as an acidogenic product (AHCL; DCAD = -190 mEq/kg DM), or alfalfa hay and polyhalite as an acidogenic product (AHPO; DCAD = -194 mEq/kg DM).

In this study, we hypothesized that alfalfa hay can be included in prepartum diets for pregnant and non-lactating cows without increasing hypocalcemia as long as a negative DCAD can be obtained through the use of acidogenic products. Based on the concentrations of CP and NDF, the AH and GH in this study would fit, respectively, the mature legume hay (NRC16F92) and the mature grass hay (NRC16F33) according to NASEM (2021). As expected, AH had a greater concentration of K than GH (2.50 vs. 1.88% K), which should demand greater inclusions of acidogenic products (based solely on the concentration of K) to obtain a similar negative DCAD. The concentration of K in the AH used in this study was 7% greater than the mean concentration reported by NASEM (2021) for mature legume hay (2.50 vs. 2.34% K), whereas the concentration of K in the GH used in this study was 15% greater than the mean concentration reported by NASEM (2021) for mature grass hay (1.63% K).

Despite the greater concentration of K, AH had a cation-anion difference similar to that of GH (294 and 289 mEq/kg DM, respectively; Table 3), which contradicts our expectations of observing a much greater cation-anion difference for AH than for GH. According to NASEM (2021) the cation-anion difference is 387 mEq/kg DM for mature legume hay and 187 mEq/kg DM for mature grass hay, which agrees with our expectation of observing a much greater cation-anion difference for alfalfa hay than for grass hay. Therefore, in this study the cation-anion difference of the AH was lower than expected and the cation-anion difference of the GH was greater than expected.

The unexpected cation-anion differences of the forages can be attributed to at least two factors, which are soil mineral concentrations (Goff et al., 2007) and fertilization (Goff et al., 2007; Penner et al., 2008). Goff et al. (2007) grew alfalfa on soils with different concentrations of Cl in the soil (3.3 and 2.2 mg Cl/kg soil) and observed that the cation-anion difference was 184 mEq/kg DM when alfalfa grew in the soil containing more Cl and 394 mEq/kg DM when alfalfa grew in the soil containing less Cl. In that same study, Goff et al. (2007) fertilized alfalfa with Cl-based fertilizers and observed that the concentration of Cl increased (0.52 vs. 0.84% Cl) and the cation-anion difference of the alfalfa decreased (288 vs. 194 mEq/kg DM). Similarly, Penner et al. (2008) evaluated the effect of fertilization of timothy field with $CaCl2$ and observed that the concentration of Cl increased (0.17 vs. 0.75% Cl) and the cation-anion difference of the timothy decreased (145 vs. 16 mEq/kg DM). Even though we ignore them, the soil mineral concentrations, the fertilization practices, or both could explain the similar cation-anion differences of the hays used in this study.

Obtaining a negative DCAD through the use of acidogenic products was paramount to show that AH can be included in prepartum diets for pregnant and non-lactating cows. Regardless of the type of hay, feeding diets with negative DCAD decreased urine pH to values below 6.5 for all diets (Figure 2), which supports our hypothesis that alfalfa can be fed as long as a strong negative DCAD is obtained. Even more, cows consuming diets with AH had a lower urine pH than cows consuming diets with GH (Figure 2), and this was attributed to the higher urine pH observed for cows consuming the GHPO diet.

Testing the inclusion of polyhalite as an acidogenic product is quite relevant in this study. Polyhalite mineral ($K2SO4·2CaSO4·MgSO4·2H2O$) is a natural and abundant mineral found in rock salt formations (Peryt et al., 1998; Wollmann et al., 2008) that contains two equivalents of K+ and four equivalents of SO42−. Despite the presence of K, Ferreira et al. (2019) and Richardson et al. (2021) evaluated the inclusion of polyhalite in prepartum diets and consistently reported reductions of urine pH when acidogenic diets (i.e., DCAD < -150 mEq/kg DM) containing polyhalite were fed to pregnant and non-lactating dairy cows. In this line, Zimpel et al. (2018) fed diets containing >1.75% K but with positive or negative DCAD (194 and -113 mEq/kg DM, respectively) to pregnant heifers and reported a urine pH equal to 7.9 for heifers consuming the positive DCAD diets and a pH less than 6.0 for the heifers consuming the negative DCAD diets. All these observations highlight that the acidogenic property of the diet is more relevant than the concentration of K in the diet to reduce urine pH.

