

# Effect of Cation-Anion Difference on Measures of Acid-Base Physiology and Performance in Beef Cattle

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## Introduction

In Florida, the dietary cation-anion balance (**DCAD**) characteristic of bahiagrass (*Paspalum notatum*) can be highly variable. This is a result of fluctuations in all four elements making up the core DCAD equation, Na, K, Cl, and S. Bahiagrass receiving annual contributions of S from ammonium sulfate fertilizer can accumulate high levels of S. In a previous study (Arthington et al. 2002), bahiagrass fertilized with ammonium sulfate (60.0 lb S/acre) contained an average of 0.50% S (DM basis). Although cow acid-base physiology was not measured, the high-S content of the ammonium sulfate fertilized pastures resulted in a decline in Cu accumulation in cows grazing these pastures. The ability of high-S content bahiagrass to alter Cu status suggests additional important metabolic responses may be occurring in cows.

Unlike dairy cows, the influence of forage DCAD on the performance of grazing beef cows has received little attention. For dairy cattle nearing parturition, nutritionists often formulate rations to contain a negative DCAD balance (-10.0 to -15.0 meq/100 g DM) to control milk fever. The resulting acidic diet leads to a decrease in urine and blood pH and a concurrent increase in the mobilization of stored Ca. Acidic diets are also correlated with noticeable decreases in DMI in both dairy cows (Vagnoni and Oetzel, 1998) and beef cattle (Ross et al., 1994a,b).

Sufficient data exists demonstrating differences in DCAD that translate to differences in blood acid-base (Pehrson et al., 1999; Roche et al., 2003; Ross et al., 1994a,b) and urinary chemistry (La Manna et al., 1999; Roche et al., 2003; Vagnoni and Oetzel, 1998). Additionally, Elrod and Butler (1993) and Elrod et al. (1993) demonstrated that excess dietary protein can significantly lower uterine pH and first service conception rate. Considering together the sensitivity of blood and urine acid-base chemistry to differences in DCAD and dietary manipulation of uterine pH, differences in uterine pH may arise from differences in dietary DCAD. Our objective was to determine if a forage-based ration DCAD would affect uterine pH in non-

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pregnant beef cows. To date we have conducted two experiments to address the issue of DCAD in beef cows. In Experiment 1 we wanted to demonstrate that differences in DCAD could indeed affect beef cow physiology. Building on the results of Experiment 1, in Experiment 2 we wanted to see if supplement treatments could alleviate the effects of a negative DCAD on beef cow physiology.

## **Materials and Methods**

### *Animal Management*

#### Experiment 1

Twenty-four non-pregnant, Brahman x British crossbred cows (initial BW = 1,148 ± 40 lb) were randomly assigned to one of two treatments. Treatments were one of two bahiagrass hay sources, one from a field fertilized with ammonium nitrate that resulted in a positive (**High-DCAD**) or from a field fertilized with ammonium sulfate that resulted in negative DCAD (**Low-DCAD**) balance. Both treatments were supplemented with ground corn and soybean meal (Table 1) to meet energy and protein requirements of the cows. Initial analysis of the hays indicated little difference in actual DCAD. Therefore, the diets were supplemented with Soy-Chlor (West Central Cooperative, Ralston, IA) or Na-sesquicarbonate to result in substantially different DCAD values.

All cows were housed in individual pens in a barn with concrete floors (161 ft<sup>2</sup>). Both hay types were ground with a tub grinder to pass a 3.5 cm screen. Cows were fed their daily ration once daily for 42 d. Hay was offered in amounts to ensure ad libitum access. Water was provided ad libitum throughout the entire experiment. Cows were withheld from feed and water for 16 h and shrunk BW of the cows was taken at the initiation of the experiment and after 42 d. Cow DMI of hay was measured daily for 5 d prior to initiation of the experiment (Period 1), d 0 to 10 (Period 2), and d 28 to 36 (Period 3) of the experiment. Two cows from the High-DCAD and one cow from the Low-DCAD were removed from the experiment because they would not consume the supplement.

Estrus cycles of the cows were synchronized. All cows were administered 25 mg IM of PGF<sub>2α</sub> (Lutylase, Pfizer, New York, NY) on d 0 and 11 of the trial. Cows were synchronized to eliminate the potential for uterine pH measurements being confounded by stage of the estrus cycle.