In this study, we expected a greater cation-anion difference for alfalfa hay than for grass hay (which did not occur). Under our hypothesis, we also expected to include greater proportions of acidogenic product in the diets containing AH than in the diets containing GH, which might have resulted in reduced DMI (Oetzel and Barmore, 1993; Lean et al., 2019; Santos et al., 2019). In this study, DMI did not differ among diets, although cows consuming the AHCL tended to consume less than all other cows (Figure 1). Concerns exist about potential reductions of DMI when feeding of acidogenic products due to palatability or the acid-base status of the diet (Zimpel et al., 2018). From a palatability perspective, cows consumed >275 g/d of CaCl2 or >420 g/d of polyhalite, as in previous studies (Ferreira et al., 2019; Richardson et al., 2021) and always via a pelleted concentrate. In other studies, commercial acidogenic products have been fed at rates greater than 420 g/d (Wu et al., 2014; Glosson et al., 2020).

Based on the concentrations of Ca in plasma, 16.7% of the cows had clinical hypocalcemia ([Ca] < 5.5 mg/dL) and 55.6% of the cows had clinical subclinical hypocalcemia (5.5 mg/dL < [Ca] < 8.0 mg/dL) in this study. Although they might seem excessive, similar incidences have been previously reported (Hendriks et al., 2020; McArt and Neves, 2020; Seely et al., 2021). It is worth highlighting that only 1 of the 13 cases of clinical hypocalcemia resulted in periparturient paresis. Similar to this study, Hendriks et al. (2020) classified 36 of 106 cows (33.9%) as clinically hypocalcemic but without signs of parturient paresis. Despite the minimal occurrence (one case only) of periparturient paresis among clinically hypocalcemic cows, 7 of the 13 cows with hypocalcemia showed signs of clinical hypocalcemia, such as cold ears or wobbly gait (Goff and Koszewski, 2018). Conversely, 9 cows that did not have Ca concentrations in plasma below 5.5 mg/d showed signs of clinical hypocalcemia (Goff and Koszewski, 2018), although they did have Ca concentrations in plasma between 5.5 and 8.0 mg/dL (several of these, slightly above the 5.5 mg/dL threshold).

Regarding diets, the concentrations of Ca in plasma did not differ among diets and neither existed an interaction between time and diets (Figure 3). From a categorical analysis, however, cows consuming diets containing AH had a greater incidence of normocalcemia (37 and 40% for AHCL and AHPO, respectively) than cows consuming diets containing GH (20 and 25% for GHCL and GHPO, respectively; Figure 5). These observations indicate that including alfalfa hay in prepartum diets for pregnant and non-lactating cows does not necessarily increase the incidence of hypocalcemia.

As expected, a decrease of Ca output in urine was observed for all diets around calving (Figure 4). Regarding diets, cows consuming the GHCL diet excreted the greatest amount of Ca in urine and cows consuming the GHPO diet excreted the least amount of Ca in urine. The low output of urinary Ca for the GHPO diet coincides with the greater urinary pH (Figure 2). The reasons for the different Ca output in urine are not totally clear but the different dietary concentrations of Ca (1.10 vs. 0.88% Ca for GHCL and GHPO, respectively) and the difference in urine pH suggest that Ca metabolism of cows was affected by consuming these diets. The source of anions (Cl-based vs. SO4-based) could also explain this difference (Goff et al., 2004) although such a difference was not observed within the AH diets.

The **overall conclusion** of this study is that the inclusion of alfalfa hay in prepartum diets for pregnant and non-lactating cows does not necessarily increase the incidence of hypocalcemia relative to prepartum diets including grass hay. Another conclusion worth highlighting is that the cation-anion difference of the specific alfalfa hay is a determinant of whether alfalfa hay fits in a prepartum feeding program for pregnant and non-lactating cows. In this regard, more research evaluating the fertility of the soil and the use of acidifying fertilizers is needed to better understand how to incorporate alfalfa hay in prepartum diets for pregnant and non-lactating dairy cows in the prepartum period.

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