#### Experiment 2

Twenty-one non-pregnant Braford cows (initial BW = 1,184 ± 90 lb). All cows were fed the same limpograss hay and a soybean meal/Soy Chlor supplement as the basal diet (Table 2). All treatments were supplemented with soybean meal and either corn or molasses to meet energy and protein requirements of the cows. Three treatments were utilized: 1) Control basal diet with corn, 2) Molasses (basal diet with molasses replacing corn), and 3) Molasses+buffer (basal diet with molasses replacing corn and buffer added).

All cows were housed in individual pens in a barn with concrete floors (161 ft<sup>2</sup>). Limpograss hay was ground with a tub grinder to pass a 3.5 cm screen. Cows were fed their daily ration once daily for 42 d. Hay was offered in amounts to ensure ad libitum access. Water was provided ad libitum throughout the entire experiment. Cows were withheld from feed and water for 16 h and shrunk BW of the cows was taken at the initiation of the experiment and after 42 d. Cow DMI of hay was measured daily during the experiment. The daily Soy-Chlor feeding was based upon the previous daily hay DMI. The amount of Soy-Chlor was fed to maintain a constant DCAD according to the treatment protocol despite variation in hay DMI.

Similar to Experiment 1, all cows were synchronized to eliminate the potential for uterine pH measurements being confounded by stage of the estrus cycle. On day -17 all cows received a CIDR and 100 µg IM of GnRH (Fertagyl®). On day -10, the CIDR were removed and all cows were injected IM with 25 mg of prostaglandin F<sub>2α</sub> (Lutalyse® Sterile Solution). Then on day 11 all cows were again administered 100 µg IM of GnRH.

### *Sample Collection and Analysis*

#### Experiment 1 and 2

Blood, urine, and uterine flush samples were collected from all cows approximately 2 h after rations were offered on d 0, 21, and 42 of the experiment. Blood was collected by jugular venipuncture into a syringe. Whole blood samples were analyzed chute-side for pH and blood gases using an Osmetech Opti CCA machine with Type B cassettes (Osmetech Inc, Roswell, GA). Urine samples were collected into plastic cups. Urine pH was determined using an Accumet AB15 pH meter and probe. Uterine flush samples were collected by passing a sterile foley 2-way, 18 fr catheter (C. R. Bard, Covington, GA) into the uterus of the cow. Sixty mL of sterile saline was gently infused into the uterus through the catheter. Saline was allowed to equilibrate in the uterus for 90 s, and then flushed out of the uterus through the catheter into a cup. Uterine flush pH was determined with similar equipment as urine. The pH of the sterile saline was used to standardize the pH calibration prior to measurement of the uterine flush.

### *Statistical Analysis*

#### Experiment 1 and 2

All BW, ADG, DMI, blood, urine, and uterine data were analyzed as a completely random design using the Mixed procedure of SAS (SAS Inst. Inc., Cary NC). The statistical model for performance parameters of BW, ADG, and DMI and the physiological parameters of blood, urine, and uterine data included treatment as the fixed effect. The experimental unit was cow, and the random term was cow within treatment. Blood, urine, and uterine data were analyzed by day of the experiment. Because of missing observations, least squares means were utilized. For all data, differences between means were considered significant if  $P < 0.05$ .

## Results and Discussion

### *Cow Performance and Dry Matter Intake*

Experiment 1. Final cow BW (Table 3) was not different ( $P = 1.00$ ) between High- and Low-DCAD treatments. Mean ADG was 0.28 lb/d during the 42 d experiment and did not differ between ( $P = 0.71$ ) treatments. Prior to the initiation of the experiment (Period 1), mean daily hay DMI was not different ( $P = 0.84$ , mean = 17.1 lb), nor was DMI, % BW ( $P = 0.95$ , mean = 1.45%) between treatments. During Period 2, hay DMI was numerically lower in both treatments because of the addition of the supplement. Hay DMI (kg and % of BW) in Period 2 did not differ ( $P = 0.70$  and 0.56, respectively) between High- and Low-DCAD cows. In Period 3, High-DCAD cows had 13.5% greater ( $P = 0.01$ ) hay DMI than Low-DCAD cows. Similarly, during Period 3, hay DMI, % of BW, was 11% greater ( $P = 0.04$ ) for High-compared with Low-DCAD cows.

Experiment 2. Initial and final cow BW (Table 4) exhibited more variation than in Experiment 1, as a result final cow BW did not differ between treatments ( $P=0.48$ ). Likewise, mean ADG was not different ( $P=0.47$ ) between treatments in Experiment 2. In Experiment 2, mean ADG was 2.22 lb/d which was considerably greater than in Experiment 1. Weekly hay DMI and hay DMI, % of BW, did not differ between treatments (data not shown). Mean hay DMI across the six weeks of the experiment was 15.4 lb/d and was not different ( $P=0.39$ ) among treatments. Similarly, hay DMI, % of mean feeding BW, was not different ( $P=0.19$ ; 1.3%) among treatments.

Differences in hay source and supplement amount likely contributed to the profound differences in BW responses between Experiment 1 and 2. Nearly twice as much corn/molasses was utilized in the supplement of Experiment 2 as was utilized in Experiment 1. The additional energy supplied likely masked any potential differences in BW response and hay DMI among the treatments in Experiment 2.

Roche et al. (2005) reported no difference in DMI of grazing cows with pasture forage DCAD values of +23 to +88 mEq/100 g of DM. These authors indicated that altering pasture forage DCAD through fertilization has resulted in inconsistent effects on the final forage DCAD. However, other research using formulated diets have reported DCAD effects on DMI. Ross et al. (1994b) using growing beef steers and finishing beef steers (Ross et al., 1994a) reported increased DMI as DCAD increased from 0 to +45 mEq/100 g of DM during 84 d in the growing and finishing stages. Similarly, Tucker et al. (1988) reported that a DCAD of -10 mEq/100 g of DM decreased DMI of lactating dairy cows compared with cows consuming rations with DCAD values of 0, +10, and +20 mEq/100 g of DM. In contrast, Jackson et al. (2001) reported no difference in DMI of young dairy calves consuming diets with DCAD values of 0 or +200 mEq/kg. However, those authors did report increased DMI for all groups over the 8-wk experiment, which was in contrast to their previous work. An optimum DCAD range of +15 to +20 mEq/100 g of DM (Roche et al., 2000) has been indicated to positively affect DMI in dairy cows (Sanchez et al., 1994; Roche et al., 2005).

The influence of DCAD on DMI has a direct effect on the supply of nutrients for maintenance, growth, gestation, and lactation. Pehrson et al. (1999) indicated that differences in DCAD resulted in decreased DMI and thus energy and protein consumed by cows. The decreased DMI associated with negative DCAD could be influenced by the resulting acid-base balance (Pehrson et al., 1999) or metabolic acidosis (Vagnoni and Oetzel, 1998).

### *Acid-Base Physiology*

Experiment 1. Blood pH (Figure 1) was greater in High-DCAD cows than Low-DCAD cows after DCAD treatments were offered. High-DCAD cows had blood pH that was 0.05 and 0.04 pH units greater ( $P=0.002$ ,  $0.04$ ) than Low-DCAD cows on d 21 and 42 respectively. Other blood acid-base parameters were affected by DCAD in Experiment 1 (Table 5). Blood bicarbonate concentration on day 21 and 42 was greater ( $P<0.001$  and  $P=0.05$ ) in High-DCAD cows compared to Low-DCAD cows. Blood base excess (BE) in High-DCAD cows was increased ( $P < 0.001$ ) 5.22 mmol/L compared to Low-DCAD cows. On day 42 High-DCAD cows continued to have a 3.33 mmol/L greater ( $P=0.03$ ) BE concentration than Low-DCAD cows.

Experiment 2. In contrast to Experiment 1, blood pH in Experiment 2 (Figure 4) did not differ after day 0 when the dietary treatments were fed. Blood pH remained remarkably consistent in the Molasses and Molasses+Buffer treatment throughout the 42-d experiment. In fact, blood pH numerically was more variable between sampling dates in the Control diet. Other blood gas measurements of BE and blood bicarbonate (Table 6) were not affected by the DCAD supplement interaction. Blood BE generally increased in all treatments with increasing days on feed. Likewise, blood bicarbonate increased after day 0 in all three treatments. This trend is similar to the High-DCAD treatment in Experiment 1, however, the Low-DCAD treatment in Experiment 1 exhibited moderate decreases in BE and bicarbonate values. In contrast, blood BE and bicarbonate concentrations of the Control diet in Experiment 2, which had a negative DCAD value, were apparently unaffected.

Beef steers during the growing phase (Ross et al., 1999b) and finishing phase (Ross et al., 1999a) had decreased blood pH by 42 days on feed as DCAD was decreased from +45 to 0 mEq/100 g of DM. Likewise, Roche et al. (2005) observed a linear decrease in blood pH as DCAD of grazing cows decreased from +88 to +23 mEq/100 g of DM. Xin et al. (1991) reported no difference in blood pH at week four of a feeding trial, but reported differences in blood pH at eight wk in dairy calves consuming rations with DCAD values of +16.87 and -10.88 mEq.

Numerous experiments have reported increased blood bicarbonate in response to increasing DCAD in growing beef steers (Ross et al., 1994a,b) and dairy cows (Roche et al., 2003, 2005). In contrast, Pehrson et al. (1998) reported no difference in blood bicarbonate after two or three wk of cows consuming diets that differed in DCAD by over 2000 mEq. Roche et al. (2005) reported a linear decline of

BE in grazing cows in response to oral drenching to elicit DCAD values of +23, +45, +70, or +88 mEq/100 g of DM. Vagnoni and Oetzel (1997) reported negative BE in cows fed diets formulated for DCAD of -51, -40, and -63 compared with positive BE in control diets with a DCAD value of +203. Interestingly, Pehrson et al. (1998) observed no decline in BE after three wk from cows on a forage based diet with DCAD values of either +2,275 or -262 mEq/d. Once DCAD values reached -1,185 mEq/d, BE was lowered by 2.75 mM.

There appears to be a lag in blood pH in response to changes in DCAD values in Experiment 1 and some previously published data. The lag in blood pH may be a result of a more rapid response observed in acid-base parameters such as pCO<sub>2</sub>, bicarbonate, and BE. Blood pH is a highly regulated physiological property and thus no effect of DCAD on blood pH has been previously reported (Vagnoni and Oetzel, 1997; Pehrson et al., 1998; Jackson et al., 2001). The observed changes in blood bicarbonate, BE, and to a lesser extent pCO<sub>2</sub> in response to differences in DCAD in the current experiment were likely adequately compensated by non-respiratory mechanisms (Vagnoni and Oetzel, 1998).

#### *Urine and Uterine Response*

Experiment 1. Urine pH (Figure 2) was responsive to DCAD in both treatments. Urine pH increased in High-DCAD cows and was decreased in Low-DCAD cows (treatment x day,  $P < 0.001$ ). By d 21, maximum and minimum urine pH was exhibited by High- and Low-DCAD cows, respectively. The decrease of urine pH by 1 pH unit in Low-DCAD cows demonstrates the capacity of the body to disseminate an acid load from the blood into other body tissues and fluids. Scant data exists for the effect of diet on uterine pH in beef cows. High-DCAD cows had greater ( $P = 0.08$ ) uterine pH compared with Low-DCAD cows (Figure 3). In High-DCAD cows, uterine pH increased ( $P = 0.11$ ) from d 0 to 42. On d 42 of the experiment, uterine pH was greater ( $P = 0.04$ ) for High-DCAD than Low-DCAD cows.

Experiment 2. Like the first experiment, urine pH of cows in Experiment 2 was responsive to the DCAD of the different treatments (Figure 5). On day 21, urine pH of the Control cows was 1.58 and 1.86 units less ( $P < 0.001$ ) than the urine of Molasses or Molasses+Buffer treatment cows, respectively. However on day 42, urine pH of Control cows and Molasses did not differ ( $P > 0.05$ ), but urine pH of Control and Molasses cows was decreased ( $P = 0.002$ ) compared to the Molasses+Buffer cows. Uterine pH was lower ( $P = 0.02$ ) for Molasses cows compared to Molasses+Buffer cows on day 0 of the experiment. Uterine pH of Molasses cows continued to be numerically less than Control or Molasses+Buffer cows. On day 42 uterine pH did not differ ( $P = 0.69$ ) among treatments. On day 42, uterine pH of Control cows was similar to the day 0 value, whereas uterine pH of Molasses and Molasses+Buffer cows had increased 0.21 and 0.09 units, respectively. The uterine pH data in Experiment 2 is within the range of those observed in Experiment 1. However, the number of experimental units was decreased in Experiment 2. The decrease in the number of cows per treatment in

Experiment 2 coupled with the tenuous measurement of uterine pH may have conspired to mask any treatment differences.

Vagnoni and Oetzel (1998) reported differences in urine pH between positive and negative DCAD diets similar to the 1.5 pH unit difference between treatments that we observed in Experiment 1 and 2 on d 21. Likewise Jackson et al. (2001) observed differences in urine pH similar to the 1.35 pH unit difference between treatments on d 42 in the Experiment 1. The increase in urine pH of Control cows from day 21 to 42 in Experiment 2 is not readily explainable in light of the results of Experiment 1, where Low-DCAD cows' urine pH decreased after day 0 and did not greatly increase across the 42 days on feed. The feeding regime implemented in Experiment 2 resulted in a constant intake of negative DCAD supplement in the basal diet to account for the differences in hay DMI. Indeed the amount of negative DCAD supplement increased with increasing days on feed during Experiment 2.

Elrod and Butler (1993) and Elrod et al. (1993) observed increases in uterine pH (6.8 to 7.1) from d 0 to 7 of the estrus cycle. The uterine pH reported in the current study is considerably lower in both treatments compared with the uterine pH values previously reported (Elrod and Butler, 1993; Elrod et al., 1993). Uterine pH differences measured in Elrod and Butler, (1993) and Elrod et al. (1993) were the result of differences in protein supplementation. Differences in uterine pH were elicited by feeding 50% greater degradable intake protein and 25% greater degradable intake protein or undegradable intake protein. In the experiments of Elrod, high protein concentrations and thus high PUN affected uterine pH in the absence of a blood pH effect through some unknown mechanism.

Additionally, the absence of an increase in uterine pH in the Low-DCAD cows with increasing days after synchronization would be of some concern. Ejaculated bull semen has a reported pH of 6.8 (Elrod and Butler, 1993). The difference of 0.58 to 0.67 pH units between the uterine environment and the ejaculate could be of potential concern in terms of sperm capacitation, sperm viability, fertilization, and embryo implantation.

### **Implications**

Grazed and harvested forages make up the majority of a mature beef cow's diet. Forage based diets of beef cows can be manipulated through supplementation to influence dietary cation-anion difference. In the one study, differences in dietary cation-anion difference elicited responses in cow hay intake, acid-base physiology, and the uterine environment. Subtle changes in blood pH may have affected uterine environmental pH in one instance. Alterations of the uterine environment could potentially affect any number of the physiological processes that are required for successful reproductive performance. Additional investigation will be required to elucidate the mechanism of how dietary cation-anion difference might affect the uterine environment and subsequent reproductive ability.

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Table 1. Diet composition of the ration fed to cows to achieve high- or low-dietary cation-anion difference (DCAD) (Experiment 1).

Ingredient, % DM	High-DCAD	Low-DCAD
Nitrate fertilized hay	87	-
Sulfate fertilized hay	-	87
Soybean meal	3.2	1.0
Ground corn	8.5	9.4
Soy-Chlor	-	2.6
Na Sesquicarbonate	1.3	-
Analysis		
TDN, %	56.5	57.5
CP, %	10.0	10.3
Ca, %	0.403	0.449
P, %	0.190	0.189
K,	1.166	1.161
Na,	0.412	0.018
Cl,	0.472	0.598
S,	0.195	0.336
Mg,	0.281	0.310
DCAD, meq/kg <sup>a</sup>	250	-9

<sup>a</sup> DCAD = (Na + K + 0.15Ca + 0.15Mg) – (Cl + 0.60S + 0.50P).

Table 2. Diet composition of the ration fed to cows to achieve high- or low-dietary cation-anion difference (DCAD) (Experiment 2)

Ingredient, % DM	Control	Molasses	Molasses +Buffer
Low DCAD hay	76.87	75.62	73.87
Molasses	-	18.0	18.0
Soybean meal	3.75	4.0	4.0
Ground corn	17.0	-	-
Soy-Chlor	2.38	2.38	2.38
Na Sesquicarbonate	-	-	1.75
Analysis			
TDN, %	58.6	57.3	56.4
CP, %	7.90	7.78	7.67
Ca, %	0.457	0.646	0.636
P, %	0.239	0.197	0.194
K,	0.947	1.877	1.860
Na,	0.025	0.043	0.568
Cl,	0.647	1.128	1.114
S,	0.194	0.355	0.352
Mg,	0.250	0.290	0.285
DCAD, meq/kg <sup>a</sup>	-31	29	258

<sup>a</sup> DCAD = (Na + K + 0.15Ca + 0.15Mg) – (Cl + 0.60S + 0.50P).

Table 3. Effect of high- and low- dietary cation-anion difference (DCAD) on mature beef cow performance and intake (Experiment 1).

Item	Dietary Treatment <sup>a</sup>		SEM <sup>b</sup>	P-value
	High-DCAD	Low-DCAD		
Initial BW, lb	1,152	1,143	40.3	0.89
Final BW, lb	1,161	1,157	41.2	1.00
ADG, lb/d	0.22	0.33	0.331	0.71
Hay DMI, lb				
Period 1	17.2	16.9	0.75	0.84
Period 2	15.8	16.4	1.01	0.70
Period 3	18.9	16.7	0.62	0.01
Hay DMI, % of BW				
Period 1	1.45	1.46	0.07	0.95
Period 2	1.34	1.41	0.09	0.56
Period 3	1.61	1.45	0.05	0.04

<sup>a</sup> High- and low-DCAD treatments = 250 and -9 meq/kg respectively.

<sup>b</sup> SEM = Standard error of mean, High-DCAD (n = 10) Low-DCAD (n = 11).

Table 4. Effect of high- and low- dietary cation-anion difference (DCAD) on mature beef cow performance and intake (Experiment 2).

Item	Dietary Treatment <sup>a</sup>			SEM <sup>b</sup>	P-value
	Control	Molasses	Molasses +Buffer		
Initial BW, lb	1,138	1,212	1,203	95	0.82
Final BW, lb	1,183	1,310	1,280	80	0.48
ADG, lb/d	2.17	2.69	1.81	0.657	0.47
Mean hay DMI, lb	15.0	14.9	16.3	0.84	0.39
Mean hay DMI, % of mean feeding BW	1.41	1.18	1.31	0.086	0.19

<sup>a</sup> Control, Molasses, Molasses+Buffer = -21, 31, 258 meq/kg respectively.

<sup>b</sup> SEM = Standard error of mean, (n = 7).

Table 5. Effect of high- and low- dietary cation-anion difference (DCAD) on mature beef cow blood acid-base physiology (Experiment 1).

Item	Dietary Treatment <sup>a</sup>		SEM <sup>b</sup>	P-value
	High-DCAD	Low-DCAD		
Base Excess, mmol/L				
Day 0	3.64	5.14	0.846	0.22
Day 21	9.59	4.37	0.898	<0.001
Day 42	6.74	3.41	1.005	0.03
Bicarbonate, mmol/L				
Day 0	27.71	29.55	0.858	0.14
Day 21	33.69	28.63	0.875	<0.001
Day 42	30.52	28.11	0.843	0.05

<sup>a</sup> High- and low-DCAD treatments = 250 and -9 meq/kg respectively.

<sup>b</sup> SEM = Standard error of mean, High-DCAD (n = 10) Low-DCAD (n = 11).

Table 6. Effect of high- and low- dietary cation-anion difference (DCAD) on mature beef cow blood acid-base physiology (Experiment 2).

Item	Dietary Treatment <sup>a</sup>			SEM <sup>b</sup>	P-value
	Control	Molasses	Molasses +Buffer		
Base Excess, mmol/L					
Day 0	-6.87	-1.93	-2.66	2.395	0.32
Day 21	-1.50	2.21	2.93	1.618	0.14
Day 42	0.34	1.12	-0.24	2.892	0.94
Bicarbonate, mmol/L					
Day 0	18.85	23.23	22.36	2.106	0.33
Day 21	23.33	27.13	28.03	1.528	0.10
Day 42	24.44	26.16	24.39	2.795	0.87

<sup>a</sup> Control, Molasses, Molasses+Buffer = -21, 31, 258 meq/kg respectively.

<sup>b</sup> SEM = Standard error of mean, (n = 7).

Figure 1. The effect of high- and low- dietary cation-anion difference (DCAD) on mature beef cow blood pH. Effect of treatment (day 0,  $P=0.85$ ; day 21,  $P=0.002$ ; day 42,  $P=0.04$ ) (Experiment 1).

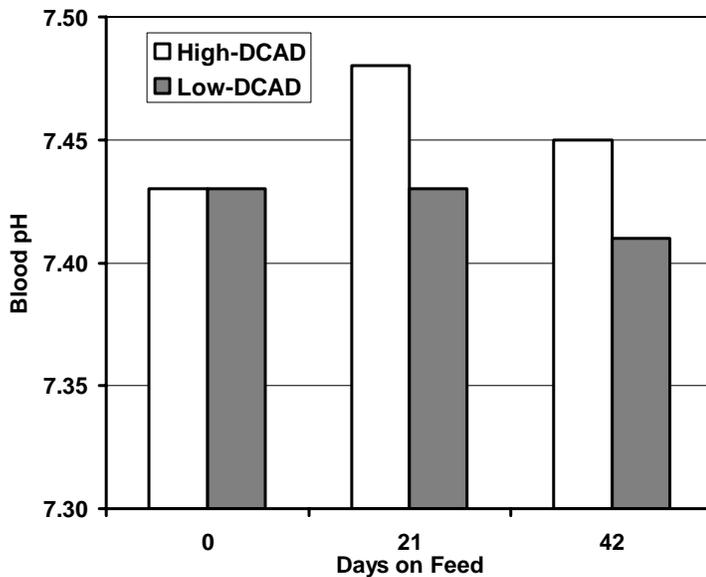


Figure 2. The effect of high- and low- dietary cation-anion difference (DCAD) on mature beef cow urine pH. Effect of treatment (day 0,  $P=0.34$ ; day 21,  $P<0.001$ ; day 42,  $P<0.001$ ) (Experiment 1).

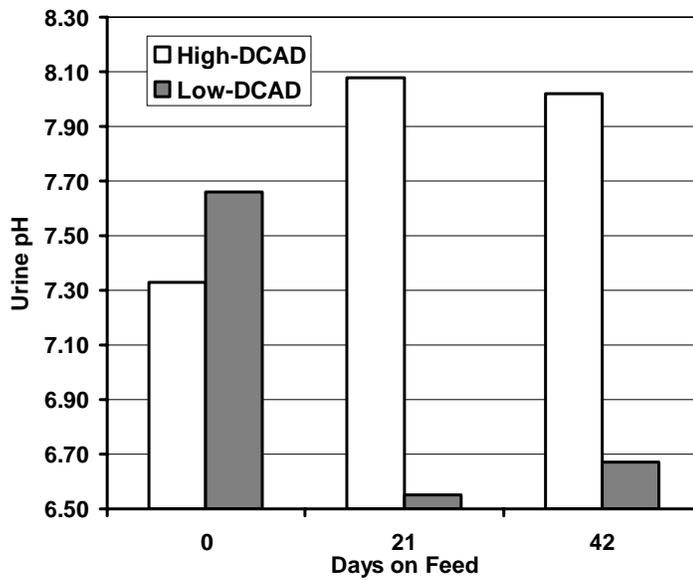


Figure 3. The effect of high- and low- dietary cation-anion difference (DCAD) on mature beef cow uterine flush pH. Effect of treatment (day 0,  $P=0.71$ ; day 21,  $P=0.11$ ; day 42,  $P=0.09$ ) (Experiment 1).

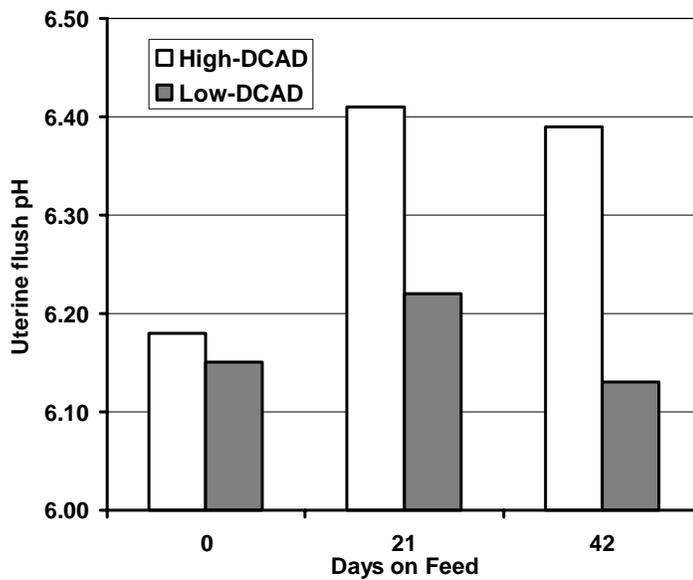


Figure 4. The effect of dietary cation-anion difference (DCAD) from different supplements on mature beef cow blood pH. Effect of treatment (day 0,  $P=0.53$ ; day 21,  $P=0.43$ ; day 42,  $P=0.83$ ) (Experiment 2).

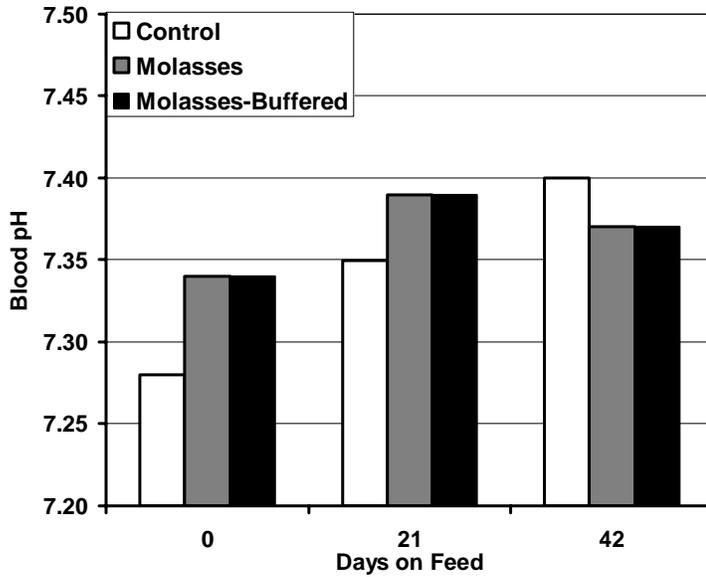
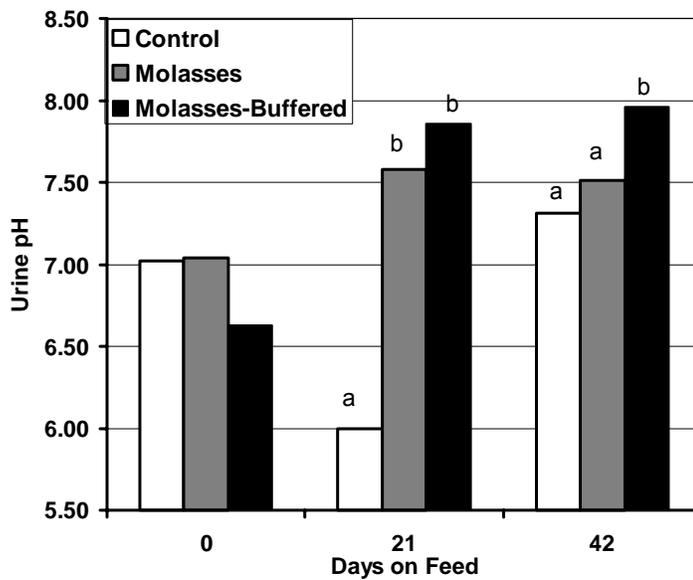
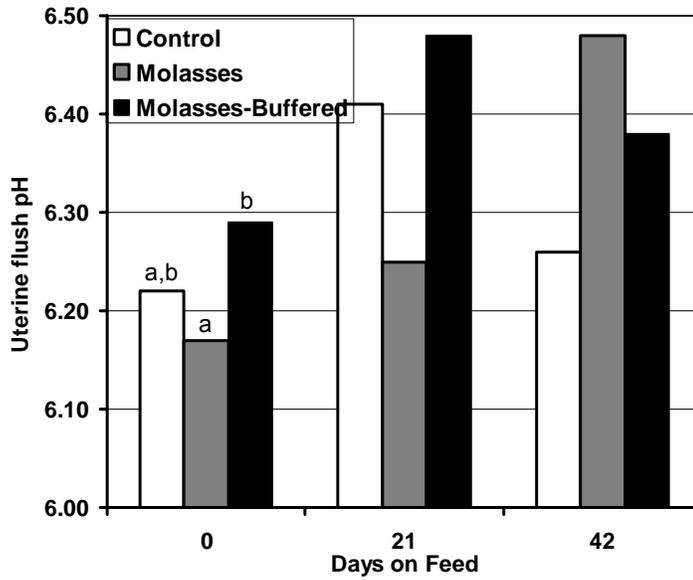


Figure 5. The effect of dietary cation-anion difference (DCAD) from different supplements on mature beef cow urine pH. Effect of treatment (day 0,  $P=0.38$ ; day 21,  $P<0.0001$ ; day 42,  $P=0.002$ ) (Experiment 2).



<sup>a,b</sup> Means with different superscripts differ,  $P<0.05$

Figure 6. The effect of dietary cation-anion difference (DCAD) from different supplements on mature beef cow uterine pH. Effect of treatment (day 0,  $P=0.02$ ; day 21,  $P=0.29$ ; day 42,  $P=0.69$ ) (Experiment 2).



<sup>a,b</sup> Means with different superscripts differ,  $P<0.05